Planning Committee for a Workshop on Potential Health Hazards Associated with Consumption of Caffeine in Food and Dietary Supplements

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Workshop Planning Committee on Potential Health Hazards Associated with Consumption of Caffeine in Food and Dietary Supplements

Statement of Task

An ad hoc committee will organize a 2-day public workshop to discuss potential health impacts stemming from the consumption of caffeine in dietary supplements and conventional foods, alone or in combination with other substances found in products commonly referred to as “energy products.” The workshop will examine cardiovascular and central nervous system (CNS) effects and other important health hazards of caffeine that may arise in at-risk populations consuming varied amounts of caffeine. The committee will develop the agenda for the workshop, select and invite speakers and discussants, and moderate the discussions. The invited presentations and discussions will be structured to explore and discuss such topics as the following:

1. Evaluating the epidemiological, toxicological, clinical, and other relevant literature to identify and describe the important health hazards association with caffeine and potential data gaps;
2. Delineating particular populations who may be at risk from caffeine exposure, taking into account interactive effects from other ingredients in “energy products” and pre-existing medical conditions such as cardiovascular diseases;
3. Describing the risk for cardiovascular or other serious important health hazards for vulnerable populations, from exposure to caffeine-containing dietary supplements and conventional foods;
4. Identifying data gaps with regard to stimulant effects such as but not limited to caffeine on the cardiovascular and CNS systems; and
5. Exploring a safe level of exposure to caffeine for general and particular populations.
Caffeine in Food and Dietary Supplements: Examining Safety

Planning Committee on Potential Health Hazards Associated with Consumption of Caffeine in Food and Dietary Supplements

The National Academies Lecture Room
2100 Constitution Avenue NW, Washington, DC 20007

August 5-6, 2013

Workshop Objectives

- Evaluate the epidemiological, toxicological, clinical, and other relevant literature to describe important health hazards associated with caffeine consumption
- Delineate vulnerable populations who may be at risk from caffeine exposure
- Describe caffeine exposure and risk of cardiovascular and other health effects on vulnerable populations, including additive effects with other ingredients and effects related to pre-existing conditions
- Explore safe caffeine exposure levels for general and vulnerable populations
- Identify data gaps on caffeine stimulant effects including but not limited to cardiovascular, central nervous system, or other health outcomes

August 5, 2013

8:00–8:45 a.m. Registration

INTRODUCTION and Opening Remarks

8:50 Welcome
Lynn Goldman, George Washington University, Chair, Planning Committee on Potential Health Hazards Associated with Consumption of Caffeine in Food and Dietary Supplements

9:00 Opening Remarks
Margaret Hamburg, Commissioner of Food and Drugs, Food and Drug Administration
SESSION 1: INTAKE AND EXPOSURE TO CAFFEINE
 Moderated by Barbara Petersen, Exponent

9:15  Examining Exposure to Caffeine in Foods, Beverage, and Supplements

Caffeine Intakes from Beverages in the U.S.
Diane Mitchell, Penn State University

Trends in Caffeine Consumption
Victor Fulgoni, Nutrition Impact, LLC

10:00  Panel Discussion with Speakers

10:30  Break

SESSION 2: SAFETY SIGNALS AND SURVEILLANCE OF POPULATIONS
 Moderated by Steve Lipshultz, University of Miami

10:50  Type and Frequency of Caffeine Toxicity: US and International Surveillance
Alvin Bronstein, Poison Control Data System

11:10  Safety Assessment of Caffeine in Foods and Beverages
Ashley Roberts, Intertek-Cantox Consulting (by WebEx)

11:30  Panel Discussion with Speakers

12:00 p.m.  Break for Lunch

SESSION 3: CAFFEINE EFFECTS ON THE CARDIOVASCULAR SYSTEM
 Moderated by Stephen Daniels, Children’s Hospital Colorado

1:00  Vascular Effects of Caffeine
John Higgins, University of Texas Health Sciences Center

1:20  Caffeine and Risk of Arrhythmia
Jeffrey Goldberger, Northwestern University

1:40  Caffeine and Risk of Hypertension
Ahmed El-Sohemy, University of Toronto (by WebEx)

2:00  Panel Discussion with Speakers
SESSION 4: CAFFEINE EFFECTS ON THE CENTRAL NERVOUS SYSTEM

Moderated by Thomas Gould, Temple University

2:20 Neuropharmacologic Effects of Caffeine Exposure
Sergi Ferre, National Institutes of Health, National Institute on Drug Abuse

2:40 Developmental Neurological Effects of Caffeine Exposure
Jennifer Temple, University of Buffalo (by WebEx)

3:00 Panel Discussion with Speakers

3:20 Break

SESSION 5: PANEL DISCUSSION: BEHAVIORAL EFFECTS ASSOCIATED WITH CAFFEINE CONSUMPTION

Moderated by Richard Adamson, TPN Associates

3:30 Dependence/Tolerance
Roland Griffiths, Johns Hopkins University
Addiction
Charles O’Brien, University of Pennsylvania
Risk-Taking
Amelia Arria, University of Maryland

PUBLIC COMMENTS AND CONCLUDING REMARKS

4:30 Public Comments

5:00 Concluding Remarks for Day 1
Lynn Goldman, George Washington University, Chair, Planning Committee on Potential Health Hazards Associated with Consumption of Caffeine in Food and Dietary Supplements

5:10 pm Adjourn for the Day

August 6, 2013

8:50 a.m. Welcome and Summary from Day 1
Lynn Goldman, George Washington University, Chair, Planning Committee on Potential Health Hazards Associated with Consumption of Caffeine in Food and Dietary Supplements

9:00 Opening Remarks
Michael Taylor, Deputy Commissioner for Foods and Veterinary Medicine, Food and Drug Administration
# Institute of Medicine
Food and Nutrition Board and Board on Health Sciences Policy

## SESSION 1: OTHER COMPOUNDS IMPACTING CAFFEINE EFFECTS
*Moderated by: James Coughlin, Coughlin & Associates*

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<tr>
<td>9:15</td>
<td>Facilitated Discussion: Other Components Impacting Caffeine Effects</td>
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<td><em>Led by: Stephen Schaffer, University of South Alabama</em></td>
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<td><strong>Summary of the Issues</strong></td>
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## SESSION 2: USE OF CAFFEINATED PRODUCTS
*Moderated by: James Coughlin, Coughlin & Associates*

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<td>10:15</td>
<td>Trends in Usage and Potential Benefits from Caffeine</td>
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<td><em>Andrew Smith, Cardiff University, UK (by WebEx)</em></td>
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<td>Q&amp;A</td>
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## SESSION 3: EXPLORING SAFE CAFFEINE EXPOSURE LEVELS

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<tr>
<td>10:45</td>
<td>Panel Discussion: Exploring Safe Caffeine Exposure Levels for Vulnerable Populations</td>
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<td><strong>Panel Moderator</strong></td>
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<td><em>Mark Feeley, Health Canada</em></td>
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<td><strong>Pregnancy/Infants</strong></td>
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<td><em>Christina Chambers, University of California, San Diego</em></td>
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<td><strong>Children/ Young Adults</strong></td>
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<td><em>Steve Lipshultz, University of Miami</em></td>
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<td>Break for Lunch</td>
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## SESSION 4: DATA GAPS
*Moderated by: Joe Rodrigs, Environ International*

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<td>12:30</td>
<td>Panel Discussion on Data Gaps and Future Research</td>
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<td><em>Stephen Schaffer, University of South Alabama</em></td>
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<td><em>Steve Lipshultz, University of Miami</em></td>
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<td><em>Regan Bailey, National Institutes of Health Office of Dietary Supplements</em></td>
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<td>1:45</td>
<td>Chair’s Summary and Final Thoughts</td>
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<td><em>Lynn Goldman, George Washington University, Chair, Planning Committee on Potential Health Hazards Associated with Consumption of Caffeine in Food and Dietary Supplements</em></td>
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<td>2:00</td>
<td>Adjourn Meeting</td>
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Planning Committee for a Workshop on Potential Health Hazards Associated with Consumption of Caffeine in Food and Dietary Supplements

Lynn R. Goldman, M.D. (Chair)
Dean
School of Public health and Health Sciences
The George Washington University

James R. Coughlin, Ph.D.
President
Coughlin & Associates

Stephen R. Daniels, M.D., Ph.D.
Professor and Chair
Department of Pediatrics
University of Colorado
Pediatrician-in-Chief
Children’s Hospital Colorado

Thomas J. Gould, Ph.D.
Professor of Psychology
Department of Psychology
Director of the Neuroscience Program
Temple University
Philadelphia, PA

Steven E. Lipshultz, M.D.
Professor and Chairman Pediatrics and
Endowed Chair in Pediatric Cardiology
Children’s Hospital of the University of Miami

Carl L. Keen, Ph.D.
Professor and Chair
Department of Nutrition
University of California, Davis

Theresa Ann Nicklas, Dr.P.H.
Professor
Department of Pediatrics
Children’s Nutrition Research Center
Baylor College of Medicine
Houston, TX

Barbara J. Petersen, Ph.D.
Principal Scientist
Exponent, Inc.
Washington, DC

Joseph V. Rodricks, Ph.D.
Principal
Environ International Corp.
Arlington, VA

Staff
Clyde J. Behney, MBA, Acting Board Director, FNB
Andrew Pope, Ph.D., Board Director, Health Sciences Policy
Ann L. Yaktine, Ph.D., R.D. Study Director
Diana Pankevich, Ph.D., Senior Program Officer
Janet Mulligan, M.S., Research Associate
Geraldine Kennedo, Administrative Assistant
Planning Committee Biographical Sketches

**Lynn R. Goldman, M.D., M.S., M.P.H.**, is Dean of the School of Public Health and Health Services and Professor of Environmental and Occupational Health at the George Washington University. As Dean of SPHHS, Dr. Goldman’s responsibilities are informed by her broad and deep public policy and academic experience. She joined the school in August 2010 from Johns Hopkins University Bloomberg School of Public Health, where she had been Professor of Environmental Health Sciences since July 2000. In that capacity, she served as principal investigator of the National Children’s Study Center at Hopkins. Dr. Goldman was also the co-PI of the National Study Center for Preparedness and Critical Event Response, which supports the mission of the Department of Homeland Security through research and educational initiatives designed to build the science of preparedness. Prior to joining Hopkins, Dr. Goldman was Assistant Administrator for Toxic Substances in the U.S. Environmental Protection Agency from 1993 to 1998 under President Bill Clinton. Under her watch, the EPA overhauled the nation’s pesticide laws, expanded right-to-know requirements for toxic chemical releases, reached consensus on an approach to testing chemicals with endocrine-disrupting potential, developed standards to implement lead screening legislation, and promoted children’s health and global chemical safety. Dr. Goldman also worked in environmental health for the California Department of Public Health Services. Among other responsibilities, she managed a statewide environmental epidemiology program that focused on childhood lead poisoning, birth defects, and occupational health. Dr. Goldman received her BS from the University of California (UC), her MPH from Johns Hopkins University, her MS in Health and Medical Services from UC-Berkeley, and her MD from UC-San Francisco School of Medicine.

**James R. Coughlin, Ph.D.**, is President of Coughlin & Associates. He has over 35 years of experience in addressing and shaping the current understanding of food and nutrition science in the US and internationally, particularly in the areas of food, chemical and environmental toxicology and safety, chemical risk assessment, risk-benefit evaluation of foods and food ingredients, GRAS food additive safety evaluations, and scientific risk communication. Before undertaking his current role as an independent consultant in 1991, he spent ten years at General Foods Corporation and Kraft Foods Inc. managing external toxicology, safety and regulatory affairs, including several years as Director of International Scientific Relations. Health and regulatory issues surrounding coffee/caffeine is one of his areas of expertise for over three decades. In the early 1990’s, he served as President of the Association for Science and Information on Coffee (ASIC). He has been deeply involved in the assessment and management of California Proposition 65 requirements since the law’s passage in 1986. He is a Fellow and Certified Food Scientist of the Institute of Food Technologists. Dr. Coughlin received his BS in Chemistry from Siena College, his MS in Food Science and Technology and his PhD in Agricultural and Environmental Chemistry from the University of California, Davis, where he also completed post-doctoral training in Environmental Toxicology.
Stephen R. Daniels, M.D., Ph.D., is Professor of Pediatrics and Chairman in the Department of Pediatrics at the University of Colorado School of Medicine. He also serves as Pediatrician-in-Chief at Children's Hospital Colorado. Dr. Daniels' area of expertise is preventive cardiology, with a longtime interest in the application of sophisticated epidemiologic and biostatistical methods to pediatric clinical research problems. He is interested in the causes of blood pressure elevation and cholesterol abnormalities in children and adolescents, particularly the role that obesity may play in these health issues; development of structural and functional abnormalities in the heart and vascular system, including cardiovascular abnormalities occurring in pediatric patients with diabetes mellitus; as well as the relationship of left ventricular hypertrophy to obesity and hypertension. The role of lifestyle factors, such as diet and physical activity, is central to many of Dr. Daniels' studies. Dr. Daniels completed his residency in Pediatrics and his fellowship in Pediatric Cardiology at the Cincinnati Children's Hospital Medical Center, in 1981 and 1984, respectively. Dr. Daniels held numerous academic and clinical appointments at the University of Cincinnati, College of Medicine and the Cincinnati Children's Hospital before joining the University of Colorado, School of Medicine in 2006. Dr. Daniels received his MD from the University of Chicago, his MPH from Harvard University, and his PhD in Epidemiology from the University of North Carolina.

Thomas J. Gould, Ph.D., is Director of the Neuroscience Program, Head of the Neurobiological Investigations of Learning & Addiction (NILA) lab, and a Professor of Psychology at Temple University. In addition to affiliations with the psychology department and the neuroscience program, Dr. Gould has a secondary appointment in the Center for Substance Abuse Research (CSAR) at the Temple University School of Medicine, and he is also an investigator and member of the Center for Interdisciplinary Research on Nicotine Addiction at the University of Pennsylvania. His current research interest is the neurobiology of learning and memory with a specific focus on identifying the cellular and molecular events that underlie the effects of nicotine, caffeine, and ethanol on learning and memory. To that end, the NILA lab uses neurobiological, behavioral, pharmacological, genetic, and molecular techniques to investigate the effects of drugs of abuse on learning, with a specific emphasis on how those effects change as drug administration transitions from acute to chronic drug use, and then to withdrawal. Current projects in the lab include an examination of the effects of nicotine on hippocampus functioning and hippocampus-dependent learning. This research also examines genetic and developmental factors that may contribute to the effects of drugs of abuse on cognitive processes. Dr. Gould received his BS in Psychology from the University of Wisconsin and his PhD in Psychology and in Neuroscience from Indiana University.

Steven E. Lipshultz, M.D., is Professor of Pediatrics, Professor of Public Health Sciences, Professor of Medicine (Oncology), Director of the Batchelor Children's Research Institute, and Member of the Sylvester Comprehensive Cancer Center at the University of Miami Miller School of Medicine. Dr. Lipshultz holds the Batchelor Family Endowed Chair in Pediatric Cardiology. He has been the principal investigator in a number of groundbreaking NIH studies on the causes and treatment of cardiomyopathies in children. He studies the efficacy, long-term side effects, and outcomes of pharmacological agents in children using cross-sectional approaches. Another major focus of his research is development of surrogate outcome measures and biomarkers of adult-onset disease, such as coronary artery disease and health failure and prenatal and postnatal factors that moderate outcomes. Dr. Lipshultz has served on the faculties at Harvard Medical School and Boston University School of Medicine. He received his MD from Dartmouth Medical School, and went to Case Western Reserve University School of Medicine for his residency in pediatrics, followed by his fellowships in clinical cardiology and research at Children's Hospital in Boston.
Carl L. Keen, Ph.D., is Professor of Nutrition and Internal Medicine at the University of California, Davis. He is the Mars Endowed Chair in Developmental Nutrition. Dr. Keen’s research group has four main areas of activity; the first concerns the influence of diet on embryonic and fetal development. A second research theme in his group is the study of gene-nutrient interactions, with an emphasis on how subtle changes in cell mineral concentrations influence the expression of select genes. The third major research theme in his laboratory is the study of how diet influences oxidant defense systems, and as a consequence the occurrence of cellular oxidative damage. The fourth research area concerns the influence of dietary flavonoids on vascular health. Dr. Keen has served on many government boards, including California's Scientific Advisory Board for the Office of Environmental Health Hazard Assessment (1993-2012), EPA Environmental Health Grant Review Panels (1990-1999), USDA Human Nutrient Requirements Study (1987-92), and the following NIH panels: Nutrition Study Section (1997-99), ALTOX Study Section (2002-2004), XNDA Study Section (2003-2007), and the NIH College of Reviewers for the Center for Scientific Review (2010-present). He has also served on numerous editorial boards. He received his PhD in Nutrition from the University of California, Davis.

Theresa A. Nicklas, Ph.D., M.P.H., is a Professor of Pediatrics at the Baylor College of Medicine. Her research interests encompass both the epidemiological and intervention aspects of chronic disease prevention and health promotion. Specifically, Dr. Nicklas investigates how eating behaviors and other lifestyles influence the development of chronic disease risk factors early in life. She also examines behavioral factors influencing the development of adverse lifestyles early in life. Dr. Nicklas has published more than 250 scientific papers, 6 book chapters and 5 monographs. She is author of the American Dietetic Association position paper on Dietary Guidance for Healthy Children Ages 2-11 years and she served on the 2005 Dietary Guidelines Advisory Committee. Her areas of expertise include cardiovascular health, nutritional epidemiology, child nutrition, and health promotion and chronic disease prevention. Dr. Nicklas received her BS in Dietetics and Nutrition from Rochester Institute of Technology, and her DrPH in Public Health from Tulane University.

Barbara J. Petersen, Ph.D., M.P.H., is a Principal Scientist in Exponent’s Health Sciences Center for Chemical Regulation and Food Safety. She is also a specialist in addressing regulatory issues involving exposure and risk assessments including FDA, EPA, EFSA. Dr. Petersen chaired the WHO working group on methods for estimating intakes of food additives, nutrients, new biochemical traits associated with foods derived from modern biotechnology (GMOs), and contaminants in foods. She also served as Principal Investigator for the National Cancer Institute's International FOODBASE project, a major effort to collect and computerize descriptive and summary information on food consumption surveys conducted in more than 40 countries. Dr. Petersen has provided statistical support to FDA's Center for Food Safety and Nutrition, including developing criteria for evaluating nutrition databases, and specifically for the International Interface Standard for food databases and to EPA's Office of Research and Development. She has provided specialized expertise in developing compliance procedures for foods derived from modern biotechnology (GMOs), heavy metals in toys, dishes, contaminants in food and other consumer products (dioxins, fumonosins, heavy metals, etc.). Dr. Petersen received her BS in Nutrition from New Mexico State University, her MPH from UC-Los Angeles, and her PhD in Biochemistry from the George Washington University.
Joseph V. Rodricks, Ph.D., is a founding Principal of ENVIRON, and an internationally recognized expert in toxicology and risk analysis. He has consulted for hundreds of manufacturers, government agencies and for the World Health Organization in the evaluation of health risks associated with human exposure to chemical substances of all types. Dr. Rodricks came to consulting after a 15-year career as a scientist at the US Food and Drug Administration (FDA). In his last four years at the FDA, he served as Associate Commissioner for Health Affairs. His experience extends from pharmaceuticals, medical devices, consumer products and foods, to occupational chemicals and environmental contaminants. He has served on the National Research Council’s Board on Environmental Studies and Toxicology, and on 30 boards and committees of the National Academy of Sciences and the Institute of Medicine. Dr. Rodricks received his BS in biochemistry from MIT, and his PhD in biochemistry from the University of Maryland.
Workshop Speakers and Moderators Biographical Sketches

Speakers: Opening Remarks

Margaret A. Hamburg, M.D., is Commissioner of the Food and Drug Administration (FDA), where she has served since May 2009. As FDA Commissioner, she is advancing regulatory science, medical product innovation and globalization of the agency, while overseeing the implementation of groundbreaking laws to curb the use of tobacco and enhance food safety. She has undertaken major efforts to streamline and modernize FDA’s regulatory pathways. Before joining FDA, Dr. Hamburg was vice president and senior scientist at the Nuclear Threat Initiative. In the 1990s, as New York City's Health Commissioner, she launched several major initiatives, including the nation's first public health bioterrorism preparedness program and an internationally recognized program to curtail the resurgence and spread of TB. President Clinton later named her Assistant Secretary for Planning and Evaluation in the U.S. Department of Health and Human Services.

Michael R. Taylor, J.D., is Deputy Commissioner for Foods and Veterinary Medicine at the U.S. Food and Drug Administration (FDA). In this position, he provides leadership and direction to the Center for Food Safety and Applied Nutrition (CFSAN) and the Center for Veterinary Medicine (CVM). He also works closely with the foods-related programs of FDA’s inspection and compliance arm, the Office of Regulatory Affairs (ORA). Mr. Taylor is responsible for establishing a modern, science-based and prevention-oriented food safety program for domestic and imported foods. He also leads critical areas such as food labeling, nutrition, animal drug safety and effectiveness and scientific capacity. Mr. Taylor served previously in senior positions at FDA and the U.S. Department of Agriculture, as a research professor in academia, and on several National Academy of Sciences expert committees.

Speakers and Moderators

Richard H. Adamson, Ph.D., is President of TPN Associates, LLC, a consulting firm specializing in toxicology, pharmacology and nutrition issues. He spent two years as a commissioned officer in the U.S. Public Health Service and then became a civil servant at the National Institutes of Health. At National Institutes of Health he was the Director of the Division of Cancer Etiology and Scientific Director of the National Cancer Institute. In 1994 Dr. Adamson joined the American Beverage Association (ABA) as Vice President for Scientific and Technical Affairs. He retired from ABA in December 2004. He has published more than 250 papers and serves on several editorial boards and has received several honors and awards.

Amelia M. Arria, Ph.D., is Associate Professor of Behavioral and Community Health and Director of the Center on Young Adult Health and Development at the University of Maryland’s School of Public Health. She is the Principal Investigator on the College Life Study, a ten-year longitudinal prospective study of college students. Dr. Arria has conducted research studies on adolescent and young adult health risk behaviors, including energy drink consumption patterns, and the relationship between energy drink use and other forms of substance use. She is currently involved in several efforts to translate research findings for parents and policy makers.
Regan L. Bailey, Ph.D., is a Nutritional Epidemiologist in the Office of Dietary Supplements, Office of Disease Prevention at the National Institutes of Health. Dr. Bailey is the Director of the annual Mary Frances Picciano Dietary Supplement Research Practicum and the Director of Career Development and Outreach. She is also an adjunct professor in the Department of Foods and Nutrition at Purdue University. The overarching goal of her research program is to prevent or lessen the risk of chronic disease through nutrition. Dr. has considerable expertise working with the National Health and Nutrition Examination Survey. She is a member of the American Society for Nutrition and the Academy of Nutrition and Dietetics. She is also on the executive board of the Nutrition Epidemiology Research Interest Section of the American Society for Nutrition. She serves as an advisor to the International Life Sciences Institute-North America both on the Fortification Committee and the Food, Nutrition & Safety Program. She is a registered dietitian.

Alvin C. Bronstein, M.D., is Medical Director of the Rocky Mountain Poison Center (RMPC), the regional poison center for the states of Colorado, Hawaii, Montana, and Nevada. He is also Associate Professor in the Department of Emergency Medicine, University of Colorado School of Medicine. In addition to his medical director responsibilities, Dr. Bronstein actively participates in the RMPC’s medical toxicology training program, oversees toxicology training for the Center’s poison information specialists and providers, and is in charge of the center’s Continuous Quality Improvement (CQI) program. His research interests include creating new methods to deliver poison information services using computer databases and poison center data surveillance and trend analysis.

Christina Chambers, Ph.D., MPH, is Professor in the Department of Pediatrics, School of Medicine, and Associate Director of the Clinical and Translational Research Institute at the University of California San Diego, La Jolla, CA. She is a perinatal epidemiologist and teratologist who specializes in research related to the effects of prenatal and breastfeeding exposure to recreational substances, medications, vaccines, chemicals and other environmental agents on the developing embryo, fetus, infant or child.

Ahmed El-Soehemy, Ph.D., is the founder of Nutrigenomix Inc. and serves as Chief Science Officer. Dr. El-Soehemy is currently an Associate Professor in the Department of Nutritional Sciences and holds a Canada Research Chair in Nutrigenomics. He joined the faculty at the University of Toronto in 2000 to establish a research program in nutrigenomics. The goal of his research is to identify biomarkers of dietary exposure and elucidate the genetic basis for variability in nutrient response and dietary preferences. He collaborates with researchers across Canada as well as the US, Costa Rica, Denmark, Italy, Switzerland, South Korea and Singapore. Dr. El-Soehemy has served on international expert advisory panels and scientific advisory boards of several organizations, and was appointed to Health Canada’s Scientific Advisory Board.

Mark Feeley, M.Sc., is Associate Director of the Bureau of Chemical Safety in the Food Directorate of Health Canada. The Bureau of Chemical Safety of Health Canada is responsible for policy, standard setting, risk assessment, research and evaluation activities with respect to chemicals in foods in Canada. Chemicals under the authority of the Bureau include food additives; food packaging materials, processing aids, and incidental additives; food allergens; food contaminants; and novel foods. Mr. Feeley is the Head of the Canadian delegation for the Codex Committee on Contaminants in Food (CCCF), a current member of Joint FAO/WHO Expert Committee on Food Additives (JECFA) Roster of Toxicological and Epidemiological Experts, and a member of the World Health Organization Expert Advisory Panel on Food Safety.
Sergi Ferre, Ph.D., M.D., is Senior Investigator and Chief of the Integrative Neurobiology Section at the National Institute on Drug Abuse Intramural Research Program. Dr. Ferre is interested in the role of receptor heteromers as targets for drug development in neuropsychiatric disorders and drug addiction. His research deals preferentially with the discovery of heteromers of receptors that are targets for addictive drugs or that are localized in brain circuits that are involved in addictive behaviors (such as dopamine, glutamate, cannabinoid and adenosine receptors) and with the analysis of their biochemical and pharmacological properties involving studies at the cellular level as well as at the in vivo level.

Victor Fulgoni, III, Ph.D., is currently Senior Vice President of Nutrition Impact, LLC, which is a consulting firm that helps food companies, develop and communicate aggressive, science-based claims about their products and services. Nutrition Impact performs analyses of government food, nutrition, and health databases like the National Health and Nutrition Examination Surveys for clients evaluating the contribution certain food and beverage products make to nutrient intake and their effect on certain health parameters. Dr. Fulgoni previously worked for the Kellogg Company as Vice President of Food and Nutrition Research where he helped develop a long-term research program and was involved in the company’s research and regulatory efforts to gain health claim approval from the US FDA regarding soluble fiber from psyllium and the risk of heart disease.

Jeffrey Goldberger, M.D., a recognized scholar and clinician, has been a practicing clinical cardiac electrophysiologist at Northwestern University for 23 years. He is an innovator in the field and has been recognized as a Top Doctor by US News and World Report and Chicago Magazine. He has been a thought leader in the problems of sudden cardiac death, atrial fibrillation, and autonomic nervous system effects on cardiac electrophysiology, leading several multidisciplinary programs. He has authored over 200 publications. He has served on multiple national committees of the American Heart Association and American College of Cardiology. Dr. Goldberger is an active participant in both undergraduate and graduate medical education and is involved in several community and national organizations in leadership roles.

Roland R. Griffiths, Ph.D., is Professor in the Departments of Psychiatry and Neurosciences at the Johns Hopkins University School of Medicine. His principal research focus in both clinical and preclinical laboratories has been on the behavioral and subjective effects of mood-altering drugs. He is also a member of the Expert Advisory Panel on Drug Dependence for the World Health Organization. Dr. Griffiths’ research provided the most thorough description of caffeine withdrawal syndrome to date including documenting clinically significant functional impairment in some people. His research was the first to rigorously demonstrate: 1) that caffeine produces reliable mood altering effects at doses far lower than previously thought possible; 2) that caffeine functions as a reliable reinforcer when administered in beverages or capsules; 3) that caffeine withdrawal potentiates the reinforcing effects of caffeine; and 4) that some people become psychiatrically dependent on caffeine in that they fulfill DSM criteria for substance dependence applied to caffeine by being unable to quit despite repeated attempts to do so, by having a medical or psychological condition that is exacerbated by caffeine, and by continuing to use caffeine to avoid caffeine withdrawal symptoms.

John P. Higgins, M.D., M.B.A., is Associate Professor of Medicine (Cardiology) at The University of Texas Medical School at Houston, Chief of Cardiology at Lyndon B. Johnson General Hospital, and Director of Exercise Physiology at the Institute for Sports Medicine and Human Performance at Memorial Hermann. Dr. Higgins was previously on staff at the Veteran’s Administration West Roxbury hospital as an Attending Cardiologist and Director of The Cardiac Stress Laboratory (Instructor in Medicine, Harvard Medical School). He currently sees patients at LBJ General Hospital. He loves to teach and has received the prestigious Dean’s Teaching
Excellence Award at The University of Texas Medical School at Houston five years in a row (2008–2013).

**Diane C. Mitchell, M.S., R.D.**, is Senior Research Scientist and the Director of the Diet Assessment Center in the Department of Nutritional Sciences at The Pennsylvania State University. In this role she is responsible for managing all external and internal research studies, including proposal development, budget administration, manuscript development, and project management. Her research interest include validating and improving various diet assessment methodologies, accuracy and sources of error in diet recall, analysis of dietary patterns, diet quality, and database development. She also serves as scientific consultant to the International Life Science Institute of North America, Caffeine Working Group to provide an update to earlier work on the caffeine intakes of the US population.

**Charles P. O'Brien, M.D., Ph.D.**, is Kenneth E. Appel Professor of Psychiatry at the University of Pennsylvania. He also established and directs a clinical research program that has had a major impact on the treatment of addictive disorders. His work involves discovery of CNS changes involved in relapse, new medications, behavioral treatments and instruments for measuring the severity of addictive disorders. He led the discovery of the effects of alcohol on the endogenous opioid system and developed a completely new treatment for alcoholism. Dr. O'Brien was elected to the Institute of Medicine of the National Academy of Sciences in 1991 and he has received numerous research and teaching awards as well as an honorary doctorate from the University of Bordeaux. Dr. O'Brien is past president of the American College of Neuropsychopharmacology and the Association for Research in Nervous and Mental Disease. In 2013 the President of France named him Chevalier dans l'Ordre National de la Légion d'Honneur for his contributions to Franco-American scientific collaboration.

**Ashley Roberts, Ph.D.**, is Senior Vice President in the Food and Nutrition Group at Intertek Cantox. In this capacity, Dr. Roberts is available to advise and assist international clients with issues that are scientific, regulatory and toxicological in nature. In addition, he is able to assist clients wishing to design and develop scientific research programmes and for those developing regulatory strategies for food additives, foods that are generally recognized as safe (GRAS), and novel foods. While in the food industry he was largely responsible for developing scientific strategies, for establishing safety and gaining regulatory approvals for new food ingredients throughout the world. Prior to working for Cantox, Dr. Roberts worked in the area of scientific and regulatory affairs for a multi-national food company for more than 10 years. Prior to this he worked in two leading European contract research organizations conducting drug metabolism and pharmacokinetics studies and phase I clinical trials.

**Stephen Schaffer, Ph.D.**, is Professor of Pharmacology at the University of South Alabama. For 11 years, he served as a member of the American Heart Association SE Regional Consortium study section. His research interests include ischemia-reperfusion injury, the effects of diabetes and insulin on the heart and the cardiac effects of the sulfur-containing amino acid, taurine. Dr. Schaffer’s work has been instrumental in establishing an important physiological role for the sulfur-containing amino acid, taurine. In 1986 he discovered that taurine depletion caused a shift in energy metabolism of the heart in favor of glucose, an effect related to impaired mitochondrial function. He recently discovered that taurine deficiency leads to a decrease in the expression of mitochondria encoded proteins, an effect that reduces the activity of the electron transport chain and enhances superoxide production by the mitochondria.

**Andrew P. Smith, Ph.D.**, is Professor in the School of Psychology and Director of the Centre for Occupational and Health Psychology at Cardiff University. His research covers the areas of Occupational and Health Psychology with the major emphasis being on well-being. Specifically,
Dr. Smith has conducted extensive research on the non-auditory effects of noise on cognition and health. In addition, he conducts research on stress and fatigue in both the workplace and life in general. Dr. Smith’s interests in health psychology cover two main themes: health-related behaviors (effects of nutrition, caffeine and chewing gum on behavior) and minor illnesses (psychosocial risk factors for susceptibility to colds and influenza; effects of upper respiratory tract infections on mood and cognition).

Jennifer Temple, Ph.D., is Associate Professor in the Departments of Exercise and Nutrition Sciences and Community Health and Health Behavior. Dr. Temple is also Director of the Nutrition and Health Research Laboratory in the School of Public Health and Health Professions at the University at Buffalo. Her research program focuses on several major areas, including physiological and behavioral effects of caffeine intake in children and adolescents, gender difference in effects of caffeine, food reinforcement and sensory system influences on eating in adults, and the relationship between food reinforcement and weight change over time. Currently, Dr. Temple is investigating the cardiovascular, cognitive, subjective, and reinforcing effects of caffeine in pre- and post-pubertal children and across the menstrual cycle.
FDA has contracted with the Institute of Medicine (IOM) to conduct a public workshop on “energy drinks” and other food products that contain caffeine. The purpose of the workshop is to obtain scientific input on dietary exposure considerations and any health hazards associated with these products. FDA anticipates obtaining information about risk to the general population and to specific subpopulations. The IOM workshop, which is scheduled for August 5-6, 2013 in Washington, DC, should address the following questions organized by technical area of expertise.

Exposure
1. Do current exposure models for caffeine accurately estimate actual dietary consumption? Do current exposure models adequately address all segments of the population including children, adolescents and adults? How can current exposure assessment models be improved?
2. Do we need data that capture acute dietary exposures of caffeine and other “energy drink” components (e.g., consumption in one sitting versus consumption over the course of a day) to more accurately assess risk?
3. What is the best way to model exposure to caffeine from products that are not traditionally caffeinated (e.g., chewing gum, popcorn, candy)? Would exposure estimates based on consumption of the non-caffeinated versions of these products be adequate?
4. How could data collection (on caffeine use and frequency of consumption) be improved to capture changing trends in caffeine exposure?
5. What evidence is there for “self-titration” of caffeine consumption in children, adolescents or adults?

Absorption, distribution, metabolism, and excretion (ADME)
6. Describe how the ADME of caffeine varies as a function of dose.
7. What effects do different caffeine-containing food products (e.g. gum, popcorn, carbonated beverage, coffee) have on ADME?
8. How does the ADME of caffeine change with age?

Cardiovascular effects
9. Considering the epidemiological, toxicological, clinical and other relevant literature, what are the cardiovascular health hazards (acute or chronic) associated with caffeine consumption in the general population? What is the strength of the dose-response relationship for any identified health hazards? Do these hazards vary by age?
10. What are the risks of caffeine consumption to cardiovascular health in the following scenarios: (a) naïve versus habitual consumers, (b) consumption of a large bolus dose of caffeine in contrast to consumption of multiple smaller doses achieving the same total exposure over the course of a day, and (c) acute versus chronic consumption?
11. Is there a definable exposure level to caffeine below which there is negligible risk of adverse cardiovascular health consequences for the population as a whole, or for any subpopulations? What health reference values were used to derive these levels (e.g., no observed adverse effect level (NOAEL), lowest observed adverse effect level (LOAEL), benchmark dose)?
Neurological and behavioral effects
12. Considering the epidemiological, toxicological, clinical and other relevant literature, what if any are the neurological and behavioral health hazards (acute or chronic) associated with caffeine consumption in the general population? What is the strength of the dose-response relationship for any identified health hazards? Do these hazards vary by age?
13. What if any are the risks of caffeine consumption to neurological health or adverse behavior changes in the following scenarios: (a) naïve versus habitual consumers, (b) consumption of a large bolus dose of caffeine in contrast to consumption of multiple smaller doses achieving the same total exposure over the course of a day, and (c) acute versus chronic consumption?
14. Is there a definable exposure level to caffeine below which there is negligible risk of adverse neurological or behavioral consequences for the population as a whole, or for any subpopulations? What health reference values were used to derive these levels (e.g., no observable adverse effect level (NOAEL), lowest observable adverse effect level (LOAEL), benchmark dose)?

Tolerance and withdrawal
15. What is the evidence for tolerance development to caffeine in children, adolescents or adults? What is the effect of tolerance to caffeine on risk?
16. What is the evidence that caffeine withdrawal does or does not present a significant health risk?

Population risk characteristics
17. Delineate any specific populations at risk from either acute or chronic caffeine exposure, taking into account variables such as age, sex, and health status (including underlying medical conditions).
18. Are there additional variables beyond individual caffeine sensitivity to consider in certain subpopulations that increase risk (e.g., strenuous physical activity, body building, or social environment)?
19. Identify and discuss any risks associated with co-exposure to caffeine and other ingredients in “energy drinks” as well as co-exposure to caffeine and various dietary supplements or medications.

Scientific data on caffeine
20. Are the scientific data including hazard characterization and exposure assessments of caffeine (a) alone or, (b) in combination with other stimulants, adequate to address the safety of caffeine for the general population and for potentially vulnerable subgroups?
21. If existing data are less than adequate, what study types and study design considerations would contribute most to our understanding of the public health consequences of ingesting caffeine?

Introduction
Caffeine is an alkaloid that occurs naturally in certain foods. It is also added to soda-type beverages, other beverages, and non-beverage foods. In spite of caffeine’s historical and widespread consumption, the safety of caffeine added to foods has been the subject of studies for decades. Various studies have raised concerns regarding the safe level of caffeine consumption, especially by pregnant women and children. FDA’s review of studies from 1980 to 1987 resulted in the agency concluding that there was no evidence to show that the use of caffeine in carbonated cola-type beverages would be a safety concern (FR 52, no.
FDA continued to review the caffeine safety literature including animal and clinical studies on developmental, reproductive, behavioral, carcinogenic, cardiovascular, and other effects. The agency again concluded in 1992 that there was no evidence to show that there was a human health hazard from the consumption of 100 milligrams (mg) caffeine per person per day (p/d) or less in cola-type beverages. Today, the market for and marketing of caffeinated products such as “energy drinks” is evolving, leading to new patterns of use and new public health concerns.

FDA is now considering the safety of adding caffeine to beverages other than cola and non-cola soda beverages in light of questions raised by both scientific experts and the general public. The majority of beverages of concern are known in the marketplace as “energy drinks” or “energy shots.” The primary ingredient in “energy drinks” is typically caffeine, but they may contain other ingredients such as taurine or glucuronolactone. The concerns stem from reports of caffeine-related effects on the cardiovascular and central nervous systems (Annex A. Adverse Event Reports, when completed).

FDA is seeking the input from an IOM scientific workshop on the human health effects of caffeine, alone or in combination with other substances found in “energy drinks” as well as other caffeinated food products. Information from the workshop will inform the agency’s policy on, and strategy for, regulating products containing added caffeine. What follows in this document is a brief overview of dietary exposure and safety data pertaining to caffeine with an emphasis on cardiovascular and neurological/behavioral endpoints. This information is not intended to serve as a comprehensive review of these specific topics; it is to provide context for the variety of questions suggested by FDA for public discussion at the IOM workshop.

**Dietary exposure**

*Natural sources of caffeine*

Coffee firstly and tea secondly are the primary sources of caffeine in the diet of the United States (U.S.) population (Frary, et al., 2005). The caffeine content in coffee beans (*Coffea sp.*) varies widely, depending on geographical origin, growing conditions, effects of processing, storage, roasting, grinding, and method of preparation (Desbrow, et al., 2012). Published chemical analyses of coffee beverages have demonstrated wide ranges of caffeine content (e.g., 58–259 mg/serving), and even in the same coffee beverage obtained from the same outlet over consecutive days (i.e., 259–564 mg per 16 fluid ounce (oz) serving) (McCusker, et al., 2003). In general, a serving of espresso (30 milliliters (mL)) provides 64 mg of caffeine and an 8 oz cup (237 mL) of automatic drip coffee provides 145 mg of caffeine (Somogyi, 2010). Tea (*Camellia sp.* ) beverages contain a variable quantity of caffeine depending on species, variety, processing method (green, black, herbal, etc.), origin, and method of preparation (e.g., water temperature and volume, steeping time, mixing) (Stavric, et al., 1988). Typically, tea beverages contain 20 to 80 mg of caffeine per 8 oz (237 mL) (Somogyi, 2010). Caffeine is also found in cocoa-based products, kola nuts, guaraná, and yerba maté (Frary, et al., 2005; Somogyi, 2010; Baumann, et al., 1995; Burdock, et al., 2009).

*Caffeine as an ingredient added to food*
Caffeine is listed as generally recognized as safe (GRAS) for use in cola-type beverages at levels not to exceed 200 parts per million (ppm) (0.02%) (21 CFR § 182.1180). This maximum level of use is equivalent to approximately 71 mg of caffeine for a 12 oz serving of cola, although colas typically contain roughly half this amount. Some non-cola soda beverages also contain added caffeine at levels common to colas. More recently, caffeine has been added to food products that have not been considered traditional sources of caffeine, such as chewing gum, candies, and “energy drinks.” Caffeine added to food can be produced synthetically; however, it is commonly obtained as a byproduct from the decaffeination of coffee.

More than 60% of soda beverages sold in the U.S. contain caffeine at approximately 30 –40 mg per 12 oz serving, or 2.5 to 3.3 mg/oz (Keast, et al., 2007; Somogyi, 2010). Soft drinks typically do not contain additional stimulants or other ingredients with purported health benefits. “Energy drinks” contain caffeine at approximately 17 to 224 mg/serving (or 1.5 to 32.5 mg/oz) (Consumer Reports, 2012). “Energy drinks” often contain vitamins, L-carnitine, taurine, glucuronolactone, guaraná, kola nut, yerba maté and/or other botanical extracts in addition to caffeine.

“Energy shots” are a specialized form of “energy drink.” “Energy shots” are usually sold in container volumes of 2 oz; however, they normally contain the same amount of caffeine as their larger-sized counterparts. “Energy shots” account for approximately 11% of the “energy drink” market, and as of June 2009, there were approximately 250 brands in the U.S. (Somogyi, 2010). “Energy shots” and “energy drinks” are marketed in the U.S. as either dietary supplements or as conventional foods or beverages.

Estimates of caffeine intake

The caffeine content of foods and beverages, as consumed, can vary significantly. The analytical procedures used to determine the caffeine content of foods have improved over time, which will directly influence the results of an intake assessment. Estimates of caffeine intake based on food and beverage consumption frequently rely on average or maximum estimates of caffeine concentration. Estimates of caffeine intake based on coffee consumption, for example, may lack information on the genetic variety and geographic source of the beans, the type or brand of coffee, method of preparation, or the actual concentration of caffeine or other physiologically active components per serving (Stavric, et al., 1988).

In calculating exposure, FDA assumes that a food ingredient will be added at the maximum use level to avoid the possibility of underestimating the intake by individuals who are frequent consumers. Current food-consumption databases used by FDA, such as the National Health and Nutrition Examination Survey (NHANES), provide data for only a limited survey period; therefore, estimates of chronic intake are generally overestimated compared to intake estimated using surveys of longer duration.

FDA typically calculates exposures based on “eaters-only” intake, which is determined from the total amounts of foods of concern consumed per day averaged over the number of days in the survey period by individuals consuming the food at least once during the survey period. The availability of food consumption data reported by each participant in a multi-day survey makes it possible to estimate the intake of caffeine by each participant in the survey. A distribution of the intakes of all individuals in the survey and specific sub-populations can be constructed if data are adequate. Statistical analyses of the
resulting distributions of data are used to calculate upper percentile intakes to account for individuals who are considered "high level" consumers of specific foods that contain caffeine. The FDA uses 90th percentile intake estimates to represent chronic intake over a lifetime for "high level" consumers.

FDA (Lee, 2007 & 2008) estimated the daily intake of caffeine for the U.S. population from foods and food categories that can be caffeinated. Caffeine intake was calculated using food consumption data from the 2003–2004 NHANES and caffeine concentration data for each food obtained from publications, internet sources, or trade associations (e.g., American Beverage Association). The 2003–2004 NHANES contains food and nutrient intake data taken from 10,122 individuals representing a cross-section of the U.S. population. Participants used a 24-hour recall of food consumption and a food frequency questionnaire to provide the data. FDA’s estimates of caffeine intake from food and beverages by various age groups based on NHANES 2-day average data are shown in Table 1. This model revealed an age-dependent increase in caffeine consumption. The increase in caffeine consumption with increasing age can be attributed to an increase in coffee and “energy drink” consumption relative to children and teenagers who tend to consume carbonated beverages or tea as their primary sources of caffeine. Women of child-bearing age (14–44 years) have a mean daily caffeine intake of 261 mg/p/d, which is lower than the corresponding mean intake for all adults.

Table 1. Two-day average intake of caffeine (mg/p/d)

<table>
<thead>
<tr>
<th>Food Category</th>
<th>All ages (2+ yr.)</th>
<th>2-5 yr. *</th>
<th>13-18 yr. *</th>
<th>18+ yr.</th>
<th>14-44 yr. **</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Natural” sources (coffee, tea, and cocoa beverages)</td>
<td>Mean</td>
<td>331</td>
<td>15</td>
<td>130</td>
<td>359</td>
</tr>
<tr>
<td></td>
<td>90th percentile</td>
<td>730</td>
<td>NA***</td>
<td>275</td>
<td>778</td>
</tr>
<tr>
<td></td>
<td>Percent eaters</td>
<td>60</td>
<td>21</td>
<td>34</td>
<td>71</td>
</tr>
<tr>
<td>“Added” sources (soft drinks, “energy” drinks, and chocolate- and coffee-containing foods)</td>
<td>Mean</td>
<td>105</td>
<td>50</td>
<td>107</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>90th percentile</td>
<td>243</td>
<td>104</td>
<td>240</td>
<td>258</td>
</tr>
<tr>
<td></td>
<td>Percent eaters</td>
<td>76</td>
<td>72</td>
<td>82</td>
<td>75</td>
</tr>
<tr>
<td>All sources (“natural” and “added” sources combined)</td>
<td>Mean</td>
<td>305</td>
<td>67</td>
<td>152</td>
<td>361</td>
</tr>
<tr>
<td></td>
<td>90th percentile</td>
<td>696</td>
<td>159</td>
<td>301</td>
<td>771</td>
</tr>
<tr>
<td></td>
<td>Percent eaters</td>
<td>91</td>
<td>74</td>
<td>87</td>
<td>93</td>
</tr>
</tbody>
</table>

*intake was not provided for the group 6-12 years of age; ** women of child-bearing age; *** not available due to insufficient data

FDA evaluates short-term intake for substances that may be associated with an acute effect. Short-term estimates of dietary intake are based on food consumption from single eating occasions or from one-day food consumption data. FDA modeled intake of caffeine for a 24-hour period (Table 2). In general, the estimated intake of a substance over a shorter exposure period is higher than chronic consumption estimates. The acute intake of caffeine for adults aged 18 years and older is approximately 8% higher than the 2-day average data (i.e., 391 vs. 361 mg/p/d). The similarity between these two intake estimates suggests that similar amounts of caffeine (e.g., from a daily cup of coffee) are consumed on a regular basis (Lee, 2007 & 2008).
In 2009, FDA contracted with an outside expert to produce an independent assessment of caffeine intake using data from all available sources (Somogyi, 2010). Daily caffeine consumption was estimated using the National Nutrient Database for Standard Reference (NDB) and NHANES food consumption surveys conducted in 1999–2000, 2001–2002, 2003–2004, and 2005–2006 along with NPD Group’s Food Consumption survey (14-day diaries) compiled in 2008. Levels of caffeine in foods were taken from the NDB, and supplemented with information from a comprehensive review of scientific publications, the Internet, trade association data, and industry sources. NPD Group data show the specific source and quantity of food consumed daily and provides a breakdown of overall consumption by age and gender. This assessment examined caffeine consumption for the U.S. population, including subpopulations of interest (e.g., children 2–13 years old, male and female teenagers 14–21 years old, and women of childbearing age 16–45 years old). Estimates of caffeine consumption were less in younger people compared with older adults because the beverages of choice in younger consumers are cola and tea, each of which contains less caffeine than coffee. Women of childbearing age consume less coffee than other adult groups and thus have a lower daily intake of caffeine. The data from these two sources were validated using market information provided by the National Coffee Association, the Tea Association of the U.S.A., and the American Beverage Association (Somogyi, 2010).

Table 2. Intake of caffeine over a 24-hour period (mg/p/d)

<table>
<thead>
<tr>
<th>Food Category</th>
<th>All ages (2+ yr.)</th>
<th>2-5 yr.*</th>
<th>13-18 yr.*</th>
<th>18+ yr.</th>
<th>14-44 yr.**</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Natural” sources (coffee, tea, and cocoa beverages)</td>
<td>Mean</td>
<td>384</td>
<td>96</td>
<td>188</td>
<td>405</td>
</tr>
<tr>
<td></td>
<td>90\textsuperscript{th} percentile</td>
<td>854</td>
<td>312</td>
<td>367</td>
<td>859</td>
</tr>
<tr>
<td></td>
<td>Percent eaters</td>
<td>52</td>
<td>15</td>
<td>24</td>
<td>62</td>
</tr>
<tr>
<td>“Added” sources (soft drinks, “energy” drinks, and chocolate- and coffee-containing foods)</td>
<td>Mean</td>
<td>132</td>
<td>68</td>
<td>133</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td>90\textsuperscript{th} percentile</td>
<td>289</td>
<td>172</td>
<td>294</td>
<td>301</td>
</tr>
<tr>
<td></td>
<td>Percent eaters</td>
<td>61</td>
<td>53</td>
<td>66</td>
<td>60</td>
</tr>
<tr>
<td>All sources (“natural” and “added” sources combined)</td>
<td>Mean</td>
<td>340</td>
<td>88</td>
<td>180</td>
<td>391</td>
</tr>
<tr>
<td></td>
<td>90\textsuperscript{th} percentile</td>
<td>785</td>
<td>206</td>
<td>351</td>
<td>855</td>
</tr>
<tr>
<td></td>
<td>Percent eaters</td>
<td>82</td>
<td>57</td>
<td>74</td>
<td>86</td>
</tr>
</tbody>
</table>

* intake was not provided for the group 6-12 years of age; ** women of child-bearing age

This model, based on 14 days of dietary consumption data, estimated caffeine intake to be 301 mg/p/d for the adult population (≥ 22 years old). This value is comparable to the 2-day estimate of 361 mg/p/d obtained by FDA. The estimated daily caffeine intake calculated by FDA is higher due to fewer survey collection days in the NHANES. Additionally, we see the trend to larger values (391 mg/p/d) in 24-hour surveys.

\footnote{The subpopulation, “women of childbearing age” differs among reports, and FDA defined this group as 14–44 years of age. These differences do not result in significantly different datasets.}
The estimates of caffeine intake provided by Lee (2007; 2008) and Somogyi (2010) do not include caffeine intake from the regular consumption of over-the-counter pharmaceuticals for alertness (e.g., NoDoz).

**Published estimates of caffeine intake**

A number of estimates of caffeine intake are presented in the literature. A published study (Barone and Roberts, 1996) compared and contrasted previously published estimates of caffeine intake based on various consumption surveys. The study included data from the USDA 1977–78 and 1987–88 Nationwide Food Consumption Survey (NFCS), the 14-day surveys conducted by the Market Research Corporation of America (MRCA) for 1972–73 and 1987–89, and the Winter Coffee Drinking Study conducted by the International Coffee Organization and the National Coffee Association. The authors developed standardized values for caffeine content of coffee, tea, cocoa-based products, and soft drinks, while noting the wide range of data that exist for a given product. The authors concluded that the estimated mean daily caffeine intake for all adults in the U.S. is approximately 3 mg per kilogram body weight (kg bw)/d and 4 mg/kg bw/d for adult consumers of caffeine-containing products. The 90th percentile caffeine intake was estimated to be 5 to 7 mg/kg bw/d. The mean and 90th percentile daily caffeine intake for children (< 18 years old) is about 1 and 2 mg/kg bw/d, respectively. The authors stated that data for pregnant/nursing women was limited; they concluded, however, that pregnant women consume less caffeine than the general adult population (approximately 1 mg/kg bw/d) (Barone and Roberts, 1996).

A published study (Knight, 2004) examined caffeine intake from beverage consumption in the U.S. population. This study was conducted by International Life Sciences Institute (ILSI) Caffeine Technical Committee. Beverage consumption data was collected by NFO WorldGroup in the 1999 U.S. Share of Intake Panel survey, which included 10,712 caffeinated beverage consumers who recorded daily intake for 14 days. Beverages included coffee, tea, cocoa and chocolate drinks, carbonated soft drinks, caffeinated fruit juices/drinks, and caffeinated bottled water. Caffeine concentrations in these beverages were based on published data or as reported by the respective manufacturer. Estimated daily intakes of caffeine based on this model are summarized in Table 3 (Knight, et al., 2004).

<table>
<thead>
<tr>
<th>Age group</th>
<th>Mean (mg/p/d)</th>
<th>90th Percentile (mg/p/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All ages</td>
<td>119.5</td>
<td>287.4</td>
</tr>
<tr>
<td>1 – 5 years</td>
<td>13.5</td>
<td>37.3</td>
</tr>
<tr>
<td>6 – 9 years</td>
<td>22.2</td>
<td>45.0</td>
</tr>
<tr>
<td>10 – 14 years</td>
<td>33.3</td>
<td>73.7</td>
</tr>
<tr>
<td>15 – 19 years</td>
<td>65.9</td>
<td>148.5</td>
</tr>
<tr>
<td>20 – 24 years</td>
<td>105.5</td>
<td>226.6</td>
</tr>
<tr>
<td>25 – 34 years</td>
<td>123.1</td>
<td>258.2</td>
</tr>
<tr>
<td>35 – 49 years</td>
<td>170.1</td>
<td>381.9</td>
</tr>
<tr>
<td>50 – 64 years</td>
<td>169.0</td>
<td>357.0</td>
</tr>
<tr>
<td>≥ 65 years</td>
<td>135.6</td>
<td>296.0</td>
</tr>
<tr>
<td>Age group</td>
<td>Mean (mg/p/d)</td>
<td>90th Percentile (mg/p/d)</td>
</tr>
<tr>
<td>------------------</td>
<td>--------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Non-pregnant women</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 – 24 years</td>
<td>90.9</td>
<td>228.7</td>
</tr>
<tr>
<td>25 – 34 years</td>
<td>108.8</td>
<td>247.3</td>
</tr>
<tr>
<td>Pregnant women</td>
<td>58.1</td>
<td>156.6</td>
</tr>
</tbody>
</table>

In a published study (Frary, 2005) caffeine intake by the U.S. population was estimated using the U.S. Department of Agriculture’s Continuing Survey of Food Intakes by Individuals (CSFII). The authors report that 87% of the survey respondents consumed caffeine containing food or beverages, and average daily intake of caffeine was 193 mg/p/d (1.2 mg/kg bw/d). Caffeine consumption increased with age, and men and women aged 35 to 64 were the highest consumers. The primary sources of caffeine were coffee (71%), soft drinks (16%), and tea (12%). The authors noted that trends in beverage consumption have continuously changed over the past 5 decades. Consumption of soft drinks has increased from 11 to 49 gallons per person per year, whereas coffee consumption fell from 16 to 9 gallons per person per year. Soft drink consumption has increased 48% in children between the 1977 and 1998. Tea and cocoa consumption has remained relatively stable. Other caffeine containing beverages are relatively new to the market, including specialty coffee drinks, caffeinated water, and “energy drinks” (Frary et al., 2005).

In Canada, average daily intakes of caffeine from all sources were reported in 1992 as about 2.4 mg/kg bw/d for adults and 1.1 mg/kg bw/d for children (5–18 years old). A more recent publication in 2001 estimated daily caffeine intakes to range from 288 to 426 mg/p/day (equivalent to 4.5 to 6.5 mg/kg bw/d for a 65 kg person) in the adult population (30–75 years old) residing in southern Ontario, Canada. Dietary intake of caffeine by the general population in the United Kingdom (U.K.) has been reported as 4 mg/kg bw/d and in Denmark as 7 mg/kg bw/d. Reported daily intakes for high-level consumers range from 5 to 15 mg/kg bw/d. For children, daily caffeine intakes are reported as <3 mg/kg bw/d in the U.K. and <2.5 mg/kg bw/d in Denmark (Nawrot et al., 2003).

Current and anticipated trends in consumption

Data are limited for assessing recent changes in the consumption of “energy drinks.” A published study on sports and “energy” drinks shows that the market continues to be a rapidly expanding segment of the beverage industry. The average daily, per capita volume of sports and “energy” drinks sold in the U.S. increased from 3.8 mL in 2000 to 41.1 mL in 2010. Data from the 2010 National Health Interview Survey showed that 31.3% of adults consumed a sports or “energy” drink in the past 7 days, with 21.5% consuming sports and “energy” drinks one or more times per week and 11.5% three or more times per week. In this model, age was the factor most strongly associated with sports and “energy” drink consumption, and highest among young adults (e.g., 24% of 18 to 24 year olds consumed sports and “energy” drinks three or more times per week) (Park, et al., 2013).

In June 2012, Mintel reported that sales of “energy drinks” increased in 2011 compared to 2010, after a decrease in 2009. This report stated there is statistically insignificant growth in new users of “energy drinks.” However, sales of “energy drinks” are forecast to nearly double (92% growth) during the period of 2011 to 2016 due to product innovation. Mintel states that “energy shots” have experienced a
slowdown in growth since 2009. Mintel notes that consumption of “energy drinks” and “energy shots” is highest among those adults aged 18 to 34. Among teenage consumers (aged 12 to 17), Mintel states that the “energy drink” consumer base fell from 27% in 2007 to 16% in 2011 (Mintel 2012).

The Mintel report notes that 19% of “energy drink” consumers use these products in the morning, likely as a replacement for other caffeine-containing beverage (Mintel 2012). This use category may continue to be a target for future market growth. On February 11, 2013, PepsiCo announced the launch of a new breakfast product line, referred to as Mountain Dew – Kickstart beverages. An Associated Press report states that a 16 oz container of the Kickstart beverage contains 92 mg of caffeine in 5% juice. This compares to 72 mg of caffeine in a 16 oz container of the regular Mountain Dew brand of carbonated beverage and 142 mg in a 16 oz can of PepsiCo's Amp “energy drink” (MSN, 2013).

Caffeine consumption continues to be of interest to various governmental bodies and stakeholders. The ILSI North America Caffeine Working Group has conducted a re-evaluation of caffeine intake data in the U.S. Their study reflects newly introduced caffeinated products and examines trends using a seven-day dietary record survey of beverage consumption (ILSI, 2013). ILSI has stated their intention to publish their caffeine intake work this summer.

Safety

FDA’s has used a two-pronged approach for its most recent consideration of caffeine safety; a literature update and an analysis of adverse event reports. For the literature update, FDA contracted with Oak Ridge National Laboratory (ORNL) to compile the literature from 1994 to 2011 on caffeine safety. Their literature report contains summaries of studies on cardiovascular and neurological/behavioral effects of caffeine, endpoints such as developmental and reproductive effects and effects on bone and dental health, and other endpoints of historical interest. The ORNL final report, dated October 27, 2011, has been provided to IOM.

FDA also compiled and analyzed adverse event reports for caffeine-containing products received through FDA’s reporting system (the Center for Food Safety and Applied Nutrition Adverse Event Reporting System (CAERS) database). FDA’s analysis of the CAERS reports will be available to the IOM.

FDA was provided with a draft summary (dated April 1, 2013) of data collected from National Poison Data System (NPDS) of the American Association of Poison Control Centers (AAPCC). The data includes the number of cases reported in which “energy drink” consumption was a potential factor. Data include statistics on the number of calls received by poison control centers related to an exposure to a substance as well as age and gender information for the exposed individual. The NPDS data includes exposure calls reported for the period between October 1, 2010 and September 30, 2012 for caffeine containing drinks and drinks with caffeine from other sources such as guaraná, kola nut, tea, yerba mate, and cocoa. Data on calls related to alcohol containing drinks were excluded.
The AAPCC-NPDS data demonstrated that the average number of cases per month for all “energy drink” product categories increased from 91 in 2010 to 144 in 2012. The age of the patients ranged from seven days (due to exposure through breast milk) to 80 years. Fifty-five percent of the patients were ten years or younger, including infants (2.6%). Approximately 0.3% of patients reported a “major effect”, 8% reported moderate effects”, 20% reported “minor effects”, and 72% were apparently less serious or determined to be not related to the “energy drink” exposure. The most common symptoms were agitation/irritability, tachycardia, nausea, and vomiting. There was one fatality reported, and a caffeine containing product was attributed to the tachycardia exhibited by the patient. The autopsy found that the cause of death was cardiac arrhythmia due to caffeine toxicity complicating mitral valve regurgitation in the setting of Ehlers-Danlos Syndrome. The AAPCC notes that comparison of data from different annual reports can be problematic due to changes in methodology over time. Furthermore, the AAPCC notes that NPDS data only covers those exposures which are reported to poison control centers and is not indicative of the actual number of exposures that occur in the population (Bronstein et al. 2012).

Based on its review of the safety literature and its review of the adverse event reports received through CAERS, and other available information, FDA has identified cardiovascular effects of caffeine as its primary interest and neurological/behavioral effects of caffeine as a secondary interest for the purpose of the IOM workshop.

Studies from the published literature, including from the ORNL contracted compilation, were used to summarize the current knowledge on cardiovascular and neurological/behavioral effects of caffeine described below. The summaries below start with a description of the known physiological metabolism pathways and mechanisms of action of caffeine, then focus on endpoints in human studies of primary interest to FDA for the purpose of the workshop. This summary is not intended to be a comprehensive review of these two areas of concern; it is intended to provide context for questions above suggested by FDA for discussion at the IOM workshop. FDA is aware of published studies on benefits associated with consuming caffeine-containing beverages such as coffee and tea; for the purpose of the IOM workshop, however, FDA is not seeking information on beneficial effects. ADME

Caffeine is absorbed rapidly and completely from the gastrointestinal tract, through the small intestine, into the portal circulation. Plasma caffeine concentration increases in a dose-dependent manner and reaches peak plasma concentrations within 30 to 60 minutes after ingestion (Grosso et al., 2005; Benowitz, 1990). Caffeine is metabolized in the liver by cytochrome P450 1A2 (CYP1A2) enzyme to paraxanthine which is the primary metabolite of caffeine in humans (Figure 1). The metabolites undergo additional demethylation and oxidation in the liver to other metabolites (e.g., 8-hydroxyparaxanthine, 1-methylxanthine, 1-methylurate, and 5-acetylamino-6-formylamino-3-methyluracil). Caffeine and its primary metabolites (including other dimethyl xanthines such as theobromine and theophylline) readily enter all body tissues and freely cross the blood-brain, placental, and blood-testicular barriers. The paraxanthine metabolites appear in the urine as fast as they are formed due to active renal tubular secretion. Only 0.5% to 2% of the ingested caffeine is excreted in the urine due to 98% renal tubular reabsorption (Grosso et al., 2005).
In a pharmacokinetics study by Cheng et al. (1990), normal subjects were administered single doses of 70, 200, or 300 mg of caffeine. The results show that caffeine clearance decreased with increasing dose (1.52, 1.14, and 1.08 mL per minute/kilogram (min/kg)), and half-life of caffeine increased (4.5, 6.0, and 6.4 hours) for caffeine doses of 70, 200, and 300 mg, respectively. Serum caffeine levels peaked at times earlier than 2 hours after dosing and decreased to near zero within 24 hours for all dose levels. However, the rates of metabolism and elimination of caffeine are variable. Studies cited in reviews by Grosso et al. (2005) and Benowitz (1990) demonstrate this variability. They report that the average half-life of caffeine in healthy adults ranges from 2 to 6 hours, but can be as long as 12 hours in some individuals.

**Variability and sensitivity**

The wide variability in the rate of caffeine metabolism is primarily due to variations in CYP1A2 enzyme activity. Some of the variability in CYP1A2 enzyme activity is due to genetic polymorphisms in the CYP1A2 gene which can cause increased or decreased induction of the enzyme. An adenine to cytosine (A→C) substitution at nucleotide position 734 in the CYP1A2 gene decreases the enzyme inducibility and activity. Carriers of the variant CYP1A2*1F allele (A/C or C/C) are reported to be slow caffeine metabolizers, whereas individuals homozygous for the CYP1A2*1A allele (A/A) are rapid caffeine metabolizers (Palatini et al., 2009).

Caffeine metabolism has also been studied in special populations and reports show that the rate of metabolism of caffeine is slow during later stages of pregnancy, with long-term use of oral contraceptives, with alcohol consumption, and in individuals with liver disorders. The rate of caffeine metabolism in the newborn is low resulting in a slow rate of elimination. However, the rate of caffeine metabolism increases with age. Cigarette smoking is reported to accelerate caffeine metabolism (Grosso et al., 2005; Benowitz, 1990; Chawla and Suleman, 2011).
Mechanism of action

Caffeine is a CNS stimulant and acts predominantly by antagonizing adenosine receptors located in the brain, blood vessels, kidneys, heart, and the gastrointestinal tract (Fisone et al., 2004, Olah and Stiles, 1995). Caffeine non-selectively blocks A1 and A2 subtypes of adenosine receptors in the brain and competitively inhibits the actions of adenosine at concentrations found in consumers of caffeine from dietary sources. Genetic studies suggest that the mechanism by which caffeine produces its stimulant effects is by blockade of the A2A receptor (Huang et al., 2005). Evidence in the literature suggests that caffeine’s weak psychomotor stimulant property is primarily due to caffeine’s ability to counteract the inhibitory control exerted by adenosine A2A receptors on striatal dopamine D2 transmission (Fisone et al., 2004). In animals, circulating plasma caffeine concentrations as low as 100 micromols (μmol) (equivalent to plasma concentrations of caffeine in humans achieved after drinking 1–3 cups of coffee) suppress most of the pharmacological effects of adenosine in the brain (Chawla and Suleman, 2011).

Caffeine causes the release of norepinephrine, dopamine, and serotonin in the brain, and increases in circulating catecholamines (Benowitz, 1990). At doses from 0.5–5.0 mg/kg bw, caffeine increases dopamine release in the caudate nucleus, and prefrontal cortex, but not in the shell of the nucleus accumbens (Nehlig, 1999).

The same mechanisms of adenosine receptor blockade and catecholamine release are operative in the peripheral vascular system. Caffeine has been seen in experimental studies to increase vascular resistance in healthy subjects. Stimulation of adenosine receptors in most vascular tissue induces vasodilation, and therefore, as caffeine is an adenosine receptor antagonist, this may be one mechanism through which it induces vasoconstriction (Riksen et al., 2009). In addition, caffeine may affect vascular tone by increasing adrenocorticotropic and cortisol (Lovallo et al., 1996; Nurminem et al., 1999).

Other general mechanisms of caffeine activity discussed in the literature, which only have effects at high concentrations include calcium (Ca) mobilization, prostaglandin antagonism and phosphodiesterase inhibition by caffeine. At high plasma concentrations (0.5–1 millimols (mmol)), caffeine is reported to interfere with the uptake and storage of Ca by the sarcoplasmic reticulum in striated muscles. This action can account for observations that such concentrations of caffeine increase the strength and duration of contractions in the skeletal and cardiac muscles. However, the extent to which caffeine is able to alter Ca binding and transport is unclear (Chawla and Suleman, 2011). Caffeine is also reported to act by potentiation of the inhibitors of prostaglandin synthesis, and as a competitive inhibitor of cyclic nucleotide phosphodiesterase isozymes in various tissues, including the brain. However, the affinity of caffeine for phosphodiesterases is low and a high plasma concentration of caffeine is required to attain significant effects (Fisone et al., 2004).
Select endpoints of concern

Caffeine and the substances with which it is co-administered may have acute or chronic effects on human health. FDA is interested in health hazards associated with both acute and chronic consumption of caffeine in the general population and in subpopulations, such as youth, pregnant women and their fetuses, and consumers with preexisting medical conditions.

1. Cardiovascular system

Experimental studies

Results from single-dose experimental studies in humans indicate that 200–250 mg caffeine may cause an increase in systolic (5–15 millimeters of mercury (mmHg)) and/or diastolic blood pressure (5–10 mmHg) (Nawrot et al., 2003, Nurminen et al., 1999). Changes in blood pressure and decreased heart rate have been seen with doses as low as 1 mg/kg bw in children and 1.4 mg/kg bw in adults, though in some studies caffeine caused an increased heart rate at doses ≥1.8 mg/kg bw (ORNL Report, Oct. 27, 2011). The effect on blood pressure was most pronounced in the elderly, individuals with hypertension and those who are not habituated to caffeine (Nurminen et al., 1999). As described above, the acute effects of caffeine on blood pressure are likely due to increased vascular resistance, and the change in heart rate may be due to either a reflex bradycardiac response to pressor action or a direct effect on the cardiac or sino-atrial node, though at higher doses caffeine can have a direct cardio-acceleratory effect and increase heart rate (Nurminen et al., 1999; ORNL Report, Oct. 27, 2011).

Epidemiological studies

In 14 case-control studies published between 1994 and 2011, 13 showed a positive association of caffeine intake with a risk of cardiotoxicity in the form of myocardial infarction (MI), sudden cardiac death, risk of coronary heart disease and ischemic stroke or cerebral hemorrhage (ORNL Report, Oct 27, 2011). However, while some of the prospective cohort studies examining caffeine consumption showed increased risk of cardiotoxicity, cardiovascular disease such as coronary artery disease and heart failure, or death (Happonen et al, 2004; 2006; Klatsky et al., 2008; Wilhelmsen et al., 2001a, 2001b; Klag et al., 2002; Stensvold et al., 1996), some showed a non-linear relationship between consumption and risk, with moderate exposure increasing risk, and low or high exposure decreasing risk (Hu et al., 2007; Winkelmayer et al., 2005; Uiterwaal et al., 2007; de Koning Gans et al., 2010; Paganini-Hill, 2011; Kleemola et al., 2000), and others showed no association or an inverse association (Chen et al., 2010, Rosner et al., 2007; Ahmed et al., 2009; Conen et al., 2010; Frost and Vestergaard, 2005; Shen et al., 2011; Lopez-Garcia et al., 2006; 2008; 2009; Mineharu et al., 2011; Sugiyama et al., 2010; Willett et al., 1996; Woodward and Tunstall-Pedoe, 1999; Zhang et al., 2009a; 2009b; Wang et al., 2011; Mukamal et al., 2004; Sillietta et al., 2007; Leurs et al., 2010). The risk of hypertension associated with acute caffeine consumption varies according to CYP1A2 genotype, with carriers of the slow CYP1A2*1F allele at increased risk (Palatini et al., 2009). The results of a meta-analysis of case-control studies showed that consumption of coffee (≥4 cups/day) was associated with an increased risk of nonfatal myocardial infarction only among individuals with slow caffeine metabolism (Cornelis et al., 2006). Several cross-sectional studies found a positive correlation between chronic caffeine exposure and plasma total homocysteine concentration, for which high concentrations have been associated with risk for
cardiovascular disease (Panagiotakos et al., 2004; Jacques et al., 2001; Stolzenberg-Solomon et al., 1999; Ulvik et al., 2008; Carlsen et al., 2005).

Co-exposures with other ingredients that have cardiovascular activity (e.g., taurine)

Cases have been reported where cardiac arrest ensued following strenuous physical activity and excessive consumption of “energy drinks” containing caffeine and taurine (Berger and Alford, 2009). Taurine has a positive inotropic effect on the myocardium, that is, it increases the force of myocardial contractility and cardiac output (Schaffer et al., 2010). Taurine also decreases blood pressure in human hypertensives and animal models of hypertension by dilation of the blood vessels. However, taurine can cause vasoconstriction or vasodilation depending on the vessel studied. The effect of taurine may involve, at least in part, an increase in the contractility of proteins via a Ca-dependent mechanism (Schaffer et al., 2010). As caffeine can increase diastolic and systolic blood pressure and may have a direct cardioaccelatory effect at higher doses, there is reason for concern about the effects of co-exposure of taurine and caffeine.

Ingredients such as guaraná add additional caffeine to “energy drinks.” Other ingredients added to “energy drinks”, such as niacin, may affect cardiovascular function.

2. Nervous system

Central nervous system (CNS) stimulation

Caffeine is described as the most widely consumed CNS stimulant, increasing wakefulness and enhancing mental focus and improving performance. Doses of caffeine ranging from 50 to 300 mg/d have been reported to be associated with effects on mood, such as feelings of increased energy, alertness, motivation and concentration (Chawla and Suleman, 2011). However, depending on an individual’s sensitivity, lower doses of caffeine may also have CNS stimulating effect. For example, Quinlan et al. (2000) reported increased energetic arousal at caffeine doses as low as 25 mg/d.

Three studies illustrate the effects of caffeine on the CNS. Quinlan et al. (2000) conducted two single-dose, cross-over studies using tea and coffee containing moderate levels of caffeine. In the first study, 17 habitual coffee consumers, who had abstained from coffee for 3 or 16 hours, were given caffeine doses of 37.5 or 75 mg/d in a single serving of 300 mL of tea (equivalent to levels in one or two cups of tea), or caffeine doses of 75 or 150 mg in a single serving of 300 mL of coffee (equivalent to levels in 1 or 2 cups of coffee). The subjects were observed for 2 hours after dosing. The results show that the tea and coffee produced mild autonomic stimulation and elevation in mood at both doses. In the second study by Quinlan et al., the subjects consumed decaffeinated tea with 25, 50, 100, or 200 mg of added caffeine. The results show that caffeine at all dose levels increased energetic arousal in the subjects. These data demonstrate that doses of caffeine as low as 25 mg can stimulate the CNS. In a repeated-dose study by Lieberman et al. (1987), using an intensified dosing regimen, 20 healthy males were administered caffeine capsules at dose levels of 32, 64, 128, or 256 mg/d, after a 12-hour fast. The results show a dose-related elevation of the plasma concentration of caffeine, with the 32 mg dose (typical of the level in one serving of a cola beverage) elevating plasma concentrations from 0.30 micrograms (μg)/mL to 0.85
μg/mL. In this third study, caffeine alone significantly improved performance in visual reaction time and auditory vigilance tests.

In their review, Chawla and Suleman (2011) state that in humans sleep seems to be the physiological function least sensitive to the effects of caffeine, since generally a caffeine dose greater than 200 mg is required to affect sleep significantly. Excessive consumption of caffeine (doses of 1,000–1,500 mg or about 10–15 cups/d of coffee) may lead to a state of intoxication known as caffeinism, which is characterized by restlessness, agitation, excitement, rambling thought and speech, and insomnia (Winston et al., 2005).

On its website, Health Canada suggests that adolescents and teens consume less than 2.5 mg/kg bw/d, a level which “would not cause adverse health effects in the majority of adolescent caffeine consumers” (http://www.hc-sc.gc.ca/fn-an/secteur/adsabs/caf/fd-cf-aliments-eng.php). In suggesting this limit, Health Canada acknowledges that this is not definitive advice as the data for adolescents 13 and older are insufficient. In support of its suggested limit for adolescents, Health Canada cites a review undertaken by Health Canada scientists (Nawrot et al., 2003) who examined the few experimental studies of caffeine in adolescents and children. Based on findings of adverse behavior, including anxiety, Health Canada suggests consumption of less than the lowest dose tested in any of the studies (Bernstein et al., 1994). This placebo-controlled cross-over design study examined 20 subjects (ages 8–12) given 0, 2.5, or 5.0 mg/kg bw caffeine for several endpoints including measures of self-reported anxiety and found a non-significant trend towards anxiety using some of the measures (Bernstein et al., 1994).

Seizure potential

Case studies have been reported in the literature describing adult onset tonic-clonic seizures following heavy consumption of “energy drinks” (Iyadurai and Chung, 2007; Calabro et al., 2012). Another case study reports seizure in a 15 year-old following consumption of two “energy shots” (Babu et al., 2011). While these data are limited, they raise a question regarding whether “energy drinks” or “energy shots” trigger seizures in susceptible people.

Tolerance and dependence

Caffeine is a substance that people often repeatedly use on a regular basis. This reinforcing property of caffeine appears to reflect the desire to obtain the acute stimulatory effect of consumption as well as the desire to avoid withdrawal symptoms (Daly and Fredholm, 1998). Caffeine stimulates D2 release in different areas of the brain than classical drugs of abuse. Both self-administration studies in animals and behavioral economic studies in people show that caffeine is less reinforcing than other stimulants, such as amphetamines (Nehlig, 1999). Chronic administration can lead to tolerance for some of the effects of caffeine, such as the effects on respiration, blood pressure (possibly incomplete), and heart rate (Daly and Fredholm, 1998). There is also some evidence for tolerance to the subjective effects such as jitters and anxiety as well as incomplete tolerance to the effects of sleep disturbances (Nehlig, 1999). Withdrawal symptoms, resulting from discontinuation of use after habituation, can include apathy, drowsiness, headaches, nausea, and anxiety (Daly and Fredholm, 1998).
REFERENCES


Consumer Reports magazine (2012): The buzz on energy-drink caffeine: Caffeine levels per serving for the 27 products we checked ranged from 6 milligrams to 242 milligrams per serving” Retrieved 2/27/2013 http://www.consumerreports.org/cro/magazine/2012/12/the-buzz-on-energy-drink-caffeine/index.htm


ADVERSE HEALTH EFFECTS OF CAFFEINE:
REVIEW AND ANALYSIS OF RECENT HUMAN AND ANIMAL RESEARCH

Final Report

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Speaker Recommended References

DAY 1, SESSION 1: INTAKE AND EXPOSURE TO CAFFEINE


Beverage caffeine intake in US consumers and subpopulations of interest: estimates from the Share of Intake Panel survey

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Abstract

Concerns exist about the potential adverse health effects of high consumption of dietary caffeine, especially in children and pregnant women. Recommended caffeine intakes corresponding to no adverse health effects have been suggested recently for healthy adults (400–450 mg/day), for women contemplating pregnancy (300 mg/day), and for young children age 4–6 years (45 mg/day). To determine whether current caffeine intake approaches these levels, intake from major dietary sources (coffee, tea and carbonated soft drinks) were measured in 10,712 caffeinated beverage consumers in the 1999 US Share of Intake Panel, a targeted beverage survey. Mean caffeine intakes in adult caffeinated beverage consumers ranged from 106 to 170 mg/day (90th percentile intake was 227–382 mg/day). In children 1–5 and 6–9 years, mean caffeine intakes were 14 and 22 mg/day, respectively; corresponding 90th percentile intakes were 37 and 45 mg/day. Pregnant women consumed an average of 58 mg/day (157 mg/day at the 90th percentile), and women of reproductive age ingested 91–109 mg/day (229–247 mg/day at the 90th percentile). These data show that while mean caffeine intakes are within recommended safe levels, heavy consumers of certain subpopulations, including young children and women contemplating pregnancy, might benefit from dietary advice.

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Keywords: Caffeine; Consumption; Beverages; Survey; US population; Children; Women

1. Introduction

Caffeine has been the subject of interest among consumers and health professionals alike because it is widely consumed in the diet by most segments of the population and can exert pharmacological effects (Mandel, 2002). In the United States, coffee, tea and carbonated soft drinks are the major sources of caffeine in the diet (Barone and Roberts, 1996). Mean daily caffeine consumption was previously reported to be approximately 3 mg/kg in the US general population, and 4 mg/kg in adult US consumers, with 90th percentile consumption at 5–7 mg/kg/day (Barone and Roberts, 1996). More recently, daily caffeine intakes were estimated to be 4 mg/kg in the US adult population (20 years and over) using the 1994–1996 US Department of Agriculture (USDA) Continuing Surveys of Food Intake by Individuals (CSFII) results (USDA, 2000).

Because of the wide consumption of caffeine, there is an interest in its potential to exert adverse health effects, especially in women and children. A recent review of the effects of caffeine on human health concluded that for the healthy adult population, moderate daily caffeine consumption at levels up to 400–450 mg/day (5.7–6.4 mg/kg/day in a 70 kg adult) was not associated with adverse effects, which included general toxicity, effects on bone status and calcium balance, cardiovascular effects, behavioral changes, increased incidence of cancer, or effects on male fertility (Nawrot et al., 2003). The
authors also reported that children and women of reproductive age were “at risk” subgroups who might require dietary advice to moderate their caffeine intake. Women contemplating pregnancy were recommended to consume a maximum caffeine amount of 300 mg/day (4.6 mg/kg for a 65 kg person) based on the potential for certain reproductive/developmental effects, while children 4–6 years should limit consumption of caffeine to 2.5 mg/kg/day or 45 mg/day based on reported behavioral effects by Bernstein et al. (1994). However, other reviewers suggest that caffeine’s behavioral effects in children are innocuous even at 3 mg/kg/day (Castellanos and Rapoport, 2002).

In order to determine any potential risk from caffeine ingestion, accurate consumption data is required. Unfortunately, there are relatively few sources of published consumption data targeting single ingredients like caffeine. One source of national food intake data is the USDA CSFII. This USDA survey is updated regularly and contains caffeine data for foods and beverages, along with quantities consumed (USDA, 2000; Smiciklas-Wright et al., 2003).

Another source of more targeted caffeine intake data from beverages is the Share of Intake (SIP) survey, which reports seasonally adjusted beverage consumption data, and is conducted by NFO WorldGroup, a marketing research company. Since the majority of caffeine (>95%) is consumed from caffeinated beverages (Barone and Roberts, 1996), it was appropriate to use the 1999 SIP data to estimate beverage caffeine intake in US consumers. The results are now reported in this paper.

2. Materials and methods

This study was undertaken by the International Life Sciences Institute (ILSI) Caffeine Technical Committee to gather current caffeine intake data in US consumers of caffeinated beverages. The beverage data was collected by NFO WorldGroup in their 1999 Share of Intake Panel (SIP) survey. Knight International and the Dietary Assessment Center at the Pennsylvania State University analyzed and reviewed the data that are now being reported.

2.1. Survey methodology

Share of Intake Panel (SIP) is a syndicated marketing research program that has monitored the consumption of all beverages, excluding tap water, since 1980. The SIP survey is a mail sample sent to a demographically balanced sample of the US population (according to the most recent US census). Mailings to panel households are staggered weekly to ensure coverage across the whole year. The sampling is done at the household level, but balanced at the individual level. SIP balances the survey going out to panel members and weights it when returned (due to the low response rate of lower income families). The data is weighted quarterly to be nationally representative of age within gender, household income and size, region and market size.

All participants in the SIP study voluntarily agreed to complete the questionnaire for the entire 14-day period, and were given a small upfront cash incentive ($3) to encourage completion of the questionnaire. In the 1999 SIP survey the response rate was 50%. Recipients are requested to have all household members record all beverage consumption information both at home and away from home for 14 days, using diaries designed to capture beverage-specific information. Parents complete the survey for younger children in the home.

The SIP diary collects the following types of information for each beverage consumed: brand and flavor of beverage; time of day; month/date; type and size of container purchased; class of trade where beverage was purchased; type of container from which the beverage was drunk; where beverage was consumed; how the beverage was consumed (hot or cold); total fluid ounces consumed at one occasion; and demographic information. Standard reporting measures are share of volume, penetration, volume per consumer, volume per occasion, frequency of consumption, and category share of consumption.

2.2. Analysis and statistics

A total of 10,712 caffeinated beverage consumers were analyzed from the 1999 SIP survey. Caffeine consumption was estimated in the total caffeinated beverage categories that included total coffee (brewed + instant + ready-to-drink), total tea (brewed + powdered + ready-to-drink), total caffeinated carbonated soft drinks (regular and diet categories of colas + non-colas), cocoa and chocolate drinks, caffeinated fruit juice/drinks and bottled water. Caffeine intakes were reported in this paper as total coffee, total tea, total caffeinated carbonated soft drinks, and other [cocoa and chocolate drinks, caffeinated fruit juice/drinks, and bottled water were combined since the caffeine contribution from these beverage categories was negligible (<1%)].

The following measures were calculated from the SIP data: number and percent of consumers; number of consuming occasions per consumer (mean over 14 days); and mean and 90th percentile caffeine consumption for age subgroups (as mg/day). The population of all caffeinated beverage drinkers was used as the base for each category calculation. Estimates of caffeine intake on a body weight basis (mg/kg/day) were also calculated. However, since body weight data is not collected in SIP surveys, the NHANES statistical tables (1988–1994)
were the source of mean body weights for males and females by age group (see footnotes in Table 4).

2.3. Caffeine content in beverages

The caffeine content of each category of beverage (Table 1) was based on both published reports and company/product specific data. If the brand-specific caffeine content of soft drinks varied within a category, the caffeine content of the category was based on an average of the category brand leaders. The caffeine value assigned to brewed coffee in the current study was 360 mg/l or 85 mg per 237 ml (8 fl oz) cup (IFIC, 1998). While these are somewhat lower than the caffeine values used by USDA and previously by Barone and Roberts (1996) (567 mg/l or 85 mg per 150 ml (5 fl oz) cup), they represent the caffeine concentration currently measured by the coffee industry and are similar on a per serving basis to caffeine values reported recently (Mandel, 2002). According to industry studies, the caffeine content of most drip/brewed coffees has decreased over the years due to changes in the coffee roasting process, and to greater use of the arabica coffee bean, which has a lower amount of caffeine (<1.5% by weight) than other coffee types such as robusta which contains about 2.4–2.8% caffeine (Illy, 2002). This has been further confirmed by Bracken et al. (2002).

3. Results

3.1. Consumers and consuming occasions

Data on the number of consuming occasions (mean over 14 days) and the number and percentage of consumers for each age group for each beverage category are summarized in Table 2. In caffeinated beverage consumers (All Ages), 77% reported consuming a caffeinated soft drink (CSD), 46% reported consuming a coffee beverage, and 41% consumed a tea beverage at least once in 14 days. In adults 25 years and over, 73% reported drinking a CSD, 63% consumed coffee, and 47% drank tea at least once in 14 days (calculated from data in Table 2).

The mean number of occasions that a caffeinated beverage was consumed by a drinker was 21 times in 14 days (All Ages). Children (≤14 years of age) consumed a caffeinated beverage the least number of occasions over
the 14-day period (≤10 occasions), whereas consumers 35 years and over consumed a caffeinated beverage the greatest number of occasions (≥26). In children and adults up through the age of 34 years, caffeinated carbonated soft drinks (CSDs) were consumed the most frequently, whereas in older adults coffee was consumed the most frequently.

The data show that there is an increased number of coffee consuming occasions in older age groups, whereas CSD drinking occasions peaked at 20–24 years and then gradually declined. Tea consuming occasions were fairly consistent over the age groups although a somewhat higher number of occasions occurred in ages 35 years and over. Pregnant women consumed caffeinated beverages considerably less frequently than reproductive aged women (an average of 12 occasions vs. 21 occasions over 14 days).

### 3.2. Caffeine intake

Caffeine intakes in all consumers and age subgroups are expressed as mean and 90th percentiles in actual mg/day (Table 3) and are estimated on a body weight basis in mg/kg/day (Table 4). Caffeine intakes in both pregnant women and representative age categories of non-pregnant women (ages 20–24 and 25–34 years) have also been included. Trends in caffeine intake for male and female subgroups generally were similar to the combined intake data for both sexes in an age group; therefore, the data are not reported by individual sex.

Mean caffeine intake from all caffeinated beverages in consumers (All Ages) was 120 mg/day or 1.73 mg/kg/day; intake at the 90th percentile was 287 mg/day or 4.03 mg/kg/day. Caffeine intake was highest in the 35–49 year old age group (mean = 170 mg/day and 90th = 382 mg/day), primarily from coffee.

In young children age 1–5 and 6–9 years, mean caffeine intake from all caffeinated beverages was 14 and 22 mg/day, or 0.82 and 0.85 mg/kg/d, respectively. Intakes at the 90th percentile were 37 and 45 mg/day; or about 2.32 and 1.70 mg/kg/day, respectively. Caffeinated CSDs accounted for the majority of the caffeine intake in these age groups, followed by tea. Coffee accounted for <2% of the total caffeine intake in these children.

From childhood into young adulthood, caffeine intake from all caffeinated beverages gradually rose and reached 106 mg/day (or about 1.54 mg/kg/day) in 20–24 year olds. The majority of caffeine was from caffeinated CSDs, followed by tea. In all adults 25 years and above, coffee was the major source of caffeine intake followed by CSDs. In older adults, tea became the secondary source of caffeine instead of CSDs.

Pregnant women consumed about one-half the amount of caffeine from caffeinated beverages than did women of reproductive age 20–34 years (mean intake = 58 mg/day in pregnant women and 91–109 mg/
4. Discussion

To estimate caffeine intake in US caffeinated beverage consumers and specific subgroups of interest (including children and pregnant women), data was evaluated from the 1999 Share of Intake Panel (SIP), a targeted beverage survey. We found that adult consumers of caffeinated beverages in the US population had caffeine intakes ranging from 106 to 170 mg/day (mean) and 227–382 mg/day (90th percentile). These data compare favorably with the most recent review of caffeine’s health effects reported by the Canadian group Nawrot et al. (2003), who proposed recommended maximum intake levels for caffeine of 400–450 mg/day in healthy adults.

The Canadian review also proposed specific maximum caffeine levels for reproductive aged women (300 mg/day) based on caffeine’s potential for reproductive/developmental effects. In contrast to this review, Leviton and Cowan (2002) reviewed the literature relating the risk of reproductive hazard to caffeine consumption in women and found that “no convincing evidence has been presented to show that caffeine consumption increases the risk of any reproductive adversity”. Longitudinal studies in pregnant women have also shown that they reduce their caffeine consumption during early pregnancy (Cnattingius et al., 2000; Lawson et al., 2002). Our study confirms that pregnant women consumed a caffeinated beverage (including coffee) on fewer occasions than women of childbearing age (Table 2), and have caffeine intakes about 1/2 those of women of reproductive age (Tables 3 and 4).

Children, including those diagnosed as hyperactive, are no more sensitive to the effects of caffeine than adults according to a review of 82 papers on the behavioral effects of caffeine in children (Leviton, 1992). Children metabolize caffeine more rapidly than adults and consume much less caffeine, even in proportion to their smaller size. One study in the literature (Bernstein et al., 1994) examined behavioral effects in children at a caffeine dose of 2.5 mg/kg/day (equivalent to 64–127 mg/child/day). Caffeine produced improvements on a vigilance task and tests of manual dexterity; a modest and non-significant relationship was found between caffeine and self-reported anxiety. A recent review of caffeine and behavioral effects in children by Castellanos and Rapoport (2002) indicated that caffeine doses of 3 mg/kg/day had negligible behavioral effects in normal
<table>
<thead>
<tr>
<th>Age group/segment</th>
<th>Caffeine, mean ± SE (mg/kg/day)</th>
<th>Caffeine, 90th percentile (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total caffeinated beverages</td>
<td>Total CSD</td>
</tr>
<tr>
<td>1–5 years</td>
<td>0.82 ± 0.12</td>
<td>0.59 ± 0.10</td>
</tr>
<tr>
<td>6–9 years</td>
<td>0.85 ± 0.14</td>
<td>0.47 ± 0.05</td>
</tr>
<tr>
<td>10–14 years</td>
<td>0.69 ± 0.05</td>
<td>0.53 ± 0.04</td>
</tr>
<tr>
<td>15–19 years</td>
<td>1.04 ± 0.07</td>
<td>0.74 ± 0.05</td>
</tr>
<tr>
<td>20–24 years</td>
<td>1.54 ± 0.18</td>
<td>0.93 ± 0.11</td>
</tr>
<tr>
<td>25–34 years</td>
<td>1.75 ± 0.09</td>
<td>0.71 ± 0.05</td>
</tr>
<tr>
<td>35–49 years</td>
<td>2.30 ± 0.05</td>
<td>0.57 ± 0.02</td>
</tr>
<tr>
<td>50–64 years</td>
<td>2.20 ± 0.03</td>
<td>0.30 ± 0.01</td>
</tr>
<tr>
<td>65+ years</td>
<td>1.92 ± 0.02</td>
<td>0.14 ± 0.00</td>
</tr>
<tr>
<td>All Ages</td>
<td>1.73 ± 0.02</td>
<td>0.53 ± 0.01</td>
</tr>
</tbody>
</table>

Non-pregnant women

<table>
<thead>
<tr>
<th></th>
<th>1–5 years</th>
<th>6–9 years</th>
<th>10–14 years</th>
<th>15–19 years</th>
<th>20–24 years</th>
<th>25–34 years</th>
<th>35–49 years</th>
<th>50–64 years</th>
<th>65+ years</th>
</tr>
</thead>
<tbody>
<tr>
<td>20–24 years</td>
<td>1.50 ± 0.31</td>
<td>0.83 ± 0.19</td>
<td>0.42 ± 0.20</td>
<td>0.24 ± 0.14</td>
<td>0</td>
<td>3.78</td>
<td>1.98</td>
<td>2.11</td>
<td>0.59</td>
</tr>
<tr>
<td>25–34 years</td>
<td>1.73 ± 0.14</td>
<td>0.68 ± 0.07</td>
<td>0.78 ± 0.12</td>
<td>0.27 ± 0.05</td>
<td>0</td>
<td>3.86</td>
<td>1.92</td>
<td>2.64</td>
<td>0.83</td>
</tr>
<tr>
<td>Pregnant women</td>
<td>0.93 ± 0.27</td>
<td>0.34 ± 0.10</td>
<td>0.42 ± 0.23</td>
<td>0.17 ± 0.14</td>
<td>0</td>
<td>2.40</td>
<td>0.88</td>
<td>1.67</td>
<td>0.51</td>
</tr>
</tbody>
</table>

---

4 Body weights are the 50th percentiles from the NHANES Statistical Tables 1988–1994; Male body weights in kg (by age) are: 11.5 (1 y), 13.5 (2 y), 15.5 (3 y), 17.4 (4 y), 19.9 (5 y), 21.9 (6 y), 25.5 (7 y), 17.9 (8 y), 32 (9 y), 36.1 (10 y), 39.7 (11 y), 48.0 (12 y), 52.8 (13 y), 60.6 (14 y), 63.5 (15 y), 65.9 (16 y), 70.0 (17 y), 67.5 (18 y), 71.4 (19 y), 75.0 (20–29 y), 80.0 (30–39 y), 82.1 (40–49 y), 84.0 (50–59 y), 82.3 (60–69 y), 77.9 (70–79 y), 70.8 (80 y+). Female body weights in kg (by age) are: 10.6 (1 y), 13.0 (2 y), 15.1 (3 y), 17.3 (4 y), 19.7 (5 y), 21 (6 y), 24.0 (7 y), 28.5 (8 y), 31.6 (9 y), 36.0 (10 y), 43.2 (11 y), 46.6 (12 y), 53.6 (13 y), 54.0 (14 y), 55.4 (15 y), 56.3 (16 y), 60.9 (17 y), 58.0 (18 y), 60.7 (19 y), 60.5 (20–29 y), 65.5 (30–39 y), 68.5 (40–49 y), 71.5 (50–59 y), 68.1 (60–69 y), 64.7 (70–79 y), 59.7 (80 y+).
children. They concluded that caffeine effects in children seemed to be modest and innocuous. In children a caffeine intake of 3 mg/kg/day would translate to 45–54 mg/day in a child age 1–5 years old (body weight 15–18 kg) and 105–150 mg/day for 10–14 year old children (body weight 35–50 kg).

Comparing these recommended maximum caffeine intake levels with the caffeine consumption levels from the SIP survey, and using the 90th percentile intake in consumers as an estimate of “heavy consumption”, it is evident that adults, pregnant women, and women of reproductive age are below these recommended intake levels. In adults, 90th percentile caffeine consumption ranged from 227 to 382 mg/day (3.2–5.2 mg/kg/day). In pregnant women, the 90th percentile caffeine intake was 157 mg/day (2.4 mg/kg/day), and was lower than the caffeine intake of women aged 20–34 years, which ranged from 229 to 247 mg/day (3.8–3.9 mg/kg/day). In this specific age group of “heavy caffeine consuming” women who might be contemplating pregnancy, it might be useful to target dietary advice since caffeine consumption was approaching 300 mg/day. Pregnant women appeared to already have limited their caffeine intake, and in fact, caffeine consumption was similar to the 90th percentile intake level of 3.7 and 4.6 mg/kg/day reported by Barone and Roberts (1996) using the 1987–1988 USDA CFSII and 1989 MRCA data for coffee, respectively.

Caffeine intake in children at the 90th percentile from SIP data was 37, 45 and 73 mg/day for ages 1–5, 6–9, and 10–14 years, respectively. This intake, expressed on a body weight basis, was 2.3, 1.7 and 1.4 mg/kg/day, again under the maximum intake of 2.5 mg/kg/day suggested by Nawrot et al. (2003) or 3 mg/kg/day derived from inspection of the review of Castellanos and Rapoport (2002).

This caffeine evaluation in children would not be complete without consideration of the same approach using the USDA CFSII data from 1994 to 1996; 1998 Supplemental Children’s survey (Table 5). Using this conservative approach of 90th percentile intake data in consumers yields caffeine intakes of 48.5 mg/day or 3 mg/kg/day in children age 1–5 years (Unpublished data, Novigen Sciences Inc., 2001). Using mean caffeine intake data from USDA instead of 90th percentile data in children, Ahuja and Perloff (2001) reported that children age 1–5 years had a caffeine intake of 19.5 mg/day (or 1.3 mg/kg/day for an average body weight of 15 kg) compared to 13.5 mg/day or 0.82 mg/kg/day for children age 1–5 years in the SIP survey. The primary reason for this difference in children’s caffeine intake between the SIP and USDA surveys is likely due to the fact that the USDA CFSII survey is a short-term survey and, as such, overestimates intake.

In conclusion, good estimates of caffeine consumption are important since caffeine is one of many constituents in foods that can exert pharmacological effects. Most experts agree that moderation and common sense are the keys for consuming caffeine-containing beverages; moderate amounts of caffeine are generally considered to be ≤300 mg/day (IFIC, 1998). The data presented here from the SIP survey demonstrate that in adults, as well as in populations of most concern—young children, women of reproductive age, and pregnant women—caffeine intakes are within the levels associated with no adverse health effects. However, it would be prudent to advise women of childbearing age who are contemplating pregnancy to consume caffeine in moderate amounts. Additionally, to be conservative in young children, educational materials could be prepared to inform parents and health professionals about caffeine amounts in beverages.

Acknowledgements

This work was supported by the Caffeine Technical Committee of the North American Branch of the International Life Sciences Institute (ILSI N.A.). Carol A. Knight, Ph.D., Ian Knight, B.Sc., and Diane C. Mitchell, M.S., R.D., served as consultants to the Caffeine Technical Committee. ILSI N.A. is a public, non-profit scientific foundation that advances the understanding and application of scientific issues related to the nutritional quality and safety of the food supply, as well as health issues related to consumer self-care products. The organization carries out its mission by sponsoring relevant research programs, professional

<table>
<thead>
<tr>
<th>Survey parameter</th>
<th>Total caffeinated beverages</th>
<th>Total CSD</th>
<th>Total coffee</th>
<th>Total tea</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean caffeine intake (mg/day)</td>
<td>22.3</td>
<td>18.1</td>
<td>19.5</td>
<td>30.4</td>
<td>3.6</td>
</tr>
<tr>
<td>Mean caffeine intake (mg/kg/day)</td>
<td>1.40</td>
<td>12.7</td>
<td>1.10</td>
<td>1.90</td>
<td>0.2</td>
</tr>
<tr>
<td>90th percentile caffeine intake (mg/kg/day)</td>
<td>48.5</td>
<td>38.6</td>
<td>44.4</td>
<td>59.8</td>
<td>6.9</td>
</tr>
<tr>
<td>90th percentile caffeine intake (mg/kg/day)</td>
<td>3.0</td>
<td>2.2</td>
<td>2.4</td>
<td>3.9</td>
<td>0.4</td>
</tr>
</tbody>
</table>

*Source of data analysis is Novigen Sciences, Inc report, June, 2001 (SE not given in report).

*Total Caffeinated Beverages include CSDs, coffee, tea, and other caffeinated beverages (which represent <1% of total caffeine intake including cocoa and chocolate drinks and fruit drinks).
education programs and workshops, seminars, and publications, as well as providing a neutral forum for
government, academic, and industry scientists to discuss
and resolve scientific issues of common concern for the
well-being of the general public. ILSI N.A.’s programs
are supported primarily by its industry membership.
(For further information about the Committee or ILSI
N.A., call 202/659-0074 or email sweiss@ilsi.org).

References

Ahuja, J., Perloff, B., 2001. Caffeine and theobromine intakes of
and Nutrition Review 13, 47–51.
Barone, J., Roberts, H., 1996. Caffeine consumption. Food and
Chemical Toxicology 34, 119–129.
Bernstein, G., Carroll, M., Crosby, R., Perwein, A., Go, F., Benowitz,
N., 1994. Caffeine effects on learning, performance and anxiety in
normal school-age children. Journal of the American Academy of
Child and Adolescent Psychiatry 33, 407–415.
Bracken, M., Triche, E., Grosso, L., Hellenbrand, K., Belanger, K.,
Leaderer, B., 2002. Heterogeneity in assessing self-reports of
caffeine exposure: implications for studies of health effects. Epidemi-
ology 13, 165–171.
Castellanos, F.X., Rapoport, J.L., 2002. Effects of caffeine on
development and behavior in infancy and childhood: a review of
published literature. Food and Chemical Toxicology 40, 1235–
1242.
Cnattingius, S., Signorello, L., Anneren, G., Claussen, B., Ekbom, A.,
Ljunger, E., Blot, W., McLaughlin, J., Peterson, G., Rane, A.,
Granath, F., 2000. Caffeine intake and the risk of first-trimester
spontaneous abortion. New England Journal of Medicine 343,
1839–1845.
International Food Information Council (IFIC). 1998. Everything you
need to know about caffeine. World Wide Web: http://www.ific.
org/proactive/newsroom/release.vtml?id=17784 [accessed 28 April
2003].
hormone metabolite patterns, pregnancy symptoms, and coffee
caffeine consumption by women to their risk of reproductive
hazards. Food and Chemical Toxicology 40, 1271–1310.
Mandel, H.G., 2002. Update on caffeine consumption, disposition and
action. Food and Chemical Toxicology 40, 1231–1234.
Nawrot, P., Jordan, S., Eastwood, J., Rotstein, J., Hughenholtz, A.,
Additives and Contaminants 20, 1–30.
Smiciklas-Wright, H., Mitchell, D.C., Micklé, S., Cook, A., Goldman,
American Dietetic Association 103, 41–47.
US Department of Agriculture (USDA), Agricultural Research Service
(ARS), 2000. Intakes of Selenium, Caffeine, and Theobromine by
bhnrc/foodsurvey/home.htm [accessed 28 April 2003].
Beverage Caffeine Intakes in Young Children in Canada and the US

CAROL A. KNIGHT, PhD, IAN KNIGHT, BS, Knight International, Chicago, IL;
DIANE C. MITCHELL, MS, RD, Department of Nutritional Sciences, Pennsylvania State University, University Park, PA

Abstract

Purpose: Throughout childhood there is a shift from predominantly milk-based beverage consumption to other types of beverages, including those containing caffeine. Although a variety of health effects in children and adults have been attributed to caffeine, few data exist on caffeine intake in children aged one to five years.

Methods: Because beverages provide about 80% of total caffeine consumed in children of this age group, beverage consumption patterns and caffeine intake were evaluated from two beverage marketing surveys: the 2001 Canadian Facts study and the 1999 United States Share of Intake Panel study.

Results: Considerably fewer Canadian children than American children consume caffeinated beverages (36% versus 56%); Canadian children consume approximately half the amount of caffeine (7 versus 14 mg/day in American children). Differences were largely because of higher intakes of carbonated soft drinks in the US.

Conclusions: Caffeine intakes from caffeinated beverages remain well within safe levels for consumption by young children. (Can J Diet Pract Res 2006;67:96-99)

Résumé

Objectif. Au cours de l’enfance, la consommation de boissons principalement à base de lait est graduellement remplacée par celle d’autres types de boissons, notamment celles qui contiennent de la caféine. Bien qu’une série d’effets sur la santé des enfants et des adultes aient été attribués à la caféine, peu de données existent sur l’apport en caféine chez les enfants de un à cinq ans.


Résultats. Les enfants canadiens sont beaucoup moins nombreux que les enfants américains à consommer des boissons renfermant de la caféine (36 % contre 56 %); les enfants canadiens consomment environ la moitié moins de caféine (7 contre 14 mg/jour chez les enfants américains). Les différences s’expliquent en grande partie par une plus forte consommation de boissons gazeuses aux États-Unis.

Conclusions. Les apports en caféine provenant des boissons renfermant de la caféine correspondent aux niveaux sains de consommation chez les jeunes enfants. (Rev can prat rech diétét 2006;67:96-99)

INTRODUCTION

Beverages contribute significantly to energy and nutrient intakes, particularly in young children. As children grow older, consumption of milk and/or 100% juices is likely to diminish. This trend has been well documented, particularly with respect to increased consumption of sweetened juice drinks and carbonated soft drinks (CSDs) among children (1-3). Soft drink portion sizes have also increased between the 1989-91 United States (US) Department of Agriculture Continuing Survey of Food Intakes of Individuals and the 1994-96 survey (4). This increase was apparent in older children aged 12 to 19 years and in adults, but not in younger children. Other studies have shown that CSD consumption decreased in children aged one to five years during the ten years from 1987-88 to 1997-98 (5).

In addition to a potential effect on nutrient intakes in young children, an increased consumption of sweetened beverages – depending on the type of beverage – might increase caffeine intakes. A recent Health Canada (HC) review on the health effects of caffeine showed that moderate daily caffeine consumption of 400 to 450 mg in adults was not associated with any adverse health effects (6). However, HC suggested that children may be at greater risk, and recommended that, as a precautionary measure, children should limit their caffeine intake to 2.5 mg/kg/day. According to HC recommendations, this translates to 45 mg/day for children aged four to six years (7).

In children, beverages provide at least 80% of all caffeine consumed (8). A reasonable assumption, therefore, is that the majority of the caffeine consumed could be estimated from targeted beverage surveys. Two such recent market research surveys, the 2001 Canadian Facts survey and the 1999 US Share of Intake Panel (SIP) survey, were used to examine caffeine intakes in a large number of children. These surveys were reviewed to determine caffeinated

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beverage consumption patterns and corresponding caffeine intakes in children aged one to five years.

**METHODS**

The US SIP survey, conducted by NFO World Group Inc. since 1980, was a mail survey sent to a demographically balanced sample of the US population. All household members were asked to complete diaries for 14 consecutive days, recording all beverages consumed except tap water. Parents completed the survey for their children. The data presented here were collected in 1999 from a representative sample of US children (n=619) between the ages of one and five years. The survey response rate was 50%.

The 2001 Canadian Facts survey, conducted by CFO, the Canadian subsidiary of NFO World Group Inc., was similar in design to the US SIP survey. A representative, randomly selected sample of Canadian households were mailed a seven-day diary to be completed by all household members. In total, 751 panel households were recruited, representing all provinces. A total of 492 completed diaries were returned (representing 658 children aged one to five years), yielding a response rate of 65.5%. All beverages consumed at and away from home were recorded, including tap water. However, because the US SIP survey did not collect data on tap water, water intake data are not included in our presentation and discussion of survey findings.

In general, both surveys collected similar data for each beverage consumed. These included brand and flavour of beverage, time of day/month consumed, type and size of container purchased and drunk from, class of trade where the drink was purchased, total amount consumed on one occasion, where the drink was consumed, how the drink was consumed (hot or cold), and demographic information.

Total volume calculated from seven beverage categories was used to examine the percentage that each beverage category contributed to the total beverage volume consumed. Beverage categories were coffee, tea, milk (including chocolate milk), 100% fruit juice, juice drinks, CSDs (including those that were decaffeinated), and other beverages. Juice drinks included all fruit drinks with some fruit juice content; other beverages included sport drinks/isotonic drinks, herb-enhanced drinks, frozen slush drinks, smoothies, meal-replacement drinks, soy (non-dairy) beverages, chocolate/cocoa drinks made with water, and fruit-flavoured drinks.

**Table 1**

<table>
<thead>
<tr>
<th>Beverage type</th>
<th>Caffeine concentration (ppm/mg/8 fl oz)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Water</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caffeinated only</td>
<td>120 (28)</td>
<td>Level of Water Joe™; most caffeinated waters are similar.</td>
</tr>
<tr>
<td><strong>Fruit juice/drinks</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caffeinated only</td>
<td>200 (47)</td>
<td>Several energy drinks have 200 mg/L of caffeine, the highest caffeine concentration permitted by the FDA. Used by many companies as a guideline.</td>
</tr>
<tr>
<td><strong>Chocolate/cocoa</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All drinks listed in this category</td>
<td>21 (5)</td>
<td>Reported by IFIC(10)</td>
</tr>
<tr>
<td><strong>Tea</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hot/cold – brewed brown (excluding decaffeinated)</td>
<td>200 (47)</td>
<td>Mean of domestic and imported teas reported by IFIC</td>
</tr>
<tr>
<td>Hot/cold – brewed green (excluding decaffeinated)</td>
<td>130 (31)</td>
<td>Caffeine content reported for green tea</td>
</tr>
<tr>
<td>Ready to drink</td>
<td>80 (19)</td>
<td>Level of major brands (Lipton®, Nestea®, Snapple®, AriZona®) Reported by IFIC</td>
</tr>
<tr>
<td>Powdered, instant</td>
<td>118 (28)</td>
<td></td>
</tr>
<tr>
<td><strong>Coffee</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brewed</td>
<td>360 (85)</td>
<td>Reported by IFIC</td>
</tr>
<tr>
<td>Instant</td>
<td>316 (75)</td>
<td>Reported by IFIC</td>
</tr>
<tr>
<td>Ready to drink</td>
<td>250 (59)</td>
<td>Level of major selling brands (Frappuccino® and Nescafé®) Reported by (10).</td>
</tr>
<tr>
<td>Decaffeinated</td>
<td>20 (5)</td>
<td></td>
</tr>
<tr>
<td><strong>CSDs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cola/Pepper</td>
<td>115 (27)</td>
<td>Weighted average market share of Coke®, Pepsi®, RC®, and other brands</td>
</tr>
<tr>
<td>Citrus – lemon-lime</td>
<td>0 (0)</td>
<td>No caffeinated lemon-lime CSDs® on the market</td>
</tr>
<tr>
<td>Citrus – orange</td>
<td>115 (27)</td>
<td>Level of Sunkist®, the leading and only orange CSD® known to be caffeinated</td>
</tr>
<tr>
<td>Citrus – heavy/cloudy citrus</td>
<td>153 (36)</td>
<td>Weighted average market share of Mountain Dew®, Mello Yello®, Sun-Drop®, and others</td>
</tr>
<tr>
<td>Root beer</td>
<td>63 (15)</td>
<td>Level of Barq’s®, the only known caffeinated root beer</td>
</tr>
<tr>
<td>Other flavours</td>
<td>100 (24)</td>
<td>Level of A&amp;W Cream®, the leading brand, and others</td>
</tr>
</tbody>
</table>

*CSDs = carbonated soft drinks; FDA = Food and Drug Administration IFIC = International Food Information Council

1) Data points without references are derived from company-specific reports provided to the International Life Sciences Institute North America Caffeine Technical Committee.

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Caffeine intake from caffeinated beverages was calculated as mean mg/day and mg/kg/day. Respondents reported the body weight of each child in the Canadian Facts study. However, to analyze the US SIP data, body weights by age from the 1988-94 National Health and Nutrition Examination Survey statistical tables were used. To evaluate caffeine intakes further, the 90th percentiles for both mg/day and mg/kg/day are also presented. The US Food and Drug Administration considers this level of exposure as representative of “heavy consumers” (9).

Caffeine intake data from beverage categories are presented using children who had consumed any caffeinated beverage during the survey sampling period as the base (Canadian Facts and US SIP surveys). Caffeine concentrations in beverages consumed in both surveys were calculated using both published reports and company-/product-specific data (Table 1).

**RESULTS**

On average, US children aged one to five years consumed 706 mL of beverages other than water per day, while Canadian children consumed 793 mL. Milk and 100% juice were the predominant beverages consumed by young children. In Canadian children, milk accounted for 48% of the total volume, with 26% from 100% juice, 12% from juice drinks, 5% from CSDs, 1% from tea, and less than 0.1% from coffee. US children consumed less milk (41% of volume) and 100% juice (21% of volume) than did Canadian children and more CSDs (13% of volume); they consumed 4% from teas, and, like the Canadian children, less than 0.1% from coffee.

Overall, in Canada 36% of children consumed some type of caffeinated beverage (2001 Canadian Facts), whereas in the US, 58% of children consumed a caffeinated beverage (1999 US SIP). Canadian and US children showed similar beverage sources of caffeine; CSDs provided the greatest amount of caffeine, and tea the second-greatest amount.

US children’s mean caffeine intake from all beverages was twice that of Canadian children; almost 50% more American children were at the 90th percentile for intake (Table 2). At the 90th percentile, caffeine intake from tea was almost as significant as from CSDs in Canadian children, while US children consumed almost three times as much caffeine from CSDs as from tea.

**DISCUSSION**

The most significant challenge in evaluating dietary patterns or exposure to food or beverage components is identifying sources that provide accurate, representative, and current data. The US SIP and Canadian Facts surveys provide comparable data, which specifically target beverages representative of the US and Canadian populations.

Other investigators have also looked at how beverage or caffeine consumption patterns vary in different populations; however, most of these studies are in relatively small groups of children in discrete subgroups of the US population (8,11-14). In none of the studies is Canadian children’s caffeine intake reported.

Should health professionals be concerned about caffeine intakes in young children? Younger children do consume caffeine, and intakes are greater in US children because of higher intakes of CSDs and, to a lesser extent, intakes of tea. Despite this difference, even at the 90th percentile in the Canadian Facts and US SIP surveys, children were consuming levels of caffeine from carbonated beverages that were well within recent HC recommendations for safe limits (2.5 mg/kg/day or 45 mg/day in children aged four to six years). These intake levels are also clearly lower than the caffeine doses of 3 mg/kg/day reported to cause negligible behavioural effects (15).

Although beverage consumption patterns in children are likely to influence their caffeine intakes, the patterns are different in US and Canadian children. Recommendations should focus on the specific population of interest.

---

**Table 2**

Caffeine intakes in beverages for children aged one to five years1,2

<table>
<thead>
<tr>
<th>Measure and survey</th>
<th>Total beverages</th>
<th>Coffee</th>
<th>Tea</th>
<th>Juice drinks</th>
<th>CSDs*</th>
<th>Chocolate drinks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean caffeine intake (mg/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canadian Facts</td>
<td>6.7</td>
<td>0.4</td>
<td>2.1</td>
<td>0.02</td>
<td>3.6</td>
<td>0.5</td>
</tr>
<tr>
<td>US SIP</td>
<td>13.5</td>
<td>0.2</td>
<td>3.4</td>
<td>0.00</td>
<td>9.7</td>
<td>0.2</td>
</tr>
<tr>
<td>Mean caffeine intake (mg/kg/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canadian Facts</td>
<td>0.42</td>
<td>0.02</td>
<td>0.14</td>
<td>0.00</td>
<td>0.23</td>
<td>0.03</td>
</tr>
<tr>
<td>US SIP</td>
<td>0.82</td>
<td>0.01</td>
<td>0.21</td>
<td>0.00</td>
<td>0.59</td>
<td>0.01</td>
</tr>
<tr>
<td>90th percentile caffeine intake (mg/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canadian Facts</td>
<td>26.7</td>
<td>2.9</td>
<td>10.4</td>
<td>0.00</td>
<td>12.7</td>
<td>0.6</td>
</tr>
<tr>
<td>US SIP</td>
<td>37.3</td>
<td>0.0</td>
<td>8.6</td>
<td>0.00</td>
<td>28.9</td>
<td>0.2</td>
</tr>
<tr>
<td>90th percentile caffeine intake (mg/kg/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canadian Facts</td>
<td>1.71</td>
<td>0.14</td>
<td>0.67</td>
<td>0.00</td>
<td>0.81</td>
<td>0.09</td>
</tr>
<tr>
<td>US SIP</td>
<td>2.32</td>
<td>0.00</td>
<td>0.59</td>
<td>0.00</td>
<td>1.73</td>
<td>0.04</td>
</tr>
</tbody>
</table>

*CSD = carbonated soft drink
1From the 2001 Canadian Facts survey and the 1999 US SIP survey
2Caffeinated beverage-consuming children only; seven-day average daily intake for Canadian children, and 14-day average daily intake for US children
RELEVANCE TO PRACTICE

These data should provide nutrition professionals with a clearer understanding of beverage consumption patterns in young children, as well as of how these patterns affect caffeine intakes. Our data indicate that children in both the US and Canada are consuming safe levels of caffeine from caffeinated beverages. The differences between Canadian and US children in terms of milk, juice, and CSD consumption may also have implications for nutrient and energy intakes. Canadian children drink mostly milk, juice, and juice drinks (86%); their level of consumption for CSDs is only 5%. This compares with 13% consumption among American children, who have a 73% consumption rate for milk, juice, and juice drinks.

Acknowledgements

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The opinions expressed herein are those of the authors and do not necessarily represent the views of ILSI North America.

References

DAY 1, SESSION 2: SAFETY SIGNALS AND SURVEILLANCE OF POPULATIONS


29th Annual Report

Alvin C. Bronstein, Daniel A. Spyker, Louis R. Cantilena, Jr.,
Barry H. Rumack, and Richard C. Dart

Available online at
Review of the regulation and safety assessment of food substances in various countries and jurisdictions

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This review compares the regulations, definitions and approval processes for substances intentionally added to or unintentionally present in human food in the following specific countries/jurisdictions: Argentina, Australia, Brazil, Canada, China, the European Union, Japan, Mexico, New Zealand, and the United States. This includes direct food additives, food ingredients, flavouring agents, food enzymes and/or processing aids, food contact materials, novel foods, and nanoscale materials for food applications. The regulatory authority of each target jurisdiction/country uses its own regulatory framework and although the definitions, regulations and approval processes may vary among all target countries, in general there are many similarities. In all cases, the main purpose of each authority is to establish a regulatory framework and maintain/enforce regulations to ensure that food consumed and sold within its respective countries is safe. There is a move towards harmonisation of food regulations, as illustrated by Australia and New Zealand and by Mercosur. The European Union has also established regulations, which are applicable for all member states, to establish a common authorisation procedure for direct food additives, flavourings and enzymes. Although the path for approval of different categories of food additives varies from jurisdiction to jurisdiction, there are many commonalities in terms of the data requirements and considerations for assessment of the safety of use of food additives, including the use of positive lists of approved substances, pre-market approval, and a separation between science and policy decisions. The principles applied are largely reflective of the early work by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) committees and JECFA assessments of the safety of food additives for human and animal foods.

Keywords: food additives; novel food; food contact substances; flavouring agents; enzymes; processing aids; nanoscale materials; regulatory framework

Introduction

The purpose of this review is to present an overview and comparison of the regulation of substances added to foods in a number of different countries/jurisdictions, as well as the efforts of internationally recognised scientific and advisory bodies, such as the Codex Alimentarius Commission (CAC) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) with respect to the safety assessment of these substances.

The specific countries/jurisdictions addressed are Argentina, Australia, Brazil, Canada, China, the European Union (EU), Japan, Mexico, New Zealand, and the United States (herein referred to as the “target countries”). The choice of jurisdictions to be included was based on a number of factors, and was intended to include both of well-established and emerging regulatory systems. The substances added to human food that are included in this review are direct food additives, common food ingredients such as sugar, food contact materials, flavouring agents, food enzymes, and/or processing aids. In addition, information pertaining to the development of regulations for nanomaterials falls under the scope of this review. Pesticide residues, drug residues or contaminants (e.g. lead) are not addressed in detail. A full list of included substances and their definitions according to each target country is provided. It should be noted that regulatory information for each country may be updated frequently and this report was written as an overview of the regulatory framework for each country. To the best of our knowledge, regulatory information is current as of June 2012. It is recognised that the definitions of terms used in this document may vary by organisation.

International scientific and advisory committees

The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) established the CAC...
jointly in 1962 to address safety and nutritional quality of foods, and develop international standards to promote trade (Codex Alimentarius Commission 2006). The CAC is an intergovernmental body composed of delegations from FAO and WHO member states that participate in developing food standards. The CAC develops standards on the basis of sound scientific evidence provided by independent FAO/WHO scientific committees. JECFA, established prior to the CAC in 1956, is the oldest and most active of these. Primary roles of the CAC include establishing international food standards for approved food additives providing maximum levels in foods, maximum limits for contaminants and toxins, maximum residue limits for pesticides and for veterinary drugs used in veterinary animals, and establishing hygiene and technological function practice codes (Codex Alimentarius Commission 2006). The collection of these standards, codes of practice, guidelines and recommendations, constitute what is known as the Codex Alimentarius, a substantial and useful reference. While the standards set by the CAC serve as guidelines to nations, the CAC has no regulatory authority and their standards are not enforceable unless they have been adopted into the regulatory framework for a nation or otherwise indicated (Codex 2011). For example, the World Trade Organization (WTO) refers to the Codex Alimentarius Sanitary and Phytosanitary practice codes in the SPS Agreement for member country food safety purposes.

Expert scientific advice is provided to the CAC by JECFA. Independent scientific committee members are appointed as experts in their own right and not as representatives of their governments or employers. Initially developed by WHO and FAO to address the safety of food additives, JECFA also has extended its activities to evaluate the safety of contaminants and veterinary drug residues in food. Although the outcome of JECFA’s evaluations does not have any direct bearing on the regulatory framework for a nation or otherwise indicated (Codex 2011). For example, the World Trade Organization (WTO) refers to the Codex Alimentarius Sanitary and Phytosanitary practice codes in the SPS Agreement for member country food safety purposes.

The Joint FAO/WHO Committee on Food Additives (JECFA)

The initial steps of the Committee were to establish general principles regarding the technical purpose of food additives as well as principles for safe use for these substances. Regarding the matter of technical purpose the Committee noted that food additives could serve a valuable technical function in food: (1) to maintain the nutritional quality of food; (2) to enhance keeping quality or stability, with resulting reductions in food wastage; (3) to make food attractive to consumers; and (4) to provide essential aids to processing.

The Committee established the following situations in which food additives should not be used: (1) to deceive the consumer; (2) to result in substantial reduction of the nutritional value of food; (3) when the desired effect can be obtained by good manufacturing practices; or (4) to disguise the use of faulty processing or handling techniques.

With respect to safety evaluation of food additives the Committee again established sound key general principles. They were: (1) it is impossible to establish absolute proof of non-toxicity for all members of the human population; (2) critically designed animal studies can provide a reasonable basis for evaluating the safety of food additives; (3) the decision as to a safe level for a food additive should be based on knowledge of the minimum dietary level that produces no unfavourable response in test animals; (4) decisions on the use of food additives must be based on the considered judgment of properly qualified scientists that the intake of the additive will be below any level which could be harmful to consumers; (5) the fate of the additive during food processing and preparation should be considered because of the possible formation of toxic substances and interaction of the food additive with components of food or other food additives; and (6) consideration should be given to groups within the population who for medical reasons may be especially vulnerable to certain food additives. The Committee went on to recommend that when a food additive is proposed for use, considerations should be given:

- to determine if there is a demonstrated benefit to consumers;
- to limit the use of food additives in the diets of infants and young children;
- to assess whether there are adequate data to derive specifications for identify and purity of the food additive; and
- to limit the level of use of food additives to the minimum required to achieve the desired technical effect.

The Committee also noted that permitted lists of food additives should be drawn up because the use of prohibited lists could entail several years of exposure to potentially harmful food additives before sufficient evidence was accumulated to place it on the prohibited list. The use of permitted lists would eliminate this danger. The Committee also agreed with the principle that consumers should be made aware of the presence of food additives in their food and noted that label declaration is the most effective method of achieving this result. With respect to regulatory control at the national level, the Committee noted that methods must be available to measure food additives in foods and that enforcement of levels of use through appropriate food legislation provided a reliable way of governing the use of food additives.
JECAF also established principles and procedures for the testing of food additives to establish the safety of food additive use (JECAF 1958). The Committee noted that food additives may be consumed over a substantial proportion of lifetime and emphasised the need for studies in appropriate animal species that would reflect the conditions of human exposure. The Committee further noted that no single pattern of tests could adequately cover the testing requirements of substances of such diverse structure and function as food additives and therefore the Committee strongly emphasised that the establishment of a uniform set of experimental procedures that would be standardised and obligatory was undesirable. For this reason, which still holds true today, the Committee decided that it was only possible to formulate general recommendations on testing procedures.

The general principles regarding the testing of food additives were identified including: (1) the selection of animal species for testing indicating that background information on species/strain, natural disease rates, tumour incidence, and duration of life was essential for proper interpretation of experimental results; (2) the importance of animal housing, diets, control groups and statistical procedures for the design of studies, and their interpretation; (3) the importance of dose selection emphasising the need to magnify the dose in experimental animals to overcome statistical limitations of the test design and to provide a means of studying dose–response relationships; (4) the need for biochemical mechanistic investigations to detect subtle physiological changes and to assist in data interpretation; and (5) the need to examine the potential for food additives to induce carcinogenesis, stating that no proven carcinogen should be considered suitable for use as a food additive in any amount (JECAF 1958). The results of the toxicological evaluations are the basis for the allocation of an acceptable daily intake (ADI) or tolerable intake for contaminants the Committee considered unavoidable.

The Committee also discussed the pivotal issue relating to the extrapolation of animal studies to humans noting that in most instances a dosage level can be identified that causes no demonstrable effect in animals. It also noted that a margin of safety of 100 could be applied in extrapolating animal data to humans to account for species differences in susceptibility, numerical differences in population ranges between the test animals and the human population, the greater variety of complicating disease processes in the human population, and the possibility of synergistic action among food additives. The Committee noted that application of a 100-fold margin of safety would limit the use of some food additives yet was an adequate margin of safety for most substances proposed for use as food additives at the time.

Additionally, the Committee established specifications for the identity and purity of food additives. From the viewpoint of industry the specifications of the substance/compound helped define suitability for use in food. Items included in the specifications document were: title or name in common usage; synonyms; chemical name (IUPAC); empirical (organic compounds) or chemical formula (inorganic compounds); structural formula; molecular weight; definition (percentage of the stated substance that should be present origin of the material if necessary); description (appearance, taste, odour and other general properties); identification tests; and purity tests (tests for impurities and their nature).

In the early 1990s the CAC recognised the need to formalise the risk analysis process. A definition of risk analysis was developed, through a series of consultations, that embodies risk assessment, risk management and risk communication (CAC 2005). Scientific committees such as JECAF are responsible for risk assessment, which includes hazard identification, hazard characterisation, exposure assessment and risk characterisation. In response to formalisation of the risk assessment process, JECAF has increased its emphasis on exposure assessment, which includes predicted intake for substances new to the market or for which few relevant data exist and estimated intake of food additives and contaminants for which sufficient information exists for such an analysis. Within each jurisdiction, responsibility for each of the three components of risk analysis is often divided among different divisions of the regulatory agencies or to separate bodies. For example, in the EU, the Directorate General for Health and Consumers has risk management responsibility whereas the European Food Safety Authority (EFSA) is an independent body with risk assessment and risk communication responsibilities. Further discussion of the process of risk analysis of components in foods, including risk management and risk communication, is outside the scope of this review.

When one looks back at this early work of the Committee, it is clear that JECAF has played a leading role in setting the worldwide agenda for how food additives, contaminants and adventitious substances in food should be evaluated. Although these deliberations occurred 50 years ago, the general principles and procedures elaborated by JECAF in early meetings have stood the test of time and are still used by the Committee at the present time. The credit for this lies with the early Committee members most of whom were outstanding research scientists and widely recognised experts in their discipline. The early work of JECAF also brought harmonisation of the approach to safety assessment of food additives on a worldwide basis. This resulted in a tremendous advantage to national governments that looked to JECAF for guidance and continue to do so. There can be no doubt that JECAF is the preeminent body dealing with food safety issues internationally.
The International Programme on Chemical Safety (IPCS)

The International Programme on Chemical Safety (IPCS) is a joint venture of the United Nations Environment Programme (UNEP), the International Labour Organisation (ILO), and the WHO. The main objective of the IPCS is to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment. In 1987, in response to numerous recommendations by JECFA, the IPCS convened a Task Force to review current knowledge and advances in toxicological science, and to develop criteria for testing and evaluation of the safety of food additives and contaminants. Thus, in 1987, the Environmental Health Criteria 70 (EHC70) was published, entitled Principles for the Safety Assessment of Food Additives and Contaminants in Food. This document was subsequently updated by the IPCS in 2009, with the publication of EHC240, entitled Principles and Methods for the Risk Assessment of Chemicals in Food (WHO 2009).

These guidelines provide a comprehensive current review of the key issues considered by JECFA during their risk assessments of food chemicals. Topics addressed include the risk assessment paradigm, chemical characterisation and specifications for food chemicals, toxicological studies used for hazard identification and characterisation, dose–response assessments, derivation of health-based guidance values such as ADI, assessment of dietary exposure to chemicals in food, risk characterisation, determination of maximum residue limits for pesticides and veterinary drugs, and approaches for assessment of specific groups of substances such as flavours and novel foods.

Regulations in different jurisdictions

To obtain an understanding of the global regulation of substances intentionally added to food, the regulatory systems and laws pertaining to their safety were reviewed and tabulated for ease of presentation and comparison. The countries that were chosen included: Argentina, Australia, Brazil, Canada, China, the EU, Japan, Mexico, New Zealand, and United States. These countries include both those with well-established regulatory systems (i.e. Australia, Canada, Japan, New Zealand, and the EU and US) and several that are currently in the process of changing and/or modernising their food regulatory systems (i.e. Argentina, Brazil, China and Mexico). Although it is acknowledged that there are many other jurisdictions that were worthy of inclusion in this review, limited resources required selection of those for which we had expertise in, and English versions or translations of regulations.

For each target country, the following information was sought and is summarised in table format (Tables 1–9):

- A brief historical overview of the main regulatory body/scientific advisory body and highlight roles and responsibilities concerning the regulation of chemicals added to food.
- A discussion of the regulatory framework.
- Pertinent regulations.
- Submission requirements/process for the approval of new food substances.
- Any pending or recent changes and the reason for the changes.

Comparison of the regulatory systems and regulations for food substances in the different jurisdictions

Overview

In general, each of the target countries has a regulatory system in place for the scientific evaluation and approval of food additives, food ingredients, and food contact substances; however, several are undergoing change, refinement and working towards harmonisation with other countries. The EU has recently adopted new regulations, which establish a common authorisation procedure for food additives and, for the first time, food enzymes and flavouring agents. Prior to the implementation of these new regulations, food enzymes and flavourings were regulated at the national level for each member state.

Similar to the EU, other regional bodies representing multiple nations have been established and are working to harmonise food standards for member countries. Australia and New Zealand have harmonised their food standards, which are maintained by Food Standards Australia New Zealand (FSANZ). Thus, Australia and New Zealand (NZ) have joint labelling and compositional standards under the Australia NZ Food Standard Code; however, there are separate standards that are not part of the joint food standards setting system, covering food safety, agricultural compounds and veterinary medicines, and primary food production and processing. These are covered in the Code but apply to Australia only. Equivalent NZ standards are developed and administered by the NZ Ministry for Primary Industries (MPI) (formerly MAF). MPI is responsible for implementation and enforcement of food standards and requirements for exported foods. Furthermore, in NZ there is a standard for supplemented foods allowing foods to be modified in a way that is beyond what is permitted under the Food Standards Code. The Supplemented Food Standard was introduced in March 2010 to regulate food type products previously sold under the NZ Dietary Supplements Regulations. However, supplemented foods must comply with most sections of the Code including the food additive requirements.

In South America, the South American Common Market, known as Mercosur, represents Brazil, Argentina, Uruguay, Paraguay and Venezuela. While these nations do
Table 1. Regulatory framework of chemicals added to food in Argentina.

<table>
<thead>
<tr>
<th>Regulatory authority</th>
<th>Name: Ministry of Health (Ministerio de Salud) and The National Administration of Drugs, Foods, and Medical Technology (Administración Nacional de Medicamentos, Alimentos y Tecnología Médica – ANMAT)</th>
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</thead>
<tbody>
<tr>
<td><strong>Website:</strong></td>
<td>- Ministry of Health: <a href="http://www.msal.gov.ar/">http://www.msal.gov.ar/</a> (Ministerio de Salud 2011)</td>
</tr>
<tr>
<td><strong>Historical overview:</strong></td>
<td>ANMAT was created by Presidential Decree 1490/92 (ANMAT 1992) (<a href="http://www.anmat.gov.ar/webanmat/Legislacion/NormasGenerales/Decreto_1490-1992.pdf">http://www.anmat.gov.ar/webanmat/Legislacion/NormasGenerales/Decreto_1490-1992.pdf</a>)</td>
</tr>
<tr>
<td><strong>Role/responsibility:</strong></td>
<td>ANMAT is a decentralised body of the National Public Administration, established by Decree 1490/92. It assists in the protection of human health, ensuring the quality of products within its jurisdiction: drugs, food, medical products, diagnostic reagents, cosmetics, dietary supplements and household products. Its jurisdiction covers the entire country. It was created in August 1992. Since then, a body of professionals and technicians work with modern technology effectively to implement the processes of authorisation, registration, regulation, monitoring and control products used in medicine, human food and cosmetics. It depends both technically and scientifically on the norms and directives given to it by the Secretary for Policies, Regulations and Institutions of the Ministry of Health, with a system of economic and financial autarchy. In this context, ANMAT’s main objective is “to ensure that medicines, food and medical devices available to the population, have proven effectiveness (achieving the therapeutic, diagnostic or nutrition targets) safety (high ratio benefit/risk) and quality (responding to the needs and expectations of citizenship) …”</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Advisory scientific body</th>
<th>Name: National Committee of Food (CONAL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Website:</strong></td>
<td><a href="http://www.conal.gov.ar">http://www.conal.gov.ar</a> (CONAL 2011)</td>
</tr>
<tr>
<td><strong>Role/responsibility:</strong></td>
<td>CONAL functions as an advisory body that provides support and monitoring to the National Food Inspection System (Sistema Nacional de Control de Alimentos – SNCA), which enforces the Argentine Food Code (also known as Código Alimentario Argentino – CAA) (<a href="http://gain.fas.usda.gov/Recent%20GAIN%20Publications/Food%20%20and%20Agricultural%20Import%20Regulations%20and%20Standards%20-%20Narrative_Buenos%20Aires_Argentina_12-22-2011.pdf">http://gain.fas.usda.gov/Recent%20GAIN%20Publications/Food%20%20and%20Agricultural%20Import%20Regulations%20and%20Standards%20-%20Narrative_Buenos%20Aires_Argentina_12-22-2011.pdf</a>) (USDA 2010a). CONAL reviews all the petitions for incorporation to the CAA of new ingredients, food additives and processing aids, as well as materials in contact with foods, etc. When pertinent because of harmonisation requirements with Mercosur, CONAL requests treatment of the matter at the Mercosur level.</td>
</tr>
</tbody>
</table>

| Framework regulations                | The CAA regulates local food production; however, as harmonised Mercosur regulations become available, the CAA incorporates those regulations into the CAA through Resoluciones Conjuntas issued by the Ministry of Health and the Ministry of Agriculture, Livestock and Fisheries (http://www.anmat.gov.ar/alimentos/normativas_alimentos_caa.asp). The CAA is a set of sanitary provisions, analytical standards and rules for commercial identification of products. It has more than 1400 articles divided into 20 chapters that include general provisions related to food factories and food trade, conservation and food processing, use of utensils, containers, packaging, standards for labelling and advertising of food, technical specifications of the different types of foods and beverages, processing aids, and food additives. |
| **Historical overview:**             | The Argentine Food Code was put into force by Law 18,284, regulated by Decree 2126/71, of which Annex I is the text of CAA. It is a regularly updated technical regulation that establishes sanitary standards, analytical standards, and authenticity and quality standards to be complied by companies and individuals, production facilities, and food products that fall into their orbit. This legislation is primarily aimed at protecting the health of the population, and reliance in commercial transactions. |

| Part of an overarching international organisation | Argentina has been a member of the World Trade Organization since 1995. It is a member of the Codex Alimentarius Commission as well as a member of Mercosur. |
| Recent and/or pending changes            | None known (continued) |
Table 1. Continued.

Regulatory overview of specific food chemical groups

| Food ingredients | Definition: As per GRUPO MERCADO COMUN (GMC) 26/03, food ingredients are defined as all substances, including the food additives, which are used in the manufacture or preparation of foods and which are present in the final product in its original or modified form. Novel foods: CAA does not define novel foods. Mercosur does not have a definition of novel foods either; however, novel foods are recognised in practice. Regulation: Currently, there are no novel food regulations in the CAA

Guidance document: GMC 26/03

Approval process for a new substances (http://www.conal.gov.ar). New food ingredients, novel foods and food additives as well as any request for modification of the CAA should be done according to this guidance. However, if the product requested is considered a novel food in Europe, its inclusion will be delayed until the novel foods chapter of the CAA is written, approved and incorporated into the CAA.

Direct food additives | Definition: Food additives are defined according to GMC 26/03 as any ingredient that is added to foods intentionally, without intent to nurture, in order to modify the physical, chemical, biological or sensory characteristics of foods during its manufacture, processing, preparation, processing, packaging, conditioning, storage, transport or during food handling; it will have, or it can be reasonably expected to have (directly or indirectly) as a result, that the additive itself or its by-products become part of that food. This term does not include contaminants, or nutrients that are incorporated into a food in order to maintain or improve its nutritional properties. GMC 26/03 is currently under revision by the Sub Work Group #3 (SGT#3) Mercosur and a new Definition 2 continues to be discussed and should be gazetted during 2012.

Regulation:

Two new regulations for Mercosur, GMC 34/10 and GMC 35/10; however, not all member states have adopted these regulations yet:
- GMC 34/10 – List of food additives according to good manufacturing practices (http://www.puntofocal.gov.ar/doc/r_gmc_34-10.pdf)

From the CAA: Article 2 of Decree §2092 of October 1991, states the following:

call foods, condiments, beverages, or their raw material and food additives which are manufactured, fractioned, preserved, transported, sold or exposed, must comply with the CAA requirements. When one of those is imported, the CAA requirements will be applied. The Argentine Government also considers products from countries which have food controls comparable to those of Argentina, or when they use the Codex Alimentarius (FAO/OMS) standards, to be in compliance with Argentine standards. (http://gain.fas.usda.gov/Recent%20GAIN%20Publications/Food%20and%20Agricultural%20Import%20Regulations%20and%20Standards%20-%20Narrative_Buenos%20Aires_Argentina_12-22-2011.pdf)

Guidance document: No authoritative guidance document found

Approval process for a new substances: All food additives that are not listed in the GMC11/06 can be submitted for evaluation to CONAL who will then submit a request to the Mercosur Sub Work Group #3 to consider the revision of 11/06 and to proceed to its incorporation. Revision of GMC 11/06 is expected in 2013. Submissions dossier should be addressed to CONAL following the general guidelines set forth at: http://www.conal.gov.ar
### Table 1. Continued.

<table>
<thead>
<tr>
<th>Food contact substances (e.g. components of packaging materials)</th>
<th><strong>Definition:</strong> According to GMC, food contact substances are defined as any primary container or primary wrapping or container (one that is in direct contact with food).</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Regulation:</strong> GMC 32/07. There is a positive list of substances (in Appendix 1 of regulation GMC 32/07) that are added to plastics to achieve a technical effect in the final product (additives) such as antioxidants, antistatic, foaming, defoaming, fillers, impact modifiers, plasticisers, lubricants, stabilisers, UV protectants, preservatives, hardeners, etc. Included within this list are the substances used to provide a suitable medium polymerisation (e.g. emulsifiers, surfactants, buffers pH, solvents).</td>
<td></td>
</tr>
<tr>
<td><strong>Guidance document:</strong> No authoritative guidance document found</td>
<td></td>
</tr>
<tr>
<td><strong>Approval process for a new substances:</strong> Same as direct food additives</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Flavouring agents</th>
<th><strong>Definition:</strong> Flavouring agents are substances or mixtures of substances with odoriferous and/or flavour properties that are able to confer or enhance the aroma and/or taste of food. For the purpose of this Technical Regulation, flavourings/flavourings are classified as either natural or synthetic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Regulation:</strong> Under Mercosur, GMC 10/06. A specified list of flavouring agents (natural or synthetic), including colourants is permitted. Exclusions from the technical regulations include:</td>
<td></td>
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<tr>
<td>● Substances that give only sweet, salty or sour</td>
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</tr>
<tr>
<td>● Substances and food products with odoriferous and/or sapid consumed without processing, with or without reconstitution</td>
<td></td>
</tr>
<tr>
<td>● Substances of plant or animal origin having inherent flavours/flavouring properties, where they are used as sources of flavourings</td>
<td></td>
</tr>
<tr>
<td><strong>Guidance document:</strong> No authoritative guidance document found</td>
<td></td>
</tr>
<tr>
<td><strong>Approval process for a new substances:</strong> Submission to CONAL and subsequent submission to Mercosur. General guidelines (<a href="http://www.conal.gov.ar">http://www.conal.gov.ar</a>)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Enzymes</th>
<th><strong>Definition:</strong> Enzymes or enzyme preparations are defined as substances of animal, plant or microbial origin that act by promoting the desirable chemical reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Regulation:</strong> CAA, Chapter XVI, Articles 1261, 1262 and 1263. Enzymes regulations are not harmonised in Mercosur</td>
<td></td>
</tr>
<tr>
<td><strong>Guidance document:</strong> No authoritative guidance document found</td>
<td></td>
</tr>
<tr>
<td><strong>Approval process for a new substances:</strong> General guidelines (<a href="http://www.conal.gov.ar">http://www.conal.gov.ar</a>)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Processing aids</th>
<th><strong>Definition:</strong> A processing aid is any substance, excluding equipment and utensils, that is not consumed by itself as a food ingredient and which is intentionally used in the processing of raw materials, foods or ingredients, for a technological purpose during treatment or processing. It must be removed from the food or inactivated; the presence of traces of the substances or their derivatives may be admitted in the final product.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Regulation:</strong></td>
<td></td>
</tr>
<tr>
<td>● GMC 18/93 Modificación RES</td>
<td></td>
</tr>
<tr>
<td>● GMC 31/92 “Definición y Principios fundamentales referente a empleo de aditivos, ingredientes, coadyuvante de elaboración, contaminantes”. Resolución GMC No. 84/93, incorporated into the CAA by Resolución MSyAS No. 003 dated 11 January 1995 defines the functions of processing aids. Processing aids are not harmonised in Mercosur; the only harmonised regulation is GMC 84/93 which establishes the definition of the functions of processing aids. Certain processing aids are listed under Chapter XVI of the CAA, but this list is not comprehensive.</td>
<td></td>
</tr>
<tr>
<td><strong>Guidance document:</strong> No authoritative guidance document found</td>
<td></td>
</tr>
<tr>
<td><strong>Approval process for a new substances:</strong> General guidelines (<a href="http://www.conal.gov.ar">http://www.conal.gov.ar</a>)</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Nanoscale materials</th>
<th><strong>Definition:</strong> No authoritative statement found</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Regulation:</strong> No authoritative statement found</td>
<td></td>
</tr>
<tr>
<td><strong>Guidance document:</strong> No authoritative guidance information found</td>
<td></td>
</tr>
<tr>
<td><strong>Approval process for a new substances:</strong> No authoritative statement found</td>
<td></td>
</tr>
</tbody>
</table>

(continued)
Table 1. Continued.

Efforts towards developing standards and regulations (Locascio et al. 2011):

- El Centro Científico Tecnológico, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) – Mendoza (Científicas y Técnicas (CONICET) – Mendoza (http://www.cricyt.edu.ar) developing assays for acute toxicity and distribution of nanostructured alumina in mammals and toxicity of nanostructured alumina in vertebrates.

Table 2. Regulatory framework of chemicals added to food in Australia/New Zealand.

<table>
<thead>
<tr>
<th>Regulatory authority</th>
<th>Name: Food Standards Australia New Zealand (FSANZ)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Website: <a href="http://www.foodstandards.gov.au">http://www.foodstandards.gov.au</a> (FSANZ 2011c)</td>
</tr>
</tbody>
</table>

**Historical overview:**

- The first national agency to regulate food in Australia (National Food Authority) was established in June 1991. This became the Australia New Zealand Food Authority in July 1996, and FSANZ was established on 1 July 2002.
- FSANZ is a statutory authority operating under the Food Standards Australia New Zealand Act 1991 (Commonwealth of Australia 2007). The act provides a focus for cooperation between governments, industry, and the community to establish and maintain uniform food regulation in Australia and New Zealand.
- FSANZ establishes food standards for Australia and New Zealand. There is an agreement (established in July 1996) between the governments of Australia and New Zealand that establishes FSANZ’s role in setting joint food standards, i.e. standards that apply in both countries.
- The agreement does not cover some areas, such as maximum residue limits, food hygiene provisions and export requirements relating to third country trade. It also contains provisions that allow New Zealand to opt out of a joint standard for exceptional reasons relating to health, safety, environmental concerns or cultural issues. In such cases, FSANZ may be asked to prepare a variation to a standard to apply only in New Zealand.
- In Australia only, FSANZ develops standards for primary production and processing and for food hygiene, as well as setting residue limits for agricultural and veterinary products. Equivalent New Zealand standards are developed and administered by the New Zealand Ministry for Primary Industries (MPI), formerly MAF. MPI is also responsible for implementation and enforcement of food standards and requirements for exported foods.

**Role/responsibility:**

- FSANZ is responsible for developing and administering the Australia New Zealand Food Standards Code (the Code) – see below.
- FSANZ is governed by a Board with a wide range of expertise and experience in food matters, with members drawn from Australia and New Zealand.
- As of February 2001, the Legislative and Governance Forum on Food Regulation (the Forum) is primarily responsible for the development of domestic food regulatory policy and guidelines for setting domestic food standards. The Forum also has the capacity to adopt, amend or reject standards and to request that these be reviewed.
- FSANZ recently implemented the Code Interpretation Service (CIS) on a cost-recovery basis to provide coordinated guidance on Chapters 1 and 2 of the Australia New Zealand Food Standards Code.
- In Australia, enforcing compliance with the Code for all foods is the responsibility of State/Territory Health Departments within Australia. For imported foods, enforcing compliance is the responsibility of the Australian Quarantine Inspection Service (AQIS).
- In New Zealand, enforcing compliance with the Code for all foods is the responsibility of the New Zealand Ministry for Primary Industries (MPI), formerly MAF.

(continued)
Table 2. Continued.

Advisory scientific body
- FSANZ Board: The FSANZ Board is selected by the Australian Minister for Health and Aging in Consultation with the Food Regulation Ministerial Council and must include qualified people from a wide range of expertise. Members of the Board have been drawn from specialist areas – public health, food science, human nutrition, consumer affairs, food allergy, medical science, microbiology, food safety, biotechnology, veterinary science, primary food production, the food industry, food processing or retailing, small business, international trade, food regulation, consumer rights and consumer affairs policy, the National Health and Medical Research Council (NHMRC) and government
- FSANZ Fellows: FSANZ Fellows provide expert advice on applications, proposals and other risk assessment activities of the agency, FSANZ Fellows, within their relevant areas of expertise, also peer review FSANZ work and provide training to FSANZ staff
- Scientific Advisory Groups (SAG): These ad hoc advisory groups are set up to advise FSANZ on particular scientific issues, e.g. health claims, iodine and folic acid fortification and dairy primary production and processing

Role/responsibility: See above

Framework regulations
Australia New Zealand Standards Code (the Code): The Code regulates all aspects of food including labelling, the addition of food additives, processing aids, nutritive substances, levels of contaminants, approval of new foods (novel foods, genetically modified (GM) foods and irradiated foods), composition of standardised and special purpose foods, and applying in Australia only, food safety programmes and food processing and primary production (http://www.foodstandards.gov.au) (FSANZ 2011a)

Part of an overarching international organisation
Australia and New Zealand are both members of the World Trade Organization since 1995, and they are both members of the Codex Alimentarius Commission

Recent and/or pending changes
No major changes to the regulatory framework anticipated. Refer to the new Science Strategy above

Regulatory overview of specific food chemical groups

Food ingredients
Food ingredients as such are not defined under the Australia New Zealand food regulations. Ingredients are generally considered to be either foods or substances added to food. FSANZ is reviewing the regulation of novel foods and nutritive substances as of March 2012. Due to problems with the definition of these terms a new approach is being considered based on criteria for “eligible foods” (i.e. foods in the wider sense including ingredients, additives and other substances added to food). Any non-eligible foods would be prohibited and will require appropriate safety assessment. Novel foods (including novel food ingredients):
- Novel foods are defined as a non-traditional food with no history of safe use and the food requires an assessment of the public health and safety considerations having regard to:
  - the potential for adverse effects in humans; or
  - the composition or structure of the food; or
  - the process by which the food has been prepared; or
  - the source from which it is derived; or patterns and levels of consumption of the food; or
  - any other relevant matters.
- A non-traditional food is defined as:
  - a food that does not have a history of human consumption in Australia/New Zealand; or
  - a substance derived from a food where that substance does not have a history of human consumption in Australia/New Zealand other than as a component of that food; or
  - any other substance, where that substance, or the source from which it is derived, does not have a history of human consumption as a food in Australia/New Zealand.
- Categories of novel foods may include but are not limited to:
  - plants or animals and their components;
  - plant or animal extracts;
  - herbs, including extracts;
  - dietary macro-components;
  - single chemical entities;
Table 2. Continued.

- microorganisms, including probiotics; and
- foods produced from new sources, or by a process not previously applied to food.

Foods produced using gene technology or irradiated foods are regulated separately from novel foods and will not be discussed further within this report.

**Regulation:**
- Novel foods and novel food ingredients are regulated under Food Standard 1.5.1. This standard prohibits the sale of these foods unless they are listed in the table to Clause 2 under Food Standard 1.5.1 and comply with any special conditions of use in that table.
- Food Standard 1.3.4 describes standards for the identity and purity of novel food ingredients.

- General application requirements are provided in Sections 2 and 3.1.
- Specific requirements for novel foods are provided in Section 3.5.2.

**Approval process for a new substances:** An application to vary the code is required to approve the use of a novel food ingredient or a novel food. All application forms must conform to the general requirements (under Section 3.1 of the Application Handbook). Section 3.5.2 Novel Foods (within the handbook) lists additional requirements specific to novel foods. A summary of the requirements are listed below:
- Will need to indicate the specific class of food and brand of the food.
- Technical information on novel food:
  - Description of novel food (if it falls under one of the major categories listed below)
  - Physical and chemical properties
  - Impurity profile
  - Manufacturing process
  - Specification for identity and purity
  - Analytical method for detection.
- Safety information of novel food:
  - Depending on which major category that novel foods falls below (plants or animals and their components; plant or animal extracts; herbs (both non-culinary and culinary) including extracts; single chemical entities; dietary macro-components; microorganisms (including probiotics); food ingredients derived from new sources; or foods produced by a process not previously applied to food), different requirements are specified (see Section 3.5.2 of handbook).
- Dietary exposure to novel food:
  - List of the foods or food groups proposed to contain the novel food.
  - Proposed use level of novel food for each food or food group.
  - Percentage of the food group in which the novel food is proposed to be used or the percentage of the market likely to use the novel food ingredient.
  - Data to indicate whether the food or the food in which the novel food ingredient is used, is likely to replace another food from the diet (if applicable).
  - Information regarding the use of the novel food/novel food ingredient in other countries (if applicable).
- Nutritional impact of the novel food.
- Information related to potential impact on consumer understanding and behaviour.
- Information related to impact on the food industry (industry applicants only).

<table>
<thead>
<tr>
<th>Nutritive substances</th>
<th>Definition: Nutritive substance means a substance not normally consumed as a food in itself and not normally used as an ingredient of food, but which, after extraction and/or refinement or synthesis, is intentionally added to a food to achieve a nutritional purpose, and includes vitamins, minerals, amino acids, electrolytes and nucleotides.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Regulation:</strong> Nutritive substances cannot be added to certain special purpose foods without express permission. These are:</td>
</tr>
<tr>
<td></td>
<td>- Standard 2.9.1 Infant Formula Products</td>
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<td>- Standard 2.9.2 Food for Infants</td>
</tr>
<tr>
<td></td>
<td>- Standard 2.9.3 Formulated Meal Replacements and formulated supplementary foods</td>
</tr>
<tr>
<td></td>
<td>- Vitamins and minerals are regulated under Standard 1.3.2</td>
</tr>
<tr>
<td></td>
<td><strong>Guidance document:</strong> Application Handbook:</td>
</tr>
<tr>
<td></td>
<td>- General application requirements are provided in Sections 2 and 3.1.</td>
</tr>
<tr>
<td></td>
<td>- Specific requirements for nutritive substances are provided in Section 3.3.3.</td>
</tr>
</tbody>
</table>
Approval process for a new substances: An application to vary the code is required to approve the use of a nutritive substance or to change the permissions of a currently used nutritive substance. All application forms must conform to the general requirements (under Section 3.1 of the Application Handbook). A summary of the requirements are listed below:

- General information on the application
- Technical information on the nutritive substance:
  - Identity of the nutritive substance
  - Chemical and physical properties
  - Impurity profile
  - Manufacturing process
  - Specifications for identity and purity
  - Analytical method for detection
- Information related to the safety of the nutritive substance:
  - Toxicokinetics or metabolism of the nutritive substance
  - Studies in animals or humans that is relevant to the toxicity of the nutritive substance
- Information on dietary exposure to the nutritive substance:
  - A list of food groups or foods proposed to contain the nutritive substance
  - The maximum proposed level of the nutritive substance for each food group or food
- Information related to the nutritive impact of a nutritive substance other than vitamins and minerals:
  - Nutritional purpose of adding the nutritive substance to each food
  - Information related to the nutritive impact of a vitamin or mineral:
    - Information to demonstrate a need to permit the addition of a vitamin or mineral
    - Information to demonstrate the permitted addition of a vitamin or mineral has the potential to address a deficit or deliver a health benefit
  - Information related to the potential impact on consumer understanding and behaviour:
    - Information to demonstrate consumer awareness and understanding
    - Information to demonstrate consumer awareness and understanding
    - Information on the actual and/or potential behaviour of consumers in response to proposed foods
    - Information that the nutritive substance will not adversely affect any subpopulation
- Information related to impact on the food industry:
  - Data on the projected impact on the food industry

Direct food additives

**Definition:**
- Direct food additive is not a term used in Australia/New Zealand
- A food additive is any substance not normally consumed as a food in itself and not normally used as an ingredient of food, but which is intentionally added to a food to achieve one or more of the technological functions specified in Schedule 5. It or its by-products may remain in the food. Food additives are distinguishable from processing aids and vitamins and minerals added to food for nutritional purposes

**Regulation:**
- Food additives may only be added to food where expressly permitted in Food Standard 1.3.1. Additives can only be added to food in order to achieve an identified technological function according to good manufacturing practice (GMP). Some additives have specific permissions and maximum use levels allowed in food and other additives are limited to GMP (see Schedules 1–5 below). Specific flavourings, sweeteners and colouring agents are regulated as food additives
- Schedule 1 of Standard 1.3.1 contains information on the permitted uses of food additives (including sweeteners) by food type
- Schedule 2 of Standard 1.3.1 contains miscellaneous food additives permitted according to GMP in processed foods specified in Schedule 1
- Schedules 3 and 4 of Standard 1.3.1 contain colouring agents permitted to GMP and to specified levels in processed foods specified in Schedule 1
- Schedule 5 lists the technological functions that may be performed by food additives
- Standard 1.3.4 describes standards for the identity and purity of food additives

**Guidance document:** Application Handbook:
- General application requirements are provided in Sections 2 and 3.1
- Specific requirements for food additives are provided in Section 3.3.1

(continued)
Table 2. Continued.

**Approval process for a new substances**: Application to vary the code is required to approve the use of a new food additive or to change the permissions of a currently used food additive. The following information are required:

- Technical information on food additive:
  - Nature and technological function
  - Identification of food additive
  - Chemical and physical properties of food additive
  - Impurity profile
  - Manufacturing process
  - Specification for identity and purity
  - Food labelling information
  - Analytical method for detection

- Safety information of food additive:
  - Toxicokinetics and metabolism of food additive and if applicable, its degradation products and/or major metabolites
  - Information on the toxicity of food additive and if applicable, its degradation products and major metabolites
  - Safety assessment reports prepared by international agencies or other national government agencies, if available

- Dietary exposure to food additive:
  - List of food groups or foods proposed to contain food additive, or changes to currently permitted foods
  - Maximum proposed level or concentration range of food additive for each food group or food, or proposed changes to the currently permitted levels
  - Percentage of the food group in which the food additive is proposed to be used or the percentage of the market likely to use the food additive
  - Information regarding the use of the food additive in other countries (if applicable)

**Food contact substances (e.g. components of packaging materials)**

**Definition**:
- Food contact substances are defined as any materials in contact with food, including packaging material, which may include materials such as moisture absorbers, mould inhibitors, oxygen absorbers, promotional materials, writing or other graphics
- Food contact substances may be placed in contact with food, provided such articles or materials, if taken into the mouth, are not capable of being swallowed or of obstructing any alimentary or respiratory passage and are not otherwise likely to cause bodily harm, distress or discomfort

**Regulation**:
- Food Standard 1.4.3
- Food Standard 1.4.1

**Guidance document**:
- Food Standard 1.4.3 deals with food contact materials in general terms, and does not specify individual packaging materials for food contact or how they should be produced or used. However, with respect to plastic packaging products, the standard refers to the Australian Standard for Plastic Materials for Food Contact Use, AS 2070-1999. This Standard provides a guide to industry about the production of plastic materials for food contact use. AS 2070, in turn, refers to regulations of the United States and European Economic Community directives relevant to the manufacture and use of plastics
- Standard 1.4.1 – Contaminants and Natural Toxicants of the Food Standard Code regulates the levels of these substances that can be present in food from any source, including as a result of contact with food packaging materials. Even if a specific contaminant or toxicant is not listed in the standards, it sets out an expectation that all other contaminants and toxicants be kept to levels as low as reasonably achievable

**Approval process for a new substances**:
- Applications for food packaging materials are generally unnecessary if there is approval in the European Union or United States. At present it is voluntary that plastic materials for food contact use comply with the Australian Standard AS 2070-1999
- FSANZ has been considering its approach to chemicals that migrate from packaging into food for several years now, and it is envisaged that their approach may change to become more prescriptive and mandatory. Thus, in future, it may become mandatory to comply with either EU or US regulations under the Standard Code pending discussions with stakeholders

(continued)
Table 2. Continued.

<table>
<thead>
<tr>
<th>Flavouring agents</th>
<th>Definition:</th>
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<tbody>
<tr>
<td></td>
<td>● Flavouring agents, as such, are not defined in the Food Standards Code</td>
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<tr>
<td></td>
<td>● Flavourings are defined as intense preparations which are added to foods to impart taste and/or odour, which are used in small amounts and are not intended to be consumed alone, but do not include herbs, spices and substances which have an exclusively sweet, sour or salt taste</td>
</tr>
<tr>
<td></td>
<td>● Some flavourings are listed on the approved list of food additives with limitations</td>
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<tr>
<td></td>
<td>● “Permitted flavouring substances are those which are either: a) listed in at least one of the following publications:</td>
</tr>
<tr>
<td></td>
<td>o Food Technology, A Publication of the Institute of Food Technologists, Generally Recognized as Safe (GRAS) lists of flavoring substances published by the Flavor and Extract Manufacturers’ Association of the United States from 1960 to August 2007; or</td>
</tr>
<tr>
<td></td>
<td>or (b) a substance that is a single chemical entity obtained by physical, microbiological, enzymatic, synthetic or chemical processes, from material of vegetable or animal origin either in its raw state or after processing by traditional preparation process including drying, roasting, and fermentation”</td>
</tr>
<tr>
<td></td>
<td>Regulation: Food Standard 1.3.1 Section 11</td>
</tr>
<tr>
<td></td>
<td>Guidance document: An application is generally not required for flavourings and therefore no guidance is provided in the Application Handbook</td>
</tr>
<tr>
<td></td>
<td>Approval process for a new substances: Flavourings are regarded as food additives. An application is generally not required for flavourings</td>
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<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Definition:</th>
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<tbody>
<tr>
<td></td>
<td>● Enzymes, as such, are not specifically defined in the Food Standards Code</td>
</tr>
<tr>
<td></td>
<td>● Enzymes are considered to be processing aids. They may be used in the course of manufacture of any food, provided the enzyme is derived from the corresponding source or sources specified in the tables of Sections 15, 16, and 17 of Food Standard 1.3.3. Refer to processing aids (below)</td>
</tr>
<tr>
<td></td>
<td>Regulation: Enzymes are regulated as processing aids (below)</td>
</tr>
<tr>
<td></td>
<td>Guidance document: Refer to processing aids (below)</td>
</tr>
<tr>
<td></td>
<td>Approval process for a new substances: Refer to processing aids (below)</td>
</tr>
</tbody>
</table>

| Processing aids | Definition: Processing aid means a substance listed in clauses 3–19 [of Standard 1.3.3] where: |
|                 | ● the substances used in the processing of raw materials, foods or ingredients, to fulfil a technological purpose relating to treatment or processing but does not perform a technological function in the final food; and |
|                 | ● the substance is used in the course of manufacture of a food at the lowest level necessary to achieve a function in the processing of that food, irrespective of any maximum permitted level specified |
|                 | Regulation: Food Standard 1.3.3 – Processing aids |
|                 | Guidance document: |
|                 | ● Refer to the Application Handbook |
|                 | ● General application guidelines are provided in Sections 2 and 3.1 |
|                 | ● Specific requirements for processing aids are provided in Section 3.3.2 |
|                 | Approval process for a new substances: Application to vary the code is required to approve the use of a new processing aid or to change the permissions of a currently used processing aid. The following information are required in the application: |
|                 | ● Technical information on processing aid: |
|                 | o Information on the type of processing aid |
|                 | o Identification of processing aid |
|                 | o Chemical and physical properties of processing aid |

(continued)
Table 2. Continued.

- Manufacturing process
- Specification for identity and purity
- Analytical method for detection
- Safety information of a chemical processing aid:
  - General information on the industrial use of the chemical
  - General information on the use of the chemical as a food processing aid in other countries
  - Toxicokinetic and metabolism data on the processing aid, and if necessary its metabolites
  - Information on the toxicity of the processing aid, and if necessary its major metabolites
  - Safety assessment reports prepared by international agencies or other national government agencies, if available
- Safety information of an enzyme processing aid:
  - General information on the use of the enzyme as a food processing aid in other countries
  - Toxicity information of the enzyme processing aid
  - Information on the potential allergenicity of the enzyme processing aid
  - Safety assessment reports prepared by international agencies or other national government agencies, if available
- Additional information related to the safety of an enzyme processing aid derived from a microorganism:
  - Information regarding the source microorganism
  - Information regarding the pathogenicity and toxicity of the source microorganism
  - Information on the genetic stability of the source organism
- Additional information related to the safety of a processing aid derived from a genetically modified microorganism:
  - Information on the methods used in the genetic modification of the source organism
- Dietary exposure to the processing aid:
  - List of foods or food groups likely to contain the processing aid or its metabolites
  - Levels of residues of the processing aid or its metabolites for each food or food group
  - Percentage of the food group in which the processing aid is likely to be present or the percentage of the market likely to use the processing aid
  - Information relating to the levels of residues in foods in other countries

Nanoscale materials

**Definition**: The term “nanotechnology” is usually applied to the process of controlling the size and shape of materials at the atomic and molecular scale. Generally, the term is defined as deliberately engineered matter less than 100 nm in size in one dimension (http://www.foodstandards.gov.au/consumerinformation/nanotechnologyandfoo4542.cfm)

**Regulation**: No authoritative statement found for food applications

**Guidance document**: No specific guidance document found for food applications

**Approval process for a new substances**:
- Any new food substances that are manufactured using nanotechnologies that may present safety concerns will have to undergo a comprehensive scientific safety assessment under the appropriate standard before they can be legally supplied in Australia (http://www.foodstandards.gov.au/consumerinformation/nanotechnologyandfoo4542.cfm)
- Applications for food additives, processing aids, novel foods and nutritive substances must include particle size, size distribution and morphology, where the substance(s) is particulate in nature and will remain so in the final food

- Active role in international activities to develop best-practice testing protocols and risk assessment methodologies
- Focused on industrial chemicals that are in the form of nanomaterials
Table 3. Regulatory framework of chemicals added to food in Brazil.

<table>
<thead>
<tr>
<th>Regulatory authority</th>
<th>Name: Ministry of Health (Ministério da Saúde) through its regulatory agency Agencia Nacional de Vigilância Sanitária (ANVISA – National Agency of Sanitary Surveillance)</th>
</tr>
</thead>
</table>
| Website:                                                                             | ● Ministry of Health: http://portal.saude.gov.br/portal/saude/default.cfm (Ministerio da Saúde 2011)  
● Mercosur: http://www.mercosul.gov.br (Mercosur 2011) |
| Historical overview:                                                                 | ANVISA was established by Law 9.782, as of 26 January 1999 |
| Role/responsibility:                                                                 | The institutional purpose of the agency is to foster protection of the health of the population by exercising sanitary control over production and marketing of products and services subject to sanitary surveillance. The latter embraces premises and manufacturing processes, as well as the range of inputs and technologies concerned with the same. In addition, the agency exercises control over ports, airports and borders and also liaises with the Brazilian Ministry of Foreign Affairs and foreign institutions over matters concerning international aspects of sanitary surveillance |

| Advisory scientific body                                                             | Name: ANVISA is designated an autonomous agency operating under a special regime. This means that it is an independently administered, financially autonomous regulatory agency with security of tenure for its directors during the period of their mandates. It is managed by a Collegiate Board of Directors, comprised of five members |
| Website:                                                                             | http://portal.anvisa.gov.br/wps/portal/anvisa/home |
| Historical overview:                                                                 | No authoritative statement found |
| Role/responsibility:                                                                 | ANVISA’s function is to evaluate the safety of use of food additives and ingredients in foods. There are specific work groups, comprised of university professors who give technical support if needed. These groups work mainly in the approval of novel foods and novel food ingredients with functional health claims |

| Framework regulations                                                                | No authoritative statement found |
| Part of an overarching international organisation                                     | ● Mercosur (which was established in 1991 and encompasses Argentina, Brazil, Uruguay, Paraguay, and as of 2006, Venezuela)  
● Mercosur standards are influenced by the European Union, the Codex Alimentarius Commission, and the USFDA  
● Brazil has been a member of the World Trade Organization since 1995  
● Brazil also is a member of the Codex Alimentarius Commission. There is also the Codex Alimentarius Group of Brazil, formed by entities and professors, whose function is to meet the demands of the Codex Committee |

| Recent and/or Pending changes                                                        | None known |

| Regulatory overview of specific food chemical groups                                 | Definition: As per GMC 26/03, food ingredients are defined as all substances, including food additives, which are used in the manufacture or preparation of foods and which are present in the final product in its original or modified form. |
| Novel foods: These are the criteria for classification of novel foods:               | 1 – The following foods must be registered in the category of novel food, if they do not bear functional and/or health claims, given the criteria set out in Resolution No. 16/99:  
1.1 – foods with no history of use in the country;  
1.2 – foods containing novel ingredients, except those listed in Table 1;  
1.3 – foods containing substances already consumed that may be added or used at levels much higher than those currently observed in the foods that constitute part of a regular diet; and  
1.4 – food offered in the form of capsules, pills, tablets and the like |

(continued)
Table 3. Continued.

| Regulation | Brazil has a specific regulation for the approval of novel ingredients or novel foods. Novel foods (Resolution No. 16):  
| List of novel foods approved by ANVISA (http://www.anvisa.gov.br/alimentos/comissoes/novos_alimentos.htm)  
| List of novel ingredients approved by ANVISA (http://www.anvisa.gov.br/alimentos/comissoes/novos_ingredientes.htm)  
| Guidance document: No authoritative guidance document found  
| Approval process for a new substances: Pre-market application is required prior to sale (http://portal.anvisa.gov.br/wps/portal/anvisa/home)  
| Direct food additives | Definition: Defined by GMC 26/03, as any ingredient which added to foods intentionally, without intent to nurture, in order to modify the physical, chemical, biological or sensory characteristics of foods, during its manufacture, processing, preparation, processing, packaging, conditioning, storage, transport or during food handling: it will have, or it can be reasonably expected to have (directly or indirectly) as a result, that the additive itself or its by-products becomes part of that food. This term does not include contaminants or nutrients that are incorporated into a food in order to maintain or improve its nutritional properties. GMC 26/03 is currently under revision by Sub Work Group #3 (SGT#3) Mercosur and continues to be discussed and should be gazetted during 2012  
| Regulation:  
| GMC 34/07 – List of food additives not permitted for use  
| Two new regulations for Mercosur, GMC 34/10 and GMC 35/10; however, not all member states have adopted these regulations  
| GMC 34/10 – List of food additives according to good manufacturing practices (already internalised in Brazil by means of Resolução RDC 45 dated 3 November 2010) (http://portal.anvisa.gov.br/wps/wcm/connect/11707300474597459fc3df3fbc4c6735/Resolu%C3%A7%C3%A3o+da+Diretoria+ Colegiada+-+RDC++n++45+-+de+03+-+de+novembro+-+de+2010.pdf?MOD=AJPERES)  
| GMC 35/10 – List of food additives permitted for use at maximum levels, internalised in Brazil by means of Resolução RDC 46 dated 3 November 2010. Website (http://portal.anvisa.gov.br/wps/wcm/connect/3664e600474597459fc4df3fbc4c6735/RESOLU%C3%87%C3%83%C3%80%E2%80%93RDC%E2%80%93N%E2%80%9346%E2%80%93DE%E2%80%93NOVEMBRO%E2%80%93DE%E2%80%932010.pdf?MOD=AJP RE)  
| Revision of GMC 11/06 is expected in 2013  
| Approval process for a new substances: All food additives that are not listed in GMC11/06 can be submitted for evaluation to ANVISA, who will then submit a request to the Mercosur Sub Work Group #3 to consider the revision of 11/06 and to proceed to its incorporation. Requests must be sent first for evaluation to ANVISA and comply with the guidance document listed on the website (http://www.anvisa.gov.br/alimentos/guia_pedidos.pdf)  
| Food contact substances (e.g. components of packaging materials) | Definition: According to GMC 26/03, food contact substances are defined as the primary container or primary wrapping or container (container that is in direct contact with food)  
| Regulation: GMC 32/07:  
| There is a positive list of substances (in Appendix 1 of regulation GMC 32/07) that are added to plastics to achieve a technical effect in the final product (additives) such as antioxidants, antistatic, foaming, defoaming, fillers, impact modifiers, plasticisers, lubricants, stabilisers, UV protectants, preservatives, hardeners, etc.  
| Included within this list are the substances used to provide a suitable medium polymersisation (e.g. emulsifiers, surfactants, buffers pH, solvents)  
| Guidance document: No authoritative guidance document found  
| Approval process for a new substances: Same as direct food additives  
| Flavouring agents | Definition: Flavouring agents are substances or mixtures of substances with odoriferous and/or flavour properties that are able to confer or enhance the aroma and/or taste of food. For the purpose of this Technical Regulation, flavourings/flavourings are classified as either natural or synthetic  
| Regulation:  
| Under Mercosur, GMC 10/06  
| Permitted flavouring agents (natural or synthetic), including colourants are listed |
Table 3. Continued.

- Exclusions from the technical regulations include:
  - The substances which give only sweet, salty or sour
  - Substances and food products with odoriferous and/or sapid consumed without processing, with or without reconstitution
  - Substances of plant or animal origin, having inherent flavours/flavouring properties, where they are used as sources of flavourings


**Approval process for a new substances:** All flavouring agents that are not listed in GMC10/06 can be submitted for evaluation to ANVISA, who will then submit a request to the Mercosur Sub Work Group #3 to consider the revision of 10/06 and to proceed to its incorporation. Requests must be sent first for evaluation to ANVISA and comply with the guidance document listed on the website ([http://www.anvisa.gov.br/alimentos/guia_pedidos.pdf](http://www.anvisa.gov.br/alimentos/guia_pedidos.pdf)).

### Enzymes

**Definition:** Enzymes or enzyme preparations are defined as substances of animal, plant or microbial origin that act by promoting the desirable chemical reactions

**Regulation:** Resolução RDC # 26 dated 05/27/2009. Enzymes regulations are not harmonised in Mercosur

**Guidance document:** No authoritative guidance document found

**Approval process for a new substances:** Submissions dossier should be addressed to ANVISA following the guidance document set forth at: [http://www.anvisa.gov.br/alimentos/guia_pedidos.pdf](http://www.anvisa.gov.br/alimentos/guia_pedidos.pdf)

### Processing aids

**Definition:** Processing aids are any substances, excluding equipment and utensils, which are not consumed as it is as a food ingredient and which is intentionally used in the processing of raw materials, foods or ingredients, for a technological purpose during treatment or processing. It must be removed from the food or inactivated; the presence of traces of the substances or their derivatives may be admitted in the final product

**Regulation:** GMC 18/93 Modificación RES. GMC 31/92 “Definición y Principios fundamentales referente a empleo de aditivos, ingredientes, coadyuvante de elaboracion, contaminantes”. Resolución GMC No. 84/93

**Guidance document:** Processing aids are not harmonised in Mercosur, the only harmonised regulation is GMC 84/93 that establishes the definitions of the functions of processing aids ([http://www.anvisa.gov.br/alimentos/guia_pedidos.pdf](http://www.anvisa.gov.br/alimentos/guia_pedidos.pdf))

**Approval process for a new substances:** Submissions dossier should be addressed to ANVISA following the guidance document set forth at: [http://www.anvisa.gov.br/alimentos/guia_pedidos.pdf](http://www.anvisa.gov.br/alimentos/guia_pedidos.pdf)

### Nanoscale materials

**Definition:** No authoritative statement found

**Regulation:** No authoritative statement found

**Guidance document:** No authoritative statement found

**Approval process for a new substances:** No authoritative statement found

Efforts towards developing standards and regulations (Locascio et al. 2011): Committee for the special study of nanotechnology ([http://www.abnt.org.br](http://www.abnt.org.br)):

- Developing practices of health, safety, and environment with a scientific basis
Table 4. Regulatory framework of chemicals added to food in Canada.

<table>
<thead>
<tr>
<th>Regulatory authority</th>
<th>Name: Health Canada (HC) Food Directorate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Website:</td>
<td><a href="http://www.hc-sc.gc.ca/fn-an/index-eng.php">http://www.hc-sc.gc.ca/fn-an/index-eng.php</a> (Health Canada 2011)</td>
</tr>
<tr>
<td><strong>Historical overview:</strong></td>
<td></td>
</tr>
<tr>
<td>● The Food and Drugs Act was introduced in 1920</td>
<td></td>
</tr>
<tr>
<td><strong>Role/responsibility:</strong></td>
<td></td>
</tr>
<tr>
<td>● Establishing policies, setting standards, and providing advice and information on the safety and nutritional value of food</td>
<td></td>
</tr>
<tr>
<td>● Promoting the nutritional health and well-being of Canadians by collaboratively defining, promoting and implementing evidence-based nutrition policies and standards</td>
<td></td>
</tr>
<tr>
<td>● Administering the provisions of the Food and Drugs Act that relate to public health, safety and nutrition</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Regulatory enforcement</th>
<th>Name: Canadian Food Inspection Agency (CFIA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Website:</td>
<td><a href="http://www.inspection.gc.ca/english/toce.shtml">http://www.inspection.gc.ca/english/toce.shtml</a> (CFIA 2011)</td>
</tr>
<tr>
<td><strong>Historical overview:</strong></td>
<td></td>
</tr>
<tr>
<td>● Established in 1997 by the Canadian Food Inspection Agency Act</td>
<td></td>
</tr>
<tr>
<td>● Prior to the establishment of the CFIA, inspection and related services for food safety and animal and plant health were provided by Agriculture and Agri-Food Canada, HC, and the Department of Fisheries and Oceans (<a href="http://www.oag-bvg.gc.ca/internet/English/parl_oag_199809_12_e_9318.html/mo">http://www.oag-bvg.gc.ca/internet/English/parl_oag_199809_12_e_9318.html/mo</a>) (OAG 1998)</td>
<td></td>
</tr>
<tr>
<td><strong>Role/responsibility:</strong></td>
<td></td>
</tr>
<tr>
<td>● Enforce the food safety and nutritional quality standards established by HC and for animal health and plant protection, to set standards and carry out enforcement and inspection (<a href="http://www.inspection.gc.ca/english/faq.shtml">http://www.inspection.gc.ca/english/faq.shtml</a>)</td>
<td></td>
</tr>
<tr>
<td>● Plans and priorities link directly to the Government of Canada’s priorities for bolstering economic prosperity, strengthening security at the border and the safety of the food supply, protecting the environment and contributing to the health of Canadians (<a href="http://www.inspection.gc.ca/english/agen/agene.shtml">http://www.inspection.gc.ca/english/agen/agene.shtml</a>)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Part of an overarching international organisation</td>
<td>● Canada has been a member of the Codex Alimentarius Commission since it was established in 1963 (<a href="http://www.hc-sc.gc.ca/fn-an/intactiv/codex/activit/strateg-codex-2008-2012-eng.php">http://www.hc-sc.gc.ca/fn-an/intactiv/codex/activit/strateg-codex-2008-2012-eng.php</a>) (Health Canada 2009a)</td>
</tr>
<tr>
<td>● Canada also has been a member of the World Trade Organization since 1995</td>
<td></td>
</tr>
</tbody>
</table>

| Recent and/or pending changes | None known |

**Regulatory overview of specific food chemical groups**

**Food ingredients**

Food ingredients are defined as an individual unit of food that is combined as an individual unit of food with one or more other individual units of food to form an integral unit of food that is sold as a pre-packaged product:

● The use of a food ingredient does not require pre-market approval unless it meets the definition of a novel food. Novel foods are defined according to Division B.28.001 as:
  ○ a substance, including a microorganism, that does not have a history of safe use as a food;
  ○ a food that has been manufactured, prepared, preserved or packaged by a process that:
    (i) has not been previously applied to that food, and
    (ii) causes the food to undergo a major change; and
  ○ a food that is derived from a plant, animal or microorganism that has been genetically modified such that:
    (i) the plant, animal or microorganism exhibits characteristics that were not previously observed in that plant, animal or microorganism;
(ii) the plant, animal or microorganism no longer exhibits characteristics that were previously observed in that plant, animal or microorganism, or
(iii) one or more characteristics of the plant, animal or microorganism no longer fall within the anticipated range for that plant, animal or microorganism


Guidance document: A guidance document is available on novel foods, whether whole foods, food products or food ingredients, that are derived from plant or microbial sources. The safety assessment criteria for novel foods derived from animals are under development. Manufacturers or importers of novel foods derived from animal sources should consult with the Food Directorate to discuss which type of information is appropriate to the evaluation of the safety of a particular product (http://www.hc-sc.gc.ca/fn-an/index-eng.php)

Approval process for a new substances: The mechanism by which HC controls the sale of novel foods in Canada is the mandatory pre-market notification requirement as described in Division 28 of Part B of the Food and Drug Regulations. Manufacturers or importers are required under these regulations to submit information to HC regarding the product in question in order to determine the product’s safety prior to sale. If the information provided in the notification for a novel food is not considered adequate to determine the novel food’s safety, additional data supporting the safety of the food will be required. The type of information required to conduct the safety assessment of a novel food will depend on a number of factors such as the nature of the food, processing methods and the intended use. The approaches used to assess the safety of novel foods are outlined in the guidance document; however, the types of studies considered appropriate to demonstrate the safety of a novel food change with scientific knowledge and development. These guidelines are to be used in conjunction with information available in the scientific literature and from research and development conducted by the manufacturer. Since novel foods represent a diverse range of products, not all types of data outlined in the guidance document will be appropriate for a specific submission. Petitioners should consider the novel characteristics of their particular product when addressing the criteria in the guidance document. Consultation with the Food Directorate is encouraged during the development phase of a product to determine the specific data necessary to demonstrate the safety of the product. Information sufficient to establish that a novel food is safe for consumption may include experimental data as well as sound scientific rationales. To enhance the efficiency of the review process, petitioners should prepare their safety assessment data packages according to the following headings, as applicable:

- History of use
- Dietary exposure
- Detail of novel process
- History of organism(s)
- Characterisation of derived line/strain
- Genetic modification considerations
- Nutritional considerations
- Toxicology considerations
- Allergenicity considerations
- Chemical considerations
- Microbiological considerations

Direct food additives

Definition: According to the Food and Drugs Regulations B.01.001, “a food additive means any substance the use of which results, or may reasonably be expected to result, in it or its by-products becoming a part of or affecting the characteristics of a food, but does not include:

- Any nutritive material that is used, recognised or commonly sold as an article or ingredient of food;
- Vitamins, mineral nutrients and amino acids, other than those listed in the tables to Division 16
- Spices, seasonings, flavouring preparations, essential oils, oleoresins and natural extractives;
- Agricultural chemicals, other than those listed in the tables to Division 16,
- Food packaging materials and components thereof; and
- Drugs recommended for administration to animals that may be consumed as food”.

(continued)
It should be noted that a substance not present in the final food but which has affected the characteristics of that food would be regulated as a food additive. The official food additive provisions are listed in the tables of B.16.100 of the Food and Drug Regulations (Department of Justice 2011). Listings of permitted food additives include the common name of the food additive, a list of the foods in which the additive may be used, and the maximum level of use. Table III lists food additives that may be used as colouring agents; Table IX lists sweeteners permitted for use as a food additive. In Canada, food contact substances are regulated separately from food additives (see “Food contact substances” section below). Enzymes that are used in food processing are regulated as food additives or may be considered processing aids, depending on their context of use. Flavouring agents and processing aids do not require a submission like a food additive but petitioners can request a letter of opinion from Health Canada (see “Flavouring agents, Enzymes, and Processing Aids” section below).

Regulation: Division B.16 (http://laws.justice.gc.ca/eng/C.R.C.-C.870/page-1.html)

Approval process for a new substances: A submission for a food additive is required if a petitioner is seeking approval for use in Canada of a new food additive not currently regulated in the Food and Drug Regulations. A petitioner is also required to present a submission for an extension of use of an existing food additive, e.g. the use of an existing food additive in a different food or the use of a food additive at a higher maximum level of use. In these latter cases, there may not be a need to resubmit data already available at HC. The four possible scenarios are summarised as follows:
- Requests for the use of a new food additive (which is a food additive that has never before been approved for use in retail foods in Canada)
- Requests to extend the use of an already permitted food additive
- Requests to change the maximum level of use of an already permitted food additive
- Requests to add a new organism to the list of permitted sources of enzymes used as food additives

In the case of submissions on new food additives (those which do not appear anywhere in the Food Additives Tables of the Food and Drug Regulations), detailed data and scientific information meeting the requirements of Section B.16.002 of the Food and Drug Regulations are required in order to support the development of specifications and verify conformity of the additive with those specifications, develop and verify analytical methods, establish claims of efficacy, demonstrate residue levels or reaction products, determine human exposure to a food additive in any given application, and demonstrate the absence of any negative health effects of the food additive when used in the prescribed manner.

General requirements include:
- Identity of the food additive
- Method of manufacture
- Chemical and physical properties
- Specifications
- Purpose/function of food additive
- Directions for use
- Efficacy data demonstrating the technical effect
- Residue data
- Proposed maximum level of use
- Analytical data (for new food additives only, not required for extension of use or changes to maximum levels of use of current food additives listed in Division 16 of the Food and Drug Regulations).
- Safety data
- Food intake data
- Toxicological data
- Pharmacokinetic studies
- Human clinical studies
- Nutritional safety considerations
- Microbiological data (if food additive is derived from microbial sources or is genetically modified or in the case of antimicrobials, efficacy of preservatives)
- Labelling information
- A sample of the food additive

(continued)
Other non-statutory requirements for submissions on food additives:

- Consumer benefits and food quality considerations
- Information on evaluations, approvals, and authorisations of other national/international bodies (such as JECFA, USFDA, EU food safety authorities, Australia and New Zealand food standards, and/or Codex Alimentarius Commission)

Environmental assessment of new food additives: The Canadian Environmental Protection Act, 1999 (CEPA 1999) is the primary federal legislation respecting the protection of the environment and human health. The goal of CEPA 1999 is to contribute to sustainable development through pollution prevention. In 2001, HC announced its intent to develop environmental assessment regulations for a new substances regulated under the Food and Drugs Act.

Food contact substances (e.g. components of packaging materials)

**Definition:**

- Food contact or packaging materials have been excluded from the food additive requirements. Food contact or packaging materials are controlled separately under Division 23, Part B of the Food and Drug Regulations (http://laws.justice.gc.ca/eng/C.R.C.-C.870/page-1.html#anchorbo-ga:l_B-gb:l_23)
- “No person shall sell any food in a package that may yield to its contents any substance that may be injurious to the health of a consumer of the food” (http://www.hc-sc.gc.ca/fn-an/legislation/guide-ld/guide_packaging-emballage01-eng.php). This link also lists the documents that can be submitted voluntarily


**Guidance document:** Packaging materials (on a trade name basis, not by generic name) intended for use with foods in Canada may be submitted voluntarily to the Health Products and Food Branch (HPFB) for a pre-market assessment of their chemical safety and subsequent issuance of an advisory opinion on their acceptability in relation to Section B.23.001 of the Food and Drugs Act and Regulations dealing with the potential transfer of harmful chemicals to foods. Any type of material, whether it is in the form of a finished product such as a laminated film, a container, etc. or a formulated product such as a plastic resin, a colour concentrate, etc. may be submitted to the branch for a pre-market assessment. In addition, suppliers of single additives such as antioxidants, ultra violet absorbers, etc. may also independently request letters of opinion for their own products before selling them to formulators or converters. Thus, in terms of data requirements, food packaging submissions may be divided into two distinct categories namely, those on formulated products and finished articles, which are normally submitted by converters and formulators and those on specific constituents or single additives which usually originate from raw material suppliers

**Approval process for a new substances:** The following information is provided as a guide to the food packaging industry to assist in the preparation of submissions to HPFB:

1. Formulated products and finished articles (formulators/converters). Two elements of information are required initially for a formulated product or a finished packaging article namely, the product’s identity and its intended food packaging uses. The information to be provided under each element is as follows:
   - **Product identity:**
     - Trade name and number (mostly for records purposes)
     - Structure (e.g. laminate)
     - Composition (in the form of a quantitative listing of all components in which each one is identified by proper chemical name and/or trade name, grade and supplier)
     - Specifications
     - Chemical/physical properties relative to proposed use
   - Proposed usage:
     - Form of finished package (e.g. bottle, film, casing, etc.)
     - Dimensions of package (volume, wall thickness, etc.)
     - Packaging ratio, i.e. weight of food/unit area of packaging material (g/in²)
     - Conditions (time, temperature) to which packaging article will be exposed during packaging, distribution and use by consumers
     - Estimate of projected market penetration
2. Specific constituents/single additives (raw material supplies). Four elements of information are required for a submission on a new additive or a single constituent in a food contact material. They include information on the product’s identity, its proposed usage, data on its extractability characteristics, and toxicological data. The information to be included under each of these four elements is as follows:

(continued)
Table 4. Continued.

<table>
<thead>
<tr>
<th>Product identity:</th>
</tr>
</thead>
<tbody>
<tr>
<td>● Chemical name</td>
</tr>
<tr>
<td>● Chemical formula (empirical and structural)</td>
</tr>
<tr>
<td>● Molecular weight</td>
</tr>
<tr>
<td>● Manufacturing process (including a detailed description and a schematic diagram)</td>
</tr>
<tr>
<td>● Purity specifications (residual reactants, by products, etc.)</td>
</tr>
<tr>
<td>● Chemical and physical properties</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Proposed usage:</th>
</tr>
</thead>
<tbody>
<tr>
<td>● Intended technical effect or purpose (e.g. antioxidant, stabiliser, ultraviolet absorber, etc.)</td>
</tr>
<tr>
<td>● Types of substrates or polymers</td>
</tr>
<tr>
<td>● Maximum use level in each type of polymer</td>
</tr>
<tr>
<td>● Efficacy data to demonstrate that the additive will do what it is intended to do at the proposed use levels</td>
</tr>
<tr>
<td>● Types of foods involved</td>
</tr>
<tr>
<td>● Conditions of use (time, temperature, etc. to which the packaging material will be exposed, whether during processing, transport or handling by consumers)</td>
</tr>
</tbody>
</table>

Migration/extraction data: Extraction studies data are required to identity and quantify the potential contaminants in foods. These studies are usually conducted using food simulants under conditions that reflect as close as possible those of the proposed end-use applications. Toxicological data: The submitted toxicological data should provide the basis for the petitioner’s safety determination for the packaging material constituent under the proposed conditions of use. The toxicological studies recommended by HPFB for its safety assessment are based on levels of concern that are determined by the level of dietary exposure. Dietary exposures that fall below 0.025 µg kg\(^{-1}\) bw are considered to be below the threshold of toxicological concern and toxicological data need not be submitted.

**Flavouring agents**

*Definition:* No authoritative statement found

*Regulation:* No specific regulations on flavouring agents. Section 4 of the Food and Drug Act applies. No application/submission required for use of flavouring agents in food within Canada. Standards of identity and composition are available in Division B.10 pertaining to specific flavouring preparations (such as essences or extracts obtained from aromatic plants) (http://laws.justice.gc.ca/eng/C.R.C.-C.870/page-1.html#anchorbo-ga:l_B-gb:l_10). Flavours or flavouring preparations not mentioned in Division 10 are considered unstandardised food ingredients. Certain prohibitions exist in Section B.01.046. The definition of food additive excludes flavouring preparations. On request, the Bureau of Chemical Safety evaluates safety of use of these ingredients and issues a letter of opinion.

*Guidance document:* No authoritative guidance document found

*Approval process for a new substances:* Applicants can voluntary request a letter of opinion on a flavouring agent (see “approval process for processing aids”)

**Enzymes**

*Definition:* Not specifically defined in the Food and Drug Regulations. Enzymes that are used in food processing are regulated as food additives or may be considered processing aids, depending on their context of use.

An enzyme meets the definition of a food additive, as described in Section B.01.001 of the Food and Drug Regulations, when it affects the characteristics of the food and/or its by-products become part of the food. It is the physical enzyme residues, not enzyme activity, that are considered in determining if enzyme residues remain in or on a food.

*Regulation:* Enzymes approved as food additives are listed within Table V of Section B.16.100 Listings of permitted enzymes as food additives include the common name of the enzyme, permitted sources of the enzyme, a list of the foods in which the enzyme may be used, and the maximum level of use.

*Guidance document:* See “Direct food additives” (above)

*Approval process for a new substances:* See “Direct food additives” (above)

(continued)
### Processing aids

**Definition:** According to the Food Directorate, “a food processing aid is a substance that is used for a technical effect in food processing or manufacture, the use of which does not affect the intrinsic characteristics of the food and results in no or negligible residues of the substance or its by-products in or on the finished food”. This definition implies the absence of residues of any given chemical and its by-product in the final food product, which is different from Australia's definition of a processing aid (in which the absence of by-products is not mentioned). Also, characteristics of the final food must not be affected by the use of any given chemical classified as a processing aid. The definition of processing aid in Canada differs from the definition used by the Codex Alimentarius Commission. The CAC definition does not have a limitation on residue levels and does not refer to affecting the characteristics of the food. These restrictions must be included in the Directorate’s definition because a substance is considered to be a food additive, under the Canadian regulatory definition of food additive, if use of the substance results in residues in the food or affects the characteristics of the food.

**Regulation:** There is no regulatory definition of food processing aid in Canada. Canadian regulators have typically used “processing aid” in an informal manner for substances used as adjuncts in food processing and manufacture. Most processing aids are not mentioned in the Regulations and unlike food additives, there is no regulatory requirement for preclearance of new processing aids by the Minister of Health. Like all substances used with food, the use of a processing aid is ultimately controlled by Section 4, Part I of the Act which states:

“No person shall sell an article of food that:
- has in or on it any poisonous or harmful substance;
- is unfit for human consumption;
- consists in whole or in part of any filthy, putrid, disgusting, rotten, decomposed or diseased animal or vegetable substance;
- is adulterated; or
- was manufactured, prepared, preserved, packaged or stored under unsanitary conditions”

**Guidance document:** Not applicable

**Approval process for a new substances:** The use of a processing aid does not require a submission like a food additive but a petitioner may seek a so-called “Letter of Opinion” from the Bureau of Chemical Safety of HC’s Food Directorate, confirming that, under its conditions of use, the substance in question is considered to be a processing aid and is acceptable for use.

### Nanoscale materials

**Definition:** Nanotechnology is described as the application of nanoscience to develop new materials and products, and involves the manipulation of matter at the nanometre scale. In the food sector, nanotechnology could be used to preserve food, improve nutritional values and enhance flavours (http://www.hc-sc.gc.ca/dhp-mps/nano-eng.php) (Health Canada 2009b). Health Canada considers any manufactured product, material, substance, ingredient, device, system or structure to be nanomaterial if:

- it is at or within the nanoscale in at least one external dimension, or has internal or surface structure at the nanoscale; or
- it is smaller or larger than the nanoscale in all dimensions and exhibits one or more nanoscale properties/phenomena.

For the purposes of this definition:

- the term “nanoscale” means 1–100 nm, inclusive;
- the term “nanoscale properties/phenomena” means properties which are attributable to size and their effects; these properties are distinguishable from the chemical or physical properties of individual atoms, individual molecules and bulk material; and

**Regulation:** No authoritative statement found. No reference to nanomaterials is stated in the acts and regulations; however, HC is using the existing legislative and regulatory framework to regulate applications of nanotechnology, but it is recognised that new approaches may be necessary in future to keep pace with the advances in this area. Within the Health Portfolio, a Nanotechnology Working Group has been established to gather information, identify areas where additional regulations may need to be considered, and to act as a discussion forum for issues related to nanotechnology (http://www.hc-sc.gc.ca/dhp-mps/nano-eng.php)

(continued)
Table 4. Continued.

**Guidance document**: Only general guidance has been given (http://www.hc-sc.gc.ca/sr-sr/pubs/nano/pol-eng.php). In order to identify and assess potential risks and benefits (where applicable) of nanomaterials, the following types of information may be required, when relevant:

- Intended use, function and purpose of the nanomaterial, and information regarding any end product in which it will be used
- Manufacturing methods
- Characteristics, and physical chemical properties of the nanomaterial such as:
  - composition, identity, purity
  - morphology
  - structural integrity
  - catalytic or photo-catalytic activity
  - particle size/size distribution
  - electrical/mechanical/optical properties
  - surface-to-volume ratio
  - chemical reactivity
  - surface area/chemistry/charge/structure/shape
  - water solubility/dispersibility
  - agglomeration/aggregation (or other properties) and descriptions of the methods used to assign these determinations
- Toxicological, eco-toxicological, metabolism and environmental fate data (both generic and specific) to the nanomaterial if applicable
- Risk assessment and risk management strategies, if considered or implemented

**Approval process for a new substances**: No authoritative statement found

Efforts towards developing standards and regulations (Locascio et al. 2011):

- Developing methods to assess the risk of nanomaterials, such as developing *in vitro* assays, *in vivo* studies and tests for systemic responses

Table 5. Regulatory framework of chemicals added to food in China.

<table>
<thead>
<tr>
<th>Regulatory authority</th>
<th>Name: Ministry of Health (MOH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Historical overview:</td>
<td>Established 21 November 1949</td>
</tr>
</tbody>
</table>

**Role/responsibility:**

- To draft health laws, regulations and policies; to propose health development programmes and strategic goals; to formulate technical protocols, health standards, and to supervise their enforcement
- To propose regional health programmes, to conduct overall planning, and to coordinate the nationwide allocation of health resources
- To supervise communicable disease prevention and treatment, food health, occupational, environmental, radiological and school health. To formulate food and cosmetics quality control protocols and be responsible for their accreditation
- To organise and guide multilateral and bilateral governmental and non-governmental health and medical cooperation and exchanges and medical aid to other countries, to participate in major health events initiated by international organisations
- To coordinate medical and health exchanges and collaborations between China and the World Health Organization and other international organisations
- To undertake other work as designated by the State Council

<table>
<thead>
<tr>
<th>Advisory scientific body</th>
<th>Name: Same as Regulatory authority (above)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Website:</td>
<td>Same as Regulatory authority (above)</td>
</tr>
<tr>
<td>Historical overview:</td>
<td>Same as Regulatory authority (above)</td>
</tr>
</tbody>
</table>

(continued)
Table 5. Continued.

<table>
<thead>
<tr>
<th>Role/responsibility: Same as Regulatory authority (above)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Framework regulations</td>
</tr>
<tr>
<td>● Food Safety Law of the People’s Republic of China (adopted at the 7th Session of the 11th Standing Committee of the National People’s Congress of the People’s Republic of China on 28 February 2009)</td>
</tr>
<tr>
<td>● Implementation Rules of Food Safety Law of the People’s Republic of China – Order of State Council of the People’s Republic of China No. 557 (adopted at the 73rd Standing Committee Meeting of the State Council on 8 July 2009)</td>
</tr>
<tr>
<td>Part of an overarching international organisation</td>
</tr>
<tr>
<td>● China has been a member of the World Trade Organization since 2001</td>
</tr>
<tr>
<td>● China is a member of the Codex Alimentarius Commission</td>
</tr>
<tr>
<td>Recent and/or pending changes</td>
</tr>
<tr>
<td>None known</td>
</tr>
</tbody>
</table>

Regulatory overview of specific food chemical groups

Food ingredients

*Definition of food ingredients:* any substance, including a food additive, used in the manufacture or preparation of a food and present in the final product although possibly in a modified form. Novel foods are referred to as new resource foods, which are defined as raw food materials or food ingredients, which do not have a significant history of consumption in China (Roberts & Rogerson 2008). Novel foods are separated into four categories:

- Animals, plants and microorganisms that are not traditionally consumed in China
- Raw food materials that are derived from animals, plants and microorganisms and are not traditionally consumed in China
- New varieties of microorganisms that are used during food processing
- Raw food materials the original composition or structures of which are changed by the adoption of new techniques during production

*Regulation:* Order No. 56 – Administrative Measures on Novel Food


*Approval process for a new substances:* Premarket application demonstrating safety of the novel food. Applications for novel food shall include the following:

- Application for hygiene administration permit of novel food (manufacturer must obtain a hygiene license before producing, marketing or using the food additive)
- Research and production report
- Brief summary and flow chart of processing techniques
- Product quality standards
- Status on research and production at home and abroad, as well as safety-related documents
- Product label and instructions
- Other materials helpful to assessment and review
- A product sample or 30 g of raw material
- For the importation of novel food, it is also required to submit certificates indicating the production (or marketing) of the food products are permitted in the exporting country (region) or documents showing the traditional consumption history of the food in the exporting country (region), which are issued by relevant departments or institutions of the exporting country (region)

Direct food additives

*Definition:* “An artificially chemosynthetic or natural substance to be added to foods in order to improve food quality, colour, fragrance and taste, and for the purpose of preservation and processing technology. Nutrition enhancers, gum-based substances in chewing gum, flavouring agents, and processing aids in the food industry are also included in food additives.” Permitted food additives (including colouring agents, sweeteners, and flavour enhancers) are classified according to their function with permitted maximum levels indicated (USDA 2008). A new food additive is a food additive that:

- Is not included in the national food safety standards
- Is not included in the public announcement of permitted use issued by the Ministry of Health
- Whose scope of use or dosage is increased

(continued)
Table 5. Continued.

**Regulation:**
- National Standard GB-2760-2007 – Hygienic Standards for the Use of Food Additives
- National Food Safety Standard – Standards for uses of food additives GB-2760-xxxx (Draft for comment; Notified to the WTO as G/SPS/N/CHN/308 on 4 August 2010)
- Administrative Licensing Regulation for New Varieties of Food Additive (Notified to the WTO as G/SPS/N/CHN/201 on 8 January 2010)


**Approval process for a new substances:** Pre-market application demonstrating technological need and safety of the additive. The application shall include:
- Generic name, functions, dosage and scope of use of additives
- Documents demonstrating technical necessity and efficiency of additives
- Quality requirements, manufacturing process and testing methods of food additives, and testing methods of such additives or relevant explanations
- Safety assessment data, including raw materials or sources, chemical constitution and physical properties, manufacturing process, toxicological safety assessment data, or test report and quality test report
- Label, specifications, and sample of food additives
- Data on permission of manufacture and use granted by other countries or regions or international organisations and other data that may be useful in safety assessment

Any application for increase in the scope of use or dosage of food additives may be exempt from provision of materials identified in the fourth point above, unless additional data are required during technical review (http://gain.fas.usda.gov/Recent%20GAIN%20Publications/Food%20Additive%20Registration_Beijing_China%20-%20Peoples%20Republic%20-%20of_5-12-2010.pdf) Any applicant applying for the initial import of new food additives shall submit the following materials in addition to those specified above:
- Supporting documents permitting manufacture or sale of such additives in the exporting country or region that are issued by relevant authority or agency thereof
- Supporting documents examining or certifying the manufacturer that are issued by relevant agency or organisation in the country or region where the manufacturer is located

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**Food contact substances (e.g. components of packaging materials)**

**Definition:** All materials in contact with the food including the food containers, packaging materials and the things which contact the food in the course of manufacture, transport, sale and serve. The licensing scope of the food-related new product varieties includes:
- Food materials or mouldings without national food safety standards that are used for food packaging or containerisation and machinery that has direct contact with the food during production or packaging
- Additives not included in the Hygienic Standards for Additives Used in Food Containers and Packaging Materials (GB 9685)
- Food materials or mouldings not included in the list of food packaging materials or containers announced by the Ministry of Health and all operational tools and equipment that have direct contact with food during food production, and their processing additives
- Additives which are included in the Hygienic Standards for Additives Used in Food Containers and Packaging Materials (GB 9685) or the list announced by the Ministry of Health but with the scope or dosage expanded upon
- New detergent materials which may have food safety risks and are used for food, food production and operational tools and equipment that have direct contact with food during food production and packaging
- New disinfectant materials which are not included in the List of Food Disinfectant Materials and are used for food, food production and operational tools and equipment that have direct contact with food during food production and packaging

**Regulation:** Hygienic Standards for Additives Used In Food Containers and Packaging Materials (GB 9685)

**Guidance document:** Rules on Administrative Licensing of Food-Related New Product Varieties (Notified to the WTO as G/SPS/N/CHN/120 on 13 August 2009)
Table 5. Continued.

**Approval process for a new substances**: Premarket application demonstrating technological need and safety of the additive. If an additive in the food containers or packaging materials meets the following conditions, such additive shall be an exemptible substance and does not need to be reported for examination and approval:
- The migration volume of the additive is less than 0.01 mg kg\(^{-1}\) (i.e. 10 ppb); the additive is not carcinogenic, mutagenic or a reproductive toxic substance; the additive migrating into food does not cause the food ingredients, structure, colour, smell or taste to change
- There is multilayer composite packaging with a functional barrier layer and the migration volume of the substance outside the barrier layer is less than 10 ppb

### Flavouring agents

**Definition**:
Flavourings (flavour compound):
- A concentrated and prepared mixture incorporated by flavouring substances and flavouring adjuncts, and used for producing flavour (excluding preparations only producing salty, sweet or sour taste), which may contain or not contain flavouring adjuncts. Usually, they are not directly used for consumption. The flavourings include food flavourings, feed flavourings, flavourings in contact with mouth cavity and lips and the flavourings for dish washing detergent:
  - Oil-soluble liquid flavourings
  - Water-soluble liquid flavourings
  - Emulsified flavourings
  - Paste flavourings
  - Blending powder flavourings
  - Encapsulated powder flavourings

Flavouring (flavour compound) adjunct:
- A food additive and food ingredient necessary for producing, preserving and applying the flavourings. The additive added to food (except flavour enhancer) does not play the role for final aromatic products.

Process flavourings:
- A product or mixture prepared for the purpose of characteristics of odour, which is a kind of product prepared with the ingredients or ingredient mixtures allowed to be used in process flavourings or process flavourings allowably used for food or natural application in food through the preparation process suitable for foods consumed by humans. It can add flavouring substances and flavouring adjuncts to process flavourings.

A list of allowable flavourings is available (Annex B, People’s Republic of China, 2010)

**Regulation**: National Standard of the People’s Republic of China – Flavourings (TBT/N/CHN/575)

**Guidance document**: See “Direct food additives” (above)

**Approval process for a new substances**: See “Direct food additives” (above)

### Enzymes

**Definition**: “Biological products directly extracted from edible or non-edible parts of a plant or animal or fermented and extracted from traditional or genetically modified microorganisms (including but not limited to bacteria, actinomycetes, and fungi) that are used in food processing and have a special catalytic function.” A list of allowable food enzyme preparations is available (Annex C, People’s Republic of China, 2010)

**Regulation**: Hygienic Standard for Enzyme Preparations Used in Food Processing (Notified to the WTO as G/SPS/N/CHN/112 on 5 January 2009)

**Guidance document**: See “Direct food additives” (above)

**Approval process for a new substances**: See “Direct food additives” (above)

### Processing aids

**Definition**: A substance or material (not including apparatus or utensils), and not consumed as a food ingredient by itself, and only used to fulfil a certain technological purpose during processing or treatment. Processing aids refers to various kinds of substances to enable food processing to go smoothly, irrelative to food itself, e.g. filtration aids, clarifiers, absorbents, lubricants, mould release agents, decolouring agents, peeling agents, extraction solvents, and nutritional substances for fermentation, etc. Processing aids are regulated under food additives

**Regulation**: See “Direct food additives” (above)

**Guidance document**: See “Direct food additives” (above)
Nanoscale materials

**Definition:** Nanoscience and nanotechnology encompass studying the characteristics (manipulating of atom and molecular) and interactions (primary quantum effect) of the matter on nanometre (1–100 nm) scale, as well as the interdisciplinary science and technology using these characteristics. It extends a human’s method and ability on understanding and changing the physical world to atomic and molecular level (http://english.nanoctr.cas.cn) (NCNST 2010)

**Regulation:** No regulations pertaining to nanoscale materials are available

**Guidance document:** No authoritative guidance document found

**Approval process for a new substances:** No authoritative statement found

Efforts towards developing standards and regulations: Ministry of Science and Technology:
- Supports standardisation activities in nanotechnology including health, safety, and environment

Bio-Environmental Health Sciences of Nanoscale Materials Laboratory, National Center for Nanoscience and Technology of the Chinese Academy of Sciences:
- Studies the nanotoxicology of manufactured nanomaterials
- Includes an “Innovative methodology for nanotoxicological studies”

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Table 6. Regulatory framework of chemicals added to food in the European Union.

<table>
<thead>
<tr>
<th>Regulatory authority</th>
<th>Name: European Commission (EC) – Directorate General for Health and Consumers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Website:</strong></td>
<td><a href="http://ec.europa.eu/food/food/index_en.htm">http://ec.europa.eu/food/food/index_en.htm</a> (EC 2011)</td>
</tr>
</tbody>
</table>

**Historical overview:**
- The Treaty of Rome, establishing the European Economic Community (EEC), signed on 25 March 1957, and entered into force on 1 January 1958 (Europa 2007a)
- The Treaty establishing the European Atomic Energy Community (also known as Euratom) was signed at the same time and the two are jointly known as the Treaties of Rome (Europa 2007b)

**Role/responsibility:**
- The EC is the European Union’s (EU) executive body.
- Their responsibilities include:
  - Proposing and enforcing regulation
  - Representing and upholding the interests of Europe as a whole
  - Drafting proposals for new European laws
  - Managing the day-to-day business of implementing EU policies and allocating EU funds
  - Ensuring that everyone abides by the European treaties and laws
- The responsibilities of the Directorate General for Health and Consumers is to ensure food and consumer goods sold in Europe are safe, that the EU’s internal market works for the benefit of consumers, and that Europe helps protect and improve its citizen’s health

<table>
<thead>
<tr>
<th>Advisory scientific body</th>
<th>Name: European Food Safety Authority (EFSA)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Website:</strong></td>
<td><a href="http://www.efsa.europa.eu/">http://www.efsa.europa.eu/</a></td>
</tr>
</tbody>
</table>

**Historical overview:**
- The establishment of EFSA (also known as the Authority) was one of the key measures contained in the Commission’s White Paper on Food Safety which was published in January 2000
- Regulation 178/2002/EC providing a legal basis for the Authority was formally adopted on 28 January 2002
- Two additional Commission Regulations were adopted: 1304/2003/EC, which set up the procedure applied by EFSA to request for scientific opinions referred to it, and 2230/2004/EC, which established rules with regard to the network of organisations operating in the fields within the EFSA’s mission

(continued)
EFSA’s work covers all stages of food production and supply, from primary production to the safety of animal feed, right through to the supply of food to consumers. It collects information and analyses new scientific developments in order to identify and assess any potential risks to the food chain. It can carry out scientific assessment on any matter that may have a direct or indirect effect on the safety of the food supply, including matters relating to animal health, animal welfare, and plant health. EFSA also gives scientific advice on non-food and feed genetically modified organisms as well as on nutrition in relation to EU legislation. It can communicate directly with the public on any issue within its area of responsibility. The five committees which were transferred to EFSA in May 2003 include:

- Scientific Committee on Food (SCF)
- Scientific Committee on Animal Nutrition
- Scientific Committee on Veterinary Measures relating to Public Health
- Scientific Committee on Plants
- Scientific Committee on Animal Health and Animal Welfare

Role/responsibility: The Authority shall provide scientific advice and scientific and technical support for the Community’s legislation and policies in all fields which have a direct or indirect impact on food and feed safety. It shall provide independent information on all matters within these fields and communicate on risks (178/2002/EC, article 22)

Framework regulations

178/2002/EC – General principles and requirements of food law, establishing the EFSA and laying down procedures in matters of food safety

Part of an overarching international organisation

- The EU has been a member of the World Trade Organization since 1995
- The EU is a member of the Codex Alimentarius Commission

Recent and/or pending changes

On 16 December 2008, the regulations of the Package on Food Improvement Agents were adopted. This includes regulations on food additives, food enzymes, and flavourings and food ingredients with flavouring properties, and an additional fourth regulation (Regulation (EC) No. 1331/2008, adopted on 16 December 2010) establishing a common authorisation procedure for additives, enzymes, and flavourings

Regulatory overview of specific food chemical groups

Food ingredients

Food ingredients are defined as:

- Any substance, including additives, used in the manufacture or preparation of a food-stuff and still present in the finished product, even if in altered form
- Where an ingredient of the foodstuff is itself the product of several ingredients, the latter shall be regarded as ingredients of the foodstuff in question
- The following shall not be regarded as ingredients:
  - the constituents of an ingredient which have been temporarily separated during the manufacturing process and later reintroduced but not in excess of their original proportions;
  - additives whose presence in a given foodstuff is solely due to the fact that they were contained in one or more ingredients of that foodstuff, provided that they serve no technological function in the finished product, which are used as processing aids; and
  - substances used in the quantities strictly necessary as solvents or media for additives or flavourings
- In certain cases, decisions may be taken in accordance with the procedure laid down in Article 20 (2) in Directive 2000/13/EC as to whether the conditions described above are satisfied.

Novel foods: Novel foods are foods and food ingredients that have not been used for human consumption to a significant degree within the Community before 15 May 1997


(continued)
Table 6. Continued.

Approval process for a new substances: Foods commercialised in at least one member state before the entry into force of the Regulation on Novel Foods on 15 May 1997, are on the EU market under the “principle of mutual recognition”. In order to ensure the highest level of protection of human health, novel foods must undergo a safety assessment before being placed on the EU market. Only those products considered to be safe for human consumption are authorised for marketing. Companies who want to place a novel food on the EU market need to submit their application in accordance with Commission Recommendation 97/618/EC, which outlines the scientific information and the safety assessment report required.

Novel foods or novel food ingredients may follow a simplified procedure, only requiring notifications from the company, when they are considered by a national food assessment body as “substantially equivalent” to existing foods or food ingredients (as regards to their composition, nutritional value, metabolism, intended use, and the level of undesirable substances contained therein).

Direct food additives

Definition: Food additives are substances that are not normally consumed as food itself but are added to food intentionally for a technological purpose described in Regulation (EC) No. 1333/2008, e.g. such as the preservation of food. All food additives should be covered by this Regulation, and therefore in the light of scientific progress and technological development the list of functional classes should be updated (currently there are 26 functional classes listed in the Annex I to Regulation (EC) No. 1333/2008). However, substances should not be considered as food additives when they are used for the purpose of imparting flavour and/or taste or for nutritional purposes, such as salt replacers, vitamins and minerals. Moreover, substances considered as foods which may be used for a technological function, such as sodium chloride or saffron for colouring, and food enzymes and minerals. Also should not fall within the scope of Regulation (EC) No. 1333/2008. However, preparations obtained from foods and other natural source material that are intended to have a technological effect in the final food and which are obtained by selective extraction of constituents (e.g. pigments) relative to the nutritive or aromatic constituents, should be considered additives within the meaning of Regulation (EC) No. 1333/2008. The new Regulation (EC) No. 1333/2008 does not apply to the following substances unless they are used as food additives:

- Processing aids
- Substances used for the protection of plants and plant products in conformity with Community rules relating to plant health
- Substances added to foods as nutrients
- Flavourings, which fall within the scope of Regulation (EC) No. 1334/2008
- Regulation (EC) No. 1333/2008 does not apply to food enzymes falling within the scope of Regulation (EC) No. 1332/2008 with effect from the date of adoption of the Community list of food enzymes in accordance with Article 17 of that Regulation

Regulation: Regulation (EC) No. 1333/2008 includes the Community list of approved food additives for use in foods and conditions of use (Annex II as amended by Commission Regulation (EU) No. 1129/2011) and the Community list of food additives approved for use in food additives, food enzymes, food flavourings and nutrients and their condition of use (Annex III as amended by Commission Regulation (EU) No. 1130/2011). Food additives in Annex II are listed on the basis of the categories of food to which they may be added. Food additives in Annex III are listed on the basis of the food additives, food enzymes, food flavourings and nutrients or categories thereof to which they may be added. Before these Community lists apply (1 June 2013) the Annexes to Directives 94/35/EC, 94/36/EC and 95/2/EC are still valid. A food additive may be included in the Community lists only if it meets general conditions: no safety concerns at the level of use proposed; there is a technological need; the consumer is not misled; and there are advantages and benefits for the consumer. Other more specific conditions exist for sweeteners and colours. Links to more information on food additives including the legislation are available at: http://ec.europa.eu/food/food/EAEF/additives/framework_reg_FA_en.htm/. All authorised food additives have to fulfil purity criteria which are set out in detail in three Commission Directives (EC) No. 10/2009 (food additives other than sweeteners and colours, amending Directive 2008/84/EC), 2008/60/EC (sweeteners), and 2008/128/EC (colours). It should be
Table 6. Continued.

noted that new regulations on purity criteria were adopted on 9 March 2012 (Regulation (EU) No. 231/2012 on specifications for food additives listed in Annexes II and III) which will repeal the mentioned directives as of 1 December 2012. Food additives must be kept under continuous observation and must be re-evaluated whenever necessary in the light of changing conditions of use and new scientific information. Therefore, when the EC is informed about new scientific evidence relating to a permitted food additive, it requests EFSA to assess the new data. In addition to this ongoing observation the EC has also asked the EFSA to undertake a re-evaluation of all currently permitted food additives (Regulation (EU) No. 257/2010). Regulation (EC) No. 1333/2008 on food additives:

- The regulation, except transitional provisions, has been in application since 20 January 2010
- The regulation strengthens the principle of food safety and consumer information. It allows a more efficient and simplified procedure for authorisation of food additives by comitology. The consolidation of all food additives legislation in one single legal instrument makes legislation user-friendly for citizens and business operators. Comitology is the procedure by which the Commission prepares the draft legislation and member states vote at the Standing Committee on the Food Chain and Animal Health. If a qualified majority is in favour, it is then passed to the European Parliament and the Council, which have 2 months to object. If they do not object, it is published in the Official Journal and then it is EU law
- In accordance with Article 30 of Regulation (EC) No. 1333/2008, additives that are permitted in food under Directives 94/35/EC, 94/36/EC, and 95/2/EC and their conditions of use were entered in the Community list of food additives in Annex II to the regulation. To that end the compliance with their general and specific conditions of use was reviewed. The new EU lists amending Annexes II and III to Regulation EC 1333/2008 were adopted on 11 November 2011
- The use of food additives already permitted in Directives 94/35/EC, 94/36/EC, and 95/2/EC will continue to be permitted until the application of Annex II as amended by Regulation (EU) No. 1129/2011 (1 June 2013)

Guidance document: The Practical Guidance for Applicants was prepared to provide applicants with information that aims at facilitating the preparation and submission of applications for establishing or updating (adding, removing or changing conditions, specifications or restrictions) the Community lists. The links to other relevant documents are made in this guidance (http://ec.europa.eu/food/food/FAEF/authorisation_application_en.htm) as well as additional guidance documents (EFSA Panel 2012)

Approval process for a new substances: Applicants who wish to introduce new additives into the EU market, or seeking to revise existing provisions regulating individual additives already authorised within the EU, or seeking confirmation that an already approved additive made from a new source or by a new method of production is acceptable, must submit an application. Beside the Practical Guidance for Applicants there is also the Guidance on Submissions for Food Additive Evaluations by the SCF which provides details on the administrative and technical data required, and the range of toxicological tests generally required for new food additives, and on the format for formal submissions on additives. It must be noted that EFSA is preparing a new guidance document, which will replace the old one. The requirements for the application are also mentioned in Regulation (EU) 234/2011, which implements Regulation (EC) No. 1331/2008. The general requirements consist of:

- Administrative data
- Risk assessment data:
  - Identity of the substance
  - Information on particle size
  - Presence of impurities
  - Microbiological characteristics
  - Proposed chemical and microbiological specifications
  - Manufacturing process
  - Methods of analysis in foods
  - Reaction and fate in food
  - Case of need and proposed uses
  - Exposure
  - Additives produced by microbiological processes
  - Additives produced from genetically modified organisms

(continued)
Table 6. Continued.

- Information on national authorisations
- Proposed normal and maximum use levels
  - Toxicological data:
    - General framework for the toxicological evaluation of food additives
    - Study protocols
    - Toxicological section of the dossier (core studies and other studies)
    - Data reporting
    - Review of results and conclusions
  - Risk management data:
    - Function and technological need
    - Investigations on the efficacy
    - Advantages and benefits for the consumer
    - Information why the use would not mislead the consumer
    - Compliance with specific conditions for sweeteners and colours

Food contact substances (e.g. components of packaging materials)

**Definition:** Food contact materials and articles are those which in their finished state are intended to be brought into contact with food, or are already brought into contact with food and intended for that purpose or can reasonably be expected to be brought into contact with food or to transfer their constituents to food under normal and foreseeable conditions of use. This includes packaging materials but also cutlery, dishes, processing machines, containers etc. The term also includes materials and articles that are in contact with water intended for human consumption but it does not cover fixed public or private water supply equipment.

**Regulation:** Framework Regulation (EC) No. 1935/2004. Food contact materials should be safe and should not transfer their components into the foodstuff in unacceptable quantities. The transfer of constituents from food contact materials into food is referred to as migration. In the context of the framework Regulation, specific Regulations on plastics (10/2011/EU), Active and intelligent (A&I) FCM substances (450/2009/EC), Recycling of plastics (282/2008/EC), Regenerate cellulose (Directive 2007/42/EC), ceramics (Directive 84/500/EEC) have been published. The specific Regulation on plastic FCM contains a positive list of monomers and additives, which can be used for their manufacture. The EU Food Contact Materials Database lists all approvals and conditions of use from the above Regulations and Directives. It is available at: https://webgate.ec.europa.eu/sanco_foods/main/?event=display/. To ensure the protection of the health of the consumer and to avoid any contamination of the foodstuff, two types of migration limits have been established for plastic materials:
- An overall migration limit (OML) of 60 mg (of substances) kg\(^{-1}\) (of foodstuff or food simulants) that applies to all substances that can migrate from food contact materials to foodstuffs
- A specific migration limit (SML) applies to individual authorised substances and is fixed on the basis of the toxicological evaluation of the substance. The SML is generally established according to the acceptable daily intake (ADI) or the tolerable daily intake (TDI) set by the SCF in the past and by EFSA since 2003. To set the limit, it is assumed that every day throughout his/her lifetime, a person weighing 60 kg eats 1 kg of food packed in plastics containing the relevant substance at the maximum permitted quantity

**Guidance document:** The guidance document on the submission of a food contact material for evaluation by EFSA by the Panel on Additives, Flavourings, Processing Aids, and Materials in Contact with Food is available at: http://www.efsa.europa.eu/en/scdocs/scdoc/21r.htm

**Approval process for a new substances:** General requirements include:
- Identity of the substance
- Physical and chemical properties of the substance
- Intended use of the substance
- Authorisation of the substance (authorisation for use of the substance in EU member states and other countries)
- Migration data on the substance
- Data on the residual content of the substance in the food contact material
- Microbiological properties of the substance
- Toxicological data
- General requirements
- Core tests (not all types of studies may be applicable for the substance of interest – all tests should be carried out according to EU or OECD guidelines, and including good laboratory practice)

(continued)
Table 6. Continued.

- Three mutagenicity studies in vitro
- 90-day oral toxicity studies, normally in two species
- Studies on absorption, distribution, metabolism and excretion
- Reproduction studies in one species
- Developmental toxicity studies, normally in two species
- Long-term toxicity/carcinogenicity, normally in two species
- Additional studies/special investigations may be required if prior knowledge, or structural considerations indicate that other biological effects such as peroxisomal proliferation, neurotoxicity, immunotoxicity or endocrinological events may occur
- Dermal or inhalation sensitisation studies, if applicable
- Depending on the chemical nature of the substance to be used in food contact materials, the list of required tests mentioned above may be modified
- Additional details available in the guidance document

Flavouring agents

**Definition:** Flavouring substances are defined chemical substances that include flavouring substances obtained by chemical synthesis or isolated using chemical processes, and natural flavouring substances. Flavourings are used to improve or modify the odour and/or taste of foods for the benefit of the consumer. Flavourings and food ingredients with flavouring properties should only be used if they fulfil the criteria specified in Regulation (EC) No. 1334/2008. They must be safe when used, and certain flavourings should therefore undergo a risk assessment before they can be permitted in food.

**Regulation:** New Regulation (EC) No. 1334 was adopted on 16 December 2008; however, as of 20 January 2011, this new regulation repeals Directives 88/388/EEC and 91/71/EEC. In order to protect human health, this Regulation should cover flavourings, source materials for flavourings and foods containing flavourings. It should also cover certain food ingredients with flavouring properties which are added to food for the main purpose of adding flavour and which contribute significantly to the presence in food of certain naturally occurring undesirable substances (hereinafter referred to as food ingredients with flavouring properties), their source material and foods containing them. The Regulation sets out flavourings and source materials for which an evaluation and approval is required. The Regulation prohibits the addition of certain substances as such to food and sets maximum levels for certain substances, which are naturally present in flavourings and in food ingredients with flavouring properties, but which may raise concern for human health. As of 20 January 2010, Regulation (EC) No. 1334/2008 on flavouring and certain food ingredients with flavouring properties amended the following: Council Regulation (EEC) No. 1601/91, Regulations (EC) No. 2232/96 and (EC) No. 110/2008, and Directive 2000/13/EC; however, Regulation (EC) No. 2232/96, laying down a Community procedure for flavouring substances, will continue to apply until the date of application of the Union list of flavourings and source materials. Smokey flavourings are regulated under Regulation (EC) No. 2065/2003.

**Guidance document:** The guidance document is available at: [http://ec.europa.eu/food/food/faEF/authorisation_application_en.htm](http://ec.europa.eu/food/food/faEF/authorisation_application_en.htm)

**Approval process for a new substances:** After the completion of the evaluation programme but at the latest by 31 December 2010, the EU list of flavourings substances for use in or on foods in the EU shall be adopted [Article 5 (1) of Regulation (EC) No. 2232/96]. New substances follow the authorisation procedure laid down in Regulation (EC) No. 1331/2008 on the common authorisation procedure for food additives, food enzymes and food flavourings. Data requirements for flavouring substance application include:
- Manufacturing process
- Specifications
- Data on dietary and non-dietary sources
- Assessment of dietary exposure
- Assessment of the genotoxic potential of the flavouring substance
- Examination for structural/metabolic similarity to flavouring substances in an existing flavouring group evaluation (if applicable)

Other requirements are specified for other categories of flavouring substances (see the guidance document for full details).

Enzymes

**Definition:** A food enzyme is defined as a product obtained from plants, animals or microorganisms or products thereof including a product obtained by a fermentation process using microorganism containing one or more enzymes capable of catalysing a specific biochemical reaction and added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.

*(continued)*
A food enzyme preparation is defined as a formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardisation, dilution or dissolution (see Article 3 of Regulation (EC) No. 1332/2008). Regulation (EC) No. 1332/2008 does not include food enzymes used in the production of food additives falling within the scope of Regulation (EC) No. 1333/2008 or processing aids. The scope of this Regulation does not extend to enzymes that are not added to food to perform a technological function but are intended for human consumption, such as enzymes for nutritional or digestive purposes.


**Approval process for a new substances:** Food enzymes shall be subject to safety evaluation by EFSA and approval via an EU list. A 2-year period has been fixed in this Regulation for submission of applications on existing enzymes and new enzymes. This period started on 11 September. The inclusion of a food enzyme in the EU list will be considered by the Commission on the basis of Article 6 of Regulation (EC) No. 1332/2008, namely the opinion from EFSA, and also other general criteria such as technological need and consumer aspects. For every food enzyme included in the positive list, specifications including the purity criteria and the origin of the food enzyme shall be laid down. Data required for risk assessment and for risk management of food enzymes are laid down in Regulation (EC) No. 234/2011

**Processing aids**

**Definition:** According to Article 3.2(b) of Regulation (EC) No. 1333/2008 a processing aid shall mean any substance which is not consumed as a food by itself, is intentionally used in the processing of raw materials, foods or their ingredients, to fulfil a certain technological purpose during treatment or processing; and may result in the unintentional but technically unavoidable presence in the final product of residues of the substance or its derivatives provided they do not present any health risk and do not have any technological effect on the final product.

**Regulation:** Processing aids are not harmonised at EU level with the exception of food enzymes used as processing aids (see above “enzymes”) and extraction solvents used in the production of foodstuffs and food ingredients (Directive 2009/32/EC). If a processing aid does not meet the criteria outlined in the definition (above), it can be regulated as a food additive, and the applicable Regulation (EC) No. 1333/2008 (regulations for food additive) would be applicable.

**Guidance document:** Refer to direct food additives.  

**Approval process for a new substances:** There is no approval process for processing aids at the EU level.

**Nanoscale materials**

**Definition:** The Commission has adopted a Recommendation for a definition of “nanomaterial” for regulatory purposes which it intends to integrate progressively and where necessary in the EU food law (http://europa.eu/rapid/pressReleasesAction.do?reference=IP/11/1202&format=HTML&aged=0&language=EN&guiLanguage=en) (Stamm et al. 2012).

**Regulation:** Existing legislation covers in principle the potential health, safety and environmental risks in relation to nanomaterials. Recently, specific provisions on the risk assessment of nanomaterials were introduced in EU legislation on food additives and food contact materials (EC 2009). A definition of “engineered nanomaterial” and a mandatory
labelling requirement for all food ingredients containing such nanomaterials were introduced in the Regulation on Food Information to consumers. For example, food additives that are prepared through nanotechnology would be considered as new additives. In Article 12 of 1333/2008/EC, “when a food additive is already included in a Community list and there is a significant change in its production methods or in the starting materials used, or there is a change in particle size, for example through nanotechnology, the food additive prepared by those new methods or materials shall be considered as a different additive and a new entry in the Community lists or a change in the specifications shall be required before it can be placed on the market”

**Guidance document:** In November 2009, the EC asked EFSA to prepare a guidance document on how to assess potential risks related to certain food-related uses of nanotechnology. Given the knowledge which is currently available, the guidance to be developed will provide practical recommendations on how to assess applications from industry to use engineered nanomaterials in food additives, enzymes, flavourings, food contact materials, novel foods, food supplements, feed additives, and pesticides. The proposed guidance document was published and comments requested. This guidance document was published in May 2011 (http://www.efsa.europa.eu/en/efsajournal/doc/2140.pdf)

**Approval process for a new substances:** No authoritative statement found

**Efforts towards developing standards and regulations:** The EC has given the Finnish Institute of Occupational Health (FIOH), and Finland as a country, the opportunity to coordinate the EU-funded NanoSafety Cluster, which is a cluster of projects promoting nanomaterial safety:

- This project includes all nanosafety-related areas, such as toxicology, ecotoxicology, exposure assessment, risk management and standardisation
- The objective of NanoSafety Cluster is to standardise and harmonise nanotoxicology research and research methods
- British Standards Institute (BSI):
- National committee NTI/1 on Nanotechnologies Safety of the Nano-Materials Interdisciplinary Research Centre (SnIRC)
- Develop internationally agreed *in vivo* and *in vitro* protocols and models for investigating the routes of exposure, bioaccumulation and toxicology of nanoparticles in humans and non-human organisms

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**Table 7. Regulatory framework of chemicals added to food in Japan.**

<table>
<thead>
<tr>
<th>Regulatory authority</th>
<th>Name: Ministry of Health, Labour and Welfare (MHLW)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Website:</strong> <a href="http://www.mhlw.go.jp/english/index.html">http://www.mhlw.go.jp/english/index.html</a> (MHLW 2011a)</td>
<td></td>
</tr>
</tbody>
</table>

**Historical overview:**
- In 1947, then MHLW enacted the Food Sanitation Law as the first comprehensive law for food safety/hygiene and introduced a positive list system for food additives. Under the system, only additives designated as safe by the MHLW may be used in foods
- Since 1947, all food additives have been regulated by this law; however, the designation system had been applied only to chemically synthesised additives until 1995 when the Food Sanitation Law was amended
- Currently, all types of additives are equally subject to the designation system, synthetic or non-synthetic with minor exceptions

**Role/responsibility:**
- To protect the health of the public through the strengthening measures for the assurance of food safety
- Responsibilities include the regulation of the manufacture, import, and sale of food, food additives, food apparatus, containers/packages, and the provision of related information to consumers and businesses (MHLW 2011c).
- The administration is under the jurisdiction of the Department of Food Safety under the Pharmaceutical and Food Safety Bureau

(continued)
Table 7. Continued.

Advisory scientific body  
Name: Food Safety Commission (FSC)  
Website: http://www.fsc.go.jp/english/index.html  

Historical overview: The FSC was established on 1 July 2003, as a part of Japanese Cabinet Office, following the founding regulation of Food Safety Basic Law  

Role/responsibility: The FSC’s core role is risk assessment of food-associated hazards that are potentially contained in food including chemical substances (e.g. food additives, pesticides and veterinary medicines), biological (e.g. microorganisms and natural toxins), and novel foods. The FSC also emphasises risk communication on food-related hazards to the general public and also provides emergency response in the case of food poisoning outbreaks  

Framework regulations  
- The food safety administrative work is based on the Food Safety Basic Law (enacted in May 2003) and related laws including the Food Sanitation Law, the Abattoir Law, and the Poultry Slaughtering Business Control and Poultry Inspection Law  
- Other related laws include the Law of Temporary Measures for Enhancing the Control Method of the Food Production Process and the Health Promotion Law  
- The Food Sanitation Law (JETRO 2006) covers various responsibilities, such as the establishment of standards/specifications for food, additives, apparatus, and food containers/packages, and inspection to determine whether these established standards are met  

Part of an overarching international organisation  
- Japan has been a member of the World Trade Organization since 1995  
- Japan is a member of the Codex Alimentarius Commission  

Recent and/or pending changes  
- The MHLW has deleted 80 substances from the list of existing food additives, as there are currently no uses in the Japanese market (WTO 2010). The list is available at: http://members.wto.org/crnattachments/2010/sps/JPN/10_2581_00_e.pdf (USDA 2010c)  
- The use of those substances as an additive is prohibited in foods produced in or exported to Japan. It should be noted that although a substance withdrawn from the list is prohibited for use as a food additive, it does not mean that the substance is prohibited for use as a food ingredient, e.g. as a health food  

Regulatory overview of specific food chemical groups  

Food ingredients  
Definition: Foods (including food ingredients) are defined as all foods and drinks; provided, however, this term does not include drugs and quasi-drugs in the Pharmaceutical Affairs Law (Article 4, Food Sanitation Law) (JETRO 2006).  

Novel foods: Novel foods are not defined under Japanese jurisdiction. There are no specific approval procedures for new food or food ingredients. The FSC conducts safety assessment for novel foods that are derived from a genetically modified organism (GMO). The MHLW may prohibit the sale of a food, which could possibly injure human health according to the opinion of the Pharmaceutical Affairs and Food Sanitation Council (Article 7 of the Food Sanitation law). This includes:  
- food that has never before generally served for human consumption;  
- any article containing the same is, or is to be newly sold as food; and  
- any article served for consumption in a different recipe from the ordinary way of consumption  

Regulation: No authoritative statement found  
Guidance document: No authoritative guidance document found  
Approval process for a new substances: No authoritative statement found  

Direct food additives  
Definition:  
- According to Food Sanitation Law under Article 4 (JETRO 2006), a food additive is defined as a substance used in or on food in the process of manufacturing food or substances used for the purpose of processing or preserving food  
- Consequently, an “additive” includes both substances remaining in the finished food products, such as food colours and preservatives, and substances not remaining in the finished products, such as infiltration-supporting agents  

(continued)
Food additives include sweeteners and food colouring agents.

For food additives produced by recombinant DNA techniques, a safety assessment is mandatory according to the Food Sanitation Act as of 2001. The “Standards for the Safety Assessment of Genetically Modified Foods (Seed Plants)” (2004) is available in English from the FSC.

Regulation:
- Food Sanitation Law (JETRO 2006) (the translated document is available at: http://www.japaneselawtranslation.go.jp/law/detail/?id=12&m=04&re=02) (Ministry of Justice 2009)

There are currently 345 designated food additives approved by the MHLW under Article 10 of the Food Sanitation Law (http://www.mhlw.go.jp/english/topics/foodsafety/foodadditives/index.html) (MHLW 2011b). The MHLW has decided to start evaluating certain food additives with intent to authorise them even when there is no application from a person who wishes to use them. These food additives are those that meet the two standards given below:
- for which safety assessments have been conducted by the JECFA and whose safety has been confirmed within a certain level; and
- that are widely used in the US and EU countries and whose need is considered to be high

This decision was made from the viewpoint of international harmonisation for substances that are internationally proven safe and widely used in the world.

Establishment of specifications and standards: Usually, people continue to consume food additives throughout their lifetime; thus, food additives must be subjected to stringent regulations. All designated chemicals, with a few exceptions, and some natural additives (existing food additives) are currently regulated by specifications and/or standards. These specifications and standards include specifications concerning chemical and physical characteristics, and standards for manufacturing, storage, and use. These standards, along with labelling and storage standards are published in an official compilation of food additives, entitled “Japan’s Specifications and Standards for use of Food Additives” (MHLW 2000).


Approval process for a new substances: The procedures required to apply for designation of a new substance intended to be used as a food additive pursuant to Article 6 of the Food Sanitation Law or for revision of standards for use of a food additive pursuant to Article 7, paragraph 1 of the Food Sanitation Law are provided within the guidance document.

A checklist is provided in the last page of the guidance document, which outlines the requirements. It should be noted that all documents should be completed in Japanese; however, all documents except for the summary may be submitted in English. A brief summary of the general requirements include:

- Summary
- Chronology on origin or development and overseas use conditions
- Physicochemical characteristics and specifications:
  - Name
  - Structural formula and rational formula
  - Molecule formula and formula weight
  - Assay
  - Manufacturing methods
  - Description
  - Identification tests
  - Specific properties
  - Purity tests
  - Loss on drying, loss on ignition or water
  - Residues on ignition
  - Method of assay
  - Stability
  - Analytical method for the food additives in foods
  - Principles to establish proposed specifications

- Effectiveness:
  - Effectiveness and comparison in effect with other similar food additives
  - Stability in foods
  - Effects on nutrients of foods

(continued)
Table 7. Continued.

- Safety information:
  - Toxicity studies
  - 28-day toxicity study
  - 90-day toxicity study
  - One-year toxicity study
  - Reproduction study
  - Teratogenicity study
  - Carcinogenicity study
  - Combined chronic toxicity/carcinogenicity study
  - Antigenicity study
  - Mutagenicity study
  - General pharmacological study
  - Metabolism/pharmacokinetic studies
  - Daily intake of food additives

- Proposed standards for use
  - It should be noted that not all of the above requirements are needed if applying for revision of standards for use of a food additive

Food contact materials (e.g. components of packaging materials)

**Definition:** No authoritative statement found

**Regulation:** No regulations are available on food contact materials. In 1973, the Japan Hygienic Olefin and Styrene Plastics Association (JHOSPA) established the industry’s voluntary standards composed of a positive list describing raw materials that can be used safely for food utensils, containers, packaging materials, and the Standard Methods of Analysis that defined specifications for each resin. JHOSPA aims to carry out activities to prevent sanitary hazards caused by food utensils, containers, and packaging materials

**Guidance document:** No authoritative guidance document found

**Approval process for a new substances:** No authoritative statement found

Flavouring agents

**Definition:** Natural flavouring agents are defined as food additives intended for use in flavouring food and are substances obtained from animals or plants, or mixtures thereof (The Food Sanitation Act, Article 4, Paragraph 3) (JETRO 2006). No specifications are established for natural flavouring agents


**Guidance document:** Refer to direct food additives (above)

**Approval process for a new substances:** Refer to direct food additives (above)

Enzymes

**Definition:** No authoritative statement found

**Regulation:** Enzymes listed as a permitted food additive can be used in food under the conditions indicated; however, for enzymes produced by genetically modified microorganisms, a safety assessment would be required under Standards for the Safety Assessment of Food (additives) Produced Using Genetically Modified Microorganisms (only available in Japanese)

**Guidance document:** No authoritative guidance document found

**Approval process for a new substances:** No authoritative statement found

Processing aids

**Definition:** Processing aids defined under labelling standards according to the Food Sanitation Law (JETRO 2006) are substances added to a food in processing the food which are (1) removed from the food before the completion of the food; (2) derived from raw materials of the food and converted into components normally included in the food but do not significantly increase the amounts of the components; or (3) present in the finished food at insignificant levels but do not have any technical or functional effect of these components on the food

**Regulation:** Processing aids are regulated under the same process as food additives. Processing aids that are listed on the permitted list of food additives are permitted for use in foods (Government of Japan 2002)

**Guidance document:** Refer to direct food additives (above)

(continued)
Nanoscale materials

| Definition: | No authoritative statement found |
| Regulation: | No authoritative statement found |
| Guidance document: | No authoritative guidance document found |

Approval process for a new substances: No authoritative statement found

Efforts towards developing standards and regulations (Locascio et al. 2011): The National Institute of Advanced Industrial Science and Technology (AIST) has sponsored several projects at the Ministry of Economy Trade and Industry (METI) since 2005 (http://www.aist.go.jp/):

- Standardisation of Nanoparticle Risk Evaluation Method with the objective to develop methods for the characterisation of nanoparticles, develop methods for assessment of health impact and safety of nanoparticles, and develop systems for data collection and data standardisation
- Risk assessment of manufactured nanomaterials

Table 8. Regulatory framework of chemicals added to food in Mexico.

<table>
<thead>
<tr>
<th>Regulatory authority</th>
<th>Name: Ministry of Health (SSA) through the Federal Commission for the Protection against Sanitary Risks (COFEPRIS 2010)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Website: <a href="http://www.cofepris.gob.mx">http://www.cofepris.gob.mx</a></td>
</tr>
</tbody>
</table>

**Historical overview:** COFEPRIS was established 5 July 2001 with the publication of the “Decreto de Creación de la Comisión Federal para la Protección contra Riesgos Sanitarios (COFEPRIS)” in the “Diario Oficial de la Federación”. This decree established the structure and organisation of a decentralised administrative office with technical, administrative and operative autonomy responsible for the implementation of its legal attributes as related to sanitary regulation, control and support as stated in the General Health Law and other applicable regulations. This new Commission is composed of different General Directions that include: The General Direction of Drugs and Health Technologies, Sanitary Control of Products and Services, Environmental Health, The National Public Health Laboratory and the Direction of Sanitary Control of Publicity.

**Role/responsibility:** According to the General Health Law, the Ministry of Health will enforce its legal attributes as related to sanitary regulation, control and support through the Federal Commission for the Protection Against Sanitary Risks as related to (Art. 17 bis):

- The control and vigilance of health establishments
- The prevention and control of adverse environmental health effects as related to human health
- Occupational health and safety
- The sanitary control of products and services including importation, exportation and of the establishments that process such products
- The sanitary control of the process, use, maintenance, importation, exportation and final disposition of medical instruments, prosthetics, functional aids, diagnostic agents, odontological supplies, surgical materials, hygienic products and the establishments where these are processed
- The sanitary control of publicity of activities, products and services
- The sanitary control of the disposition of human organs, tissue and their components as well as cell cultures
- International sanitary controls
- The sanitary control of human organ, tissue and cell donations

<table>
<thead>
<tr>
<th>Advisory scientific body</th>
<th>Name: See “regulatory authority” (above)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Website: See “regulatory authority” (above)</td>
</tr>
</tbody>
</table>

**Historical overview:** See “regulatory authority” (above)

**Role/responsibility:** See “regulatory authority” (above)
Table 8. Continued.

<table>
<thead>
<tr>
<th>Framework regulations</th>
<th>For foods the following regulations apply:</th>
</tr>
</thead>
<tbody>
<tr>
<td>General Health Law (Ley General de Salud)</td>
<td></td>
</tr>
<tr>
<td>The Regulation on Sanitary Control of Products and Services (Reglamento de Control Sanitario de Productos y Servicios)</td>
<td></td>
</tr>
<tr>
<td>An agreement that establishes the substances allowed as additives and processing aids in foods, beverages and nutritional supplements (ACUERDO por el que se determinan las sustancias permitidas como aditivos y coadyuvantes en alimentos, bebidas y suplementos alimenticios) (Secretaría de Salud México 1999)</td>
<td></td>
</tr>
<tr>
<td>Applicable Official Mexican Norms (Normas Oficiales Mexicanas)</td>
<td></td>
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</table>

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<thead>
<tr>
<th>Part of an overarching international organisation</th>
<th>Mexico has been a member of the World Trade Organization since 1995</th>
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<tbody>
<tr>
<td>Mexico is a member of the Codex Alimentarius Commission</td>
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<table>
<thead>
<tr>
<th>Recent and/or pending changes</th>
<th>Pending (since 2007–2008) update and publication of a new Food Additive Positive List</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recently implemented (June 2011) labelling regulation NOM-051-SSA1-2010 mandates nutritional labelling for foods and non-alcoholic beverages as well as quantitative ingredient declaration</td>
<td></td>
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<tr>
<td>Upcoming review and publication of NOM-086-SSA1-1994 Foods with Modified Composition to homologate it with the new labelling regulation</td>
<td></td>
</tr>
<tr>
<td>Upcoming publication (2012) of a new food additive and processing aid positive list that will include limits and approved applications for each compound</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Regulatory overview of specific food chemical groups</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Food ingredients</td>
<td>Definition of a food ingredient: Any substance or product including the additives that is used in the manufacture, elaboration, preparation or treatment of a food or non-alcoholic beverage and that is present in the final product transformed or not. There are no regulations pertaining to new food ingredients or novel foods</td>
</tr>
<tr>
<td></td>
<td>Regulation: No authoritative statement found</td>
</tr>
<tr>
<td></td>
<td>Guidance document: No authoritative guidance document found</td>
</tr>
<tr>
<td></td>
<td>Approval process for new substances: No authoritative statement found</td>
</tr>
</tbody>
</table>

Definition: Food additives are defined as those substances added directly to food and drinks during its manufacture in order to provide or intensify aroma, colour or flavour, to improve its stability or its preservation. The term does not include contaminants, substances added to foods to maintain or to improve the nutritional quality, or sodium chloride. Additives always perform a technological function in the final product (USDA 2009)

Regulation:
- General Health Law
- The Regulation on Sanitary Control of Products and Services (Reglamento de Control Sanitario de Productos y Servicios) contains some specific limits for additives in specific products
- An agreement that establishes the substances allowed as additives and processing aids in foods, beverages and nutritional supplements (ACUERDO por el que se determinan las sustancias permitidas como aditivos y coadyuvantes en alimentos, bebidas y suplementos alimenticios). This is only a positive list and does not specify limits or specific applications. However, the new 2012 updated list includes limits and specific applications and will list processing aids separately
- Applicable Official Mexican Norms (Normas Oficiales Mexicanas) (NOM) may establish limits and applications
- Authorised food additives including colorants and sweeteners provided in English are available in the GAIN report MX6058 (http://www.fas.usda.gov/gainfiles/200607/14208414.pdf) (USDA 2006a)
- The authorised additives must follow the established specifications in the provisions included in the Appendix Chapter VIII of the “Regulations for the Sanitary Control of Goods and Services”, which are provided on the SSA's website (http://www.ssa.gob.mx)
- In addition, the list of authorised purified substances, enzymes, and synthetic flavours provided in English are available in the GAIN report MX6070 (USDA 2006b)
- The list of permitted food additives is not available in English
- It is important to consider all levels of regulations. The major challenge is that the documents are not all updated so limits and substances listed in specific NOMs may not be updated with the most recently approved additives

(continued)
### Table 8. Continued.

**Guidance document**: Agreement that establishes the substances allowed as additives and processing aids in foods, beverages and nutritional supplements

**Approval process for a new substances**: Through technical consultation presenting a technical dossier to request approval and inclusion in the positive list. After an official approval is obtained, it is important to follow up to ensure that the substance is included in the next update of the positive list (agreement) and any updated applicable NOMs.

<table>
<thead>
<tr>
<th>Category</th>
<th>Definition</th>
<th>Regulation</th>
<th>Guidance document</th>
<th>Approval process for a new substances</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food contact substances (e.g. components of packaging materials)</td>
<td>No authoritative statement found</td>
<td>The Regulation on Sanitary Control of Products and Services (Reglamento de Control Sanitario de Productos y Servicios – 24th Title)</td>
<td>No authoritative guidance document found Voluntary Mexican Norms (NMX) may be available for specific products</td>
<td>No specific process. Any approval should be obtained through technical consultation. Although there is no specific, formal process, most major customers will require legal confirmation that a specific compound is allowed. If a company wants to be sure that the product is allowed, then they should consult with the authorities</td>
</tr>
<tr>
<td>Flavouring agents</td>
<td>Substance or blend of substances of natural origin, identical to natural or synthetic with or without solvents and with or without the addition of other additives that are used to give or intensify flavours or aromas to products</td>
<td>Same as food additives</td>
<td>Same as food additives</td>
<td>Same as food additives. Usually when the positive list is updated, the FEMA-GRAS lists are reviewed for inclusion</td>
</tr>
<tr>
<td>Enzymes</td>
<td>Biological catalyser protein substance produced by live cells that catalyse specific reactions in diverse production processes. Source: The Regulation on Sanitary Control of Products and Services</td>
<td>Same as food additives</td>
<td>Same as food additives</td>
<td>Same as food additives</td>
</tr>
<tr>
<td>Processing aids</td>
<td>Substance or material, excluding instruments, utensils and additives that is not consumed as a food ingredient by itself and is used intentionally in the production of raw materials, foods or their ingredients to achieve a technological function during the treatment or processing and that can lead to the unintentional presence of residues or derivatives in the final product. Colour aid is the substance that is used to intensify, retain or develop colour</td>
<td>Same as food additives</td>
<td>Same as food additives</td>
<td>Same as food additives</td>
</tr>
<tr>
<td>Nanoscale materials</td>
<td>No authoritative statement found</td>
<td>No authoritative statement found</td>
<td>No authoritative guidance document found</td>
<td>No authoritative statement found Any specific approval should be obtained through technical consultation</td>
</tr>
</tbody>
</table>

**Efforts towards developing standards and regulations**: None known
Table 9. Regulatory framework of chemicals added to food in the USA.

<table>
<thead>
<tr>
<th>Regulatory authority</th>
<th>Name: US Food and Drug Administration (USFDA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Website: <a href="http://www.fda.gov/Food/FoodIngredientsPackaging/default.htm">http://www.fda.gov/Food/FoodIngredientsPackaging/default.htm</a></td>
</tr>
<tr>
<td><strong>Historical overview:</strong></td>
<td>- Began as the Bureau of Chemistry, as part of the US Department of Agriculture in 1862  &lt;br&gt;  - The Food and Drugs Act was passed in 1906  &lt;br&gt;  - Bureau of Chemistry is split into the Food Drug and Insecticide Administration and the Bureau of Chemistry and Soils in 1927  &lt;br&gt;  - Food Drug and Insecticide Administration is renamed the Food and Drug Administration in 1930  &lt;br&gt;  - The Federal Food Drug and Cosmetic Act was passed in 1938  &lt;br&gt;  - The Food Additives Amendment of 1958 was passed in 1958  &lt;br&gt;  - <a href="http://www.fda.gov/AboutFDA/WhatWeDo/History/Milestones/ucm081229.htm">http://www.fda.gov/AboutFDA/WhatWeDo/History/Milestones/ucm081229.htm</a></td>
</tr>
<tr>
<td><strong>Role/responsibility:</strong></td>
<td>- Protect the public health by ensuring that foods are safe, wholesome, sanitary, and properly labelled  &lt;br&gt;  - Promote the public health by promptly and efficiently reviewing clinical research and taking appropriate action on the marketing of regulated products in a timely manner  &lt;br&gt;  - Participate through appropriate processes with representatives of other countries to reduce the burden of regulation, harmonise regulatory requirements, and achieve appropriate reciprocal arrangements  &lt;br&gt;  - Where appropriate, carry out its mission in consultation with experts in science, medicine, and public health, and in cooperation with consumers, users, manufacturers, importers, packers, distributors, and retailers of regulated products</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Advisory Scientific body</th>
<th>Name: USFDA Science Board and USFDA Food Advisory Committee</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Website: &lt;br&gt;  - Science Board to the US Food and Drug Administration (Science Board): <a href="http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/ScienceBoardtotheFoodandDrugAdministration/default.htm">http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/ScienceBoardtotheFoodandDrugAdministration/default.htm</a>  &lt;br&gt;  - Food Advisory Committee: <a href="http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/FoodAdvisoryCommittee/default.htm">http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/FoodAdvisoryCommittee/default.htm</a></td>
</tr>
<tr>
<td><strong>Historical overview:</strong></td>
<td>Both the Science Board and the Food Advisory Committee were authorised by the Federal Advisory Committee Act (Pub. L. 92-463) passed in 1972. The Science Board was established on 26 June 1992. The Food Advisory Committee was established on 15 December 1991 (21 CFR §14.100)</td>
</tr>
<tr>
<td><strong>Role/responsibility:</strong></td>
<td>- Science Board: Provides advice to the Commissioner and other appropriate officials on a wide variety of food and drug related issues including:  &lt;br&gt;  - specific complex scientific and technical issues important to the agency and its mission  &lt;br&gt;  - emerging issues within the scientific community  &lt;br&gt;  - keeping pace with technical and scientific developments, including in regulatory science  &lt;br&gt;  - agency research  &lt;br&gt;  - upgrading the agency’s scientific and research facilities and training opportunities  &lt;br&gt;  As of 2012, the Board is made up of a committee with a core of 21 voting members knowledgeable in fields including food science, safety, and nutrition; chemistry; pharmacology; toxicology; public health and epidemiology; international health and regulation; and nanotechnology.  &lt;br&gt;  - Food Advisory Committee: Provides advice to the Commissioner and other appropriate officials on emerging food safety, food science, nutrition, and other food-related health issues and may be tasked with making recommendations on matters including:  &lt;br&gt;  - broad scientific and technical food or cosmetic related issues  &lt;br&gt;  - the safety of new foods and food ingredients  &lt;br&gt;  - labelling of foods  &lt;br&gt;  - nutrient needs and nutritional adequacy and  &lt;br&gt;  - safe exposure limits for food contaminants  &lt;br&gt;  As of 2012, the Committee consists of 17 standing members knowledgeable in the fields of physical sciences, biological and life sciences, food science, risk assessment, nutrition, food technology, molecular biology, and other relevant scientific and technical disciplines</td>
</tr>
</tbody>
</table>

(continued)
Table 9. Continued.

|------------------------|----------------------------------------------------------------------------------------------------------------|

**Part of an overarching international organisation**
- The US has been a member of the Codex Alimentarius Commission since it was established in 1963 (http://www.codexalimentarius.org/members-observers/members/en/?no_cache=1)
- The US also has been a member of the World Trade Organization since 1995 (http://www.wto.org/english/tratop_e/whatis_e/tif_e/org6_e.htm)
- http://www.fda.gov/InternationalPrograms/HarmonizationInitiatives/default.htm

**Recent and/or pending changes**
The Food Safety Modernization Act was signed into law on 4 January 2011. Changes include requiring food facilities to document in writing that all substances at the facility that are intended to be added to food are allowed by the food additive regulatory programme (http://www.fda.gov/food/foodsafety/fsma/default.htm)

**Regulatory overview of specific food chemical groups**

The US does not define the term “food ingredient”. Instead, it defines food. Food means, “(1) articles used for food or drink for man or other animals, (2) chewing gum, and (3) articles used for components of any such article” (21 USC §321(f)). Food includes human food, pet food, animal feed and substances migrating to food from food contact articles (21 CFR §170.3(m)). Any substance that is reasonably expected to become a component of food is a food additive that is subject to premarket approval by the USFDA, unless the substance is generally recognised as safe (GRAS) among experts qualified by scientific training and experience to evaluate its safety under the conditions of its intended use, or meets one of the other exclusions from the food additive definition in section 201(s) of the Federal Food, Drug, and Cosmetic Act (FDCA).

**Novel foods**: The US does not define novel foods. New substances are regulated through a variety of mechanisms described below


**Guidance document**: USFDA guidance regarding substances allowed in food, including new substances is available at: http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/FoodIngredientsandPackaging/default.htm

**Approval process for a new substances**: There are three ways a food manufacturer can get clearance to add a new substance to its products:
- A manufacturer or trade association can decide on its own that a substance’s use is “generally recognised as safe”, which is commonly known as a “GRAS substance”. This determination must be based on the opinion of “experts qualified by scientific training and experience to evaluate the safety …”. Notice to the USFDA or the public of the safety decision or its use is not required
- The USFDA approves the use of a substance by issuing a new or amended regulation. The USFDA usually makes this safety determination in response to a petition by a manufacturer or its representative. It provides the public with the opportunity to comment before the chemical use is approved and a regulation is issued. Since 2000, use of this method has fallen dramatically
- A manufacturer voluntarily asks the USFDA to review its safety assessment of a chemical it wants to use in food by submitting a notification. If the agency’s review raises no concerns, the USFDA sends a letter stating that it has “no objections” or “no questions” to the manufacturer’s decision

A determination that a particular use of a substance is GRAS (unless established by common use prior to 1958) requires both technical evidence of safety and a basis to conclude that this technical evidence of safety is generally known and accepted (i.e. general recognition of safety). 21 CFR 170.30. In contrast, a determination that a particular use of a food additive is safe via premarket approval requires only technical evidence of safety

(continued)
A substance added directly to food can fall into one of several categories: food additive, prior sanctioned substance, colour additive, or the use of the substance is considered GRAS, depending on its intended use and the mechanism through which approval is sought.

**Definition:**
- A food additive is a substance “the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food (including any substance intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting or holding food; and including any source of radiation intended for any such use); if such substance is not GRAS or sanctioned prior to 1958 or otherwise excluded from the definition of food additives
- Direct food additives are a subcategory of this larger category. They are substances intentionally added directly to food whose use has been expressly approved by the USFDA, usually in response to a food additive petition from a manufacturer or manufacturer’s representative
- Prior-sanctioned substances are chemicals that were government-approved for use in food prior to 1958
- GRAS substances are substances “generally recognized among experts qualified by scientific training and experience to evaluate their safety, as having been adequately shown through scientific procedures (or, in the case of a substance used in food prior to 1 January 1958, through either scientific procedures or experience based on common use in food) to be safe under the conditions of its intended use”. GRAS substances are distinguished from food additives by the type of information that supports the GRAS determination, that it is publicly available and generally accepted by the scientific community, but should be the same quantity and quality of information that would support the safety of a food additive. USFDA notification of the GRAS determination of a chemical’s use is voluntary
- Colour additives are substances that are capable (alone or through reaction with other substances) of imparting colour when added or applied to food. Substances intended to be used solely for purposes other than colouring, that may also impart colour, do not fall within this category. The USFDA must approve all colour additives, typically in response to a colour additive petition. Colour additives cannot be GRAS

**Regulation:**
- GRAS Notifications: http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/default.htm

**Guidance documents:** http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/FoodIngredientsPackaging/default.htm#food

**Approval process for new substances:** Same as described for food ingredients above. In this case, the petitions submitted would be food or colour additive petitions. Regarding voluntary notifications, the notification submitted by a manufacturer would be known as a GRAS notification and if the agency’s review of this notification raises no concerns, the USFDA would send a letter stating that it has “no questions” regarding the manufacturer’s decision

### Food contact substances (e.g. components of packaging materials)

In the US, substances added to food indirectly may be known as indirect additives, food contact substances (FCS), FCS below the threshold of regulation, or GRAS substances depending on their use and the method of clearance for use in food. Since 2000, FCSs are the most applicable category. Food contact substances are chemicals not intended to be added directly to or have a technical effect on the food, but which may reasonably be expected to become a component of food. These include substances used in packaging, transporting or the production of food

**Regulation:**
- General: 21 CFR §170–171
- FCS notification regulation: 21 CFR 170.3(e)(3)
- FCS threshold of regulation: 21 CFR 170.39
- See also http://www.fda.gov/Food/FoodIngredientsPackaging/FoodContactSubstancesFCS/default.htm

(continued)
Table 9. Continued.

<table>
<thead>
<tr>
<th>Flavouring agents</th>
<th>Flavouring agents are regulated as both food additives and GRAS substances (see “Direct food additives” above for a description of both). Most flavouring agents in the US have been found to be GRAS by the Flavor and Extract Manufacturers Association (FEMA) expert panel, which makes safety decisions, publishes them, and submits them to the USFDA for review. <a href="http://www.femaflavour.org/gras">http://www.femaflavour.org/gras</a>. USFDA has also approved a number of flavours as food additives and GRAS substances</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Regulation:</strong> No specific regulations on flavouring agents. They are regulated as direct food additives or GRAS substances:</td>
<td></td>
</tr>
<tr>
<td>• Regulations regarding labelling of flavours: 21 CFR §101.22</td>
<td></td>
</tr>
<tr>
<td>• Lists of flavours approved in the regulations can be found at: 21 CFR §§172, 182 and 184</td>
<td></td>
</tr>
<tr>
<td>• GRAS Notifications: (<a href="http://www.fda.gov/FoodFoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/default.htm">http://www.fda.gov/FoodFoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/default.htm</a>)</td>
<td></td>
</tr>
<tr>
<td><strong>Guidance document:</strong> <a href="http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/FoodIngredientsandPackaging/default.htm">http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/FoodIngredientsandPackaging/default.htm</a></td>
<td></td>
</tr>
<tr>
<td><strong>Approval process for a new substances:</strong> Same as described for direct food additives above. Manufacturers can also seek a determination by FEMA</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Definition: There is no specific regulation governing enzymes. They would be regulated as direct or secondary direct additives, or GRAS substances depending on their intended use and the method used to allow the substances in food</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Regulation:</strong> See “Direct food additives” (above)</td>
<td></td>
</tr>
<tr>
<td><strong>Guidance document:</strong> See “Direct food additives” (above)</td>
<td></td>
</tr>
<tr>
<td><strong>Approval process for new substances:</strong> See “Direct food additives” (above)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Processing aids</th>
<th>Definition: A subcategory of direct food additives, are what are known as “secondary direct additives”. These are food additives that have a technical effect in food during processing but that are not intended to have an ongoing technical effect in the food. These are sometimes more commonly known as processing aids. Direct food additives are described above. The USFDA may also consider processing aids to be food contact substances in some cases. Food contact substances are described above</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Regulation:</strong> See “Direct food additives” and “Food contact substances” (above)</td>
<td></td>
</tr>
<tr>
<td><strong>Guidance document:</strong> See “Direct food additives” and “Food contact substances” (above)</td>
<td></td>
</tr>
<tr>
<td><strong>Approval process for new substances:</strong> See “Direct food additives” and “Food contact substances” (above)</td>
<td></td>
</tr>
</tbody>
</table>

| Nanoscale materials | Definition: There is no formal definition for “nanotechnology” or “nanoscale” at this time. However the USFDA has indicated that “In the absence of a formal definition, when considering whether a USFDA-regulated product contains nanomaterials or otherwise involves the application of nanotechnology, the USFDA will ask: (1) whether an engineered material or end product has at least one dimension in the nanoscale range (approximately 1–100 nm); or (2) whether an engineered material or end product exhibits properties or phenomena, including physical or chemical properties or biological effects, that are attributable to its dimension(s), even if these dimensions fall outside the nanoscale range, up to one micrometer”. This definition is contained in the USFDA’s Draft Guidance for Industry: Assessing the Effects of Significant Manufacturing Process Changes, Including Emerging Technologies, on the Safety and Regulatory Status of Food Ingredients and Food contact substances, Including Food Ingredients that are Colour Additives, which was issued in April 2012 (FDA 2012). |

(continued)
have their own regulatory systems in place, many of their food standards are gradually being replaced by official Mercosur standards as they are developed.

**Regulatory authorities**

Table 10 outlines the regulatory authorities and the Advisory Scientific Body/Regulatory Enforcement Agency for each of the target countries. Labelling and compositional standards for both Australia and New Zealand are developed by FSANZ; thus, these regulations discussed within this report will be identical for both countries. The EU regulations apply verbatim in all member states.

**Participation in international organisations**

All the target countries (Argentina, Australia, Brazil, Canada, China, Japan, Mexico, New Zealand, the EU and US) are members of the WTO and CAC. In addition, Argentina and Brazil follow Mercosur standards. Mercosur was established in 1991 and encompasses Argentina, Brazil, Uruguay, Paraguay and, as of 2006, Venezuela. Mercosur standards are influenced by the EU, CAC and USFDA. For further details, see the individual summary Tables 1–9. A summary of the target countries and their participation in international organisations is presented in Table 11.

**Direct food additives**

Food additives are defined and regulated among Argentina, Australia/New Zealand, Brazil, Canada, China, the EU, Japan, Mexico and the US. Although, the precise definition of a food additive differs among the target countries, in general a food additive is defined as a substance added to foods intentionally and to achieve a technological function in the final product. In each of these countries a permitted list of food additives is published and available to the public. The permitted list contains food additives, which are deemed safe for human consumption under the specified conditions of use. In all target countries except the US, if a food additive is not on the permitted list or its use is not permitted in a particular food and an applicant wants to use the food additive, the applicant must submit an application to approve its use in the respective country, following the conditions and requirements laid out by the respective authoritative body.

In Argentina and Brazil, food additives are regulated under Mercosur standards (GMC 11/06, 34/10 and 35/10). For new food additives, applicants must submit to the Comisión Nacional de Alimentos (CONAL) or Agência Nacional de Vigilância Sanitária (ANVISA) (for Argentina and Brazil, respectively), which will subsequently forward the application to Mercosur’s Sub Work Group #3. In Canada, all new food additives or changes to the permitted uses of already approved food additives under Division 16 of the Food and Drug Regulations must undergo a pre-market assessment focused on safety. Similarly, in Japan and China a safety assessment was conducted on all currently permitted food additives. In all the target countries, a pre-market application is generally required for new food additives and for changes pertaining to an existing permitted food additive (such as changing the maximum permitted level, revision of a standard of use, etc.).

In the EU, on 16 December 2008, new regulations were adopted, which include food additives (1333/2008/
Table 10. Authoritative bodies among the target countries.

<table>
<thead>
<tr>
<th>Regulatory authority</th>
<th>Argentina</th>
<th>Australia/New Zealand</th>
<th>Brazil</th>
<th>Canada</th>
<th>China</th>
<th>European Union</th>
<th>Japan</th>
<th>Mexico</th>
<th>USA</th>
</tr>
</thead>
<tbody>
<tr>
<td>National Committee of Food (CONAL)</td>
<td>Food Standards Australia New Zealand (FSANZ); New Zealand Ministry for Primary Industries (MPI) (formerly Ministry of Agriculture and Forestry – MAF)</td>
<td>National Health Surveillance Agency; Agencia Nacional de Vigilancia Sanitaria (ANVISA)</td>
<td>Same as above</td>
<td>Same as above</td>
<td>Same as above</td>
<td>Food Safety Commission</td>
<td>USFDA Science Board; USFDA Food Advisory Committee</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
EC), food enzymes (1332/2008/EC), and flavourings and food ingredients with flavouring properties (1334/2008/EC). The regulation on food additives applies to food additives used for a technological purpose in the manufacture, processing, preparation, treatment, packaging, transport or storage of food, excluding those substances used as processing aids. Under the regulation, food additives shall be subject to a safety evaluation by the EFSA and approval via a Community list. The inclusion of a food additive in the Community list is considered by the Commission on the basis of the opinion on its safety from EFSA and a demonstrated case of need. The Commission takes into account other general criteria such as technological need and consumer aspects when considering whether to include the food additive in the Community list. For every food additive included in the positive list, specifications, including the criteria on purity and the origin of the food additive, shall be laid down. In order to increase consistency in common areas of the procedural aspects of food additives’ approval, guidelines for evaluation by EFSA and decision-making by the Commission, are provided in Regulation (EC) No. 1331/2008 (adopted 16 December 2010) which establishes a common authorisation procedure for food additives, food enzymes and food flavourings. In June 2012, new guidance for the submission of food additive applications was published outlining a tiered approach, in which the extent of toxicological testing is determined by the results of initial testing, with key issues and triggers described that can result in additional required testing (EFSA Panel 2012).

In the US, substances to be added to food are subject to a premarket approval requirement unless they are exempt as outlined below. Rulis and Levitt (2009) provide an excellent detailed description of the food additive approval process in the US. The 1958 Food Additive Amendments of the Federal Food, Drug and Cosmetic Act (FFDCA) required demonstration of the safety of food additives, but also included two clauses to exempt food additives currently in use from safety assessments by making them “grandfathered” ingredients. This included food additives that had been previously sanctioned for use in foods, and food additives that were “generally recognised as safe” (GRAS) for use as food. These ingredients became known as GRAS substances and were permitted to remain on the market, although in later years subsequent reviews of the safety of many of the grandfathered GRAS substances were undertaken and the USFDA affirmed their GRAS status. In 1997, the USFDA issued a proposed rule to eliminate the GRAS affirmation petition process and replace it with a voluntary notification procedure. Thus, the USFDA no longer accepts GRAS affirmation petitions. Additional details on the history of GRAS are available on the USFDA website.

As a result of these amendments to the FFDCA, a food additive pre-market approval is not required for a new food additive or use if the use is GRAS. The use of the substance can be determined to be GRAS by experts qualified by scientific training and experience to evaluate its safety, and having been adequately shown through scientific procedures (or in the case of a substance used in food prior to 1 January 1958, through either scientific procedures or experience based on common use in food) to be safe under the conditions of intended use. The requirement for “general recognition” of safety is often satisfied through publication of the pivotal safety data in the peer-reviewed literature, and cited in the GRAS dossier or notification submission. A list of GRAS substance notifications reviewed by the USFDA and the agency response can be found in an online database maintained by the agency. In general, the USFDA’s response is one of the following: (1) the USFDA has no questions about the notifier’s conclusion of GRAS status; (2) the notice does not provide a basis for a conclusion of GRAS status; or (3) at the notifier’s request, the USFDA ceased to evaluate the notice.

Several important points about GRAS substances should be noted. Firstly, the GRAS status of a compound is based on the intended use(s) and levels of use documented in the determination dossier, which will determine the anticipated exposure and thus safety. Thus, it is the specific use, and not the substance in general, that is determined to be GRAS. Other uses of that substance in foods are not GRAS. Secondly, as USFDA notification of the GRAS determination is voluntary, there is no publicly available list of the uses of substances that have been “self-determined” to be GRAS and thus no opportunity for public scrutiny of safety

### Table 11. Participation of target countries in international organisations.

<table>
<thead>
<tr>
<th>Member of CODEX</th>
<th>Argentina</th>
<th>Australia/New Zealand</th>
<th>Brazil</th>
<th>Canada</th>
<th>China</th>
<th>European Union</th>
<th>Japan</th>
<th>Mexico</th>
<th>USA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Member of the World Trade Organization</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Additional participation in international organisations</td>
<td>–</td>
<td>Mercosur</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

Note: This table provides a summary of the participation of various target countries in international organisations.
Table 12. Comparison of the regulations on direct food additives among the target countries.

<table>
<thead>
<tr>
<th>Definition of a food additive includes:</th>
<th>Argentina</th>
<th>Australia/New Zealand</th>
<th>Brazil</th>
<th>Canada</th>
<th>China</th>
<th>European Union</th>
<th>Japan</th>
<th>Mexico</th>
<th>USA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any ingredient added to foods intentionally, without intent to nurture, in order to modify the physical, chemical, biological or sensory characteristics of foods, during its manufacture, processing, preparation, packaging, conditioning, storage, transport or during food handling: it will have, or it can be reasonably expected to have (directly or indirectly) as a result, the additive itself or its by-products become part of that food.</td>
<td>Any ingredient added to foods intentionally, without intent to nurture, in order to modify the physical, chemical, biological or sensory characteristics of foods, during its manufacture, processing, preparation, packaging, conditioning, storage, transport or during food handling: it will have, or it can be reasonably expected to have (directly or indirectly) as a result, the additive itself or its by-products become part of that food.</td>
<td>Any substance not normally consumed as a food in itself and not normally used as an ingredient of food, but which is intentionally added to a food to achieve one or more of the technological functions specified in Schedule 5. It or its by-products may remain in the food.</td>
<td>Any substance not normally consumed as a food in itself and not normally used as an ingredient of food, but which is intentionally added to a food to achieve one or more of the technological functions specified in Schedule 5. It or its by-products may remain in the food.</td>
<td>Same as for Argentina.</td>
<td>Any substance the use of which results, or may reasonably be expected to result, in it or its by-products becoming a part of or affecting the characteristics of a food. It should be noted that a substance not present in the final food but which has affected the characteristics of that food would be regulated as a food additive.</td>
<td>Any substance not normally consumed as a food in itself and not normally used as an ingredient of food, but which is intentionally added to a food to achieve one or more of the technological functions specified in Schedule 5. It or its by-products may remain in the food.</td>
<td>An artificially chemosynthetic or natural substance to be added to foods in order to improve food quality, colour, fragrance and taste, and for the purpose of preservation and processing technology.</td>
<td>Nutrition enhancers, gum-based substances in chewing gum, flavouring agents, and processing aids in the food industry are also included in food additives.</td>
<td>Substances that are not normally consumed as food itself but are added to food intentionally for a technological purpose. Preparations obtained from foods and other natural source material that are intended to have a technological effect in the final food and which are obtained by selective extraction of constituents relative to the nutritive or aromatic constituents, should be considered additives.</td>
</tr>
</tbody>
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Table 12. Continued.

<table>
<thead>
<tr>
<th></th>
<th>Argentina</th>
<th>Australia/New Zealand</th>
<th>Brazil</th>
<th>Canada</th>
<th>China</th>
<th>European Union</th>
<th>Japan</th>
<th>Mexico</th>
<th>USA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definition of a food additive does not include:</td>
<td>Contaminants or nutrients that are incorporated into a food in order to maintain or improve its nutritional properties</td>
<td>Same as for Argentina</td>
<td>Any nutritive material used, recognised or commonly sold as an article or ingredient of food; vitamins, mineral nutrients and amino acids, other than those listed in Division 16; spices, seasonings, flavouring preparations, essential oils, oleoresins and natural extractives; agricultural chemicals, other than those listed in Division 16, food packaging materials and components thereof; and drugs for administration to animals that may be food</td>
<td>Substances used for the purpose of imparting flavour and/or taste or for nutritional purposes. Substances considered as foods which may be used for a technological function, such as sodium chloride or saffron for colouring, and food enzymes</td>
<td>Contaminants, substances added to foods to maintain or to improve the nutritional quality, or sodium chloride</td>
<td>Contaminants or substances that are not intended to be added directly to food or have a technical effect, but which may reasonably be expected to become a component of food</td>
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</table>

Regulation | Mercosur regulations, GMC 11/06, GMC 34/10, and GMC 35/10 | Same as for Argentina | Same as for Argentina | Order No. 73: Measures for Administration of New Food Additives | 1333/2008/EC | Food Sanitation Law | Regulation on Sanitary Control of Products and Services | 21 CFR §§70–82, and §§170–189. Proposed rule published in 1997 (62 FR 18938) | (continued)
Table 12. Continued.

<table>
<thead>
<tr>
<th>Additional regulation details</th>
<th>Argentina</th>
<th>Australia/New Zealand</th>
<th>Brazil</th>
<th>Canada</th>
<th>China</th>
<th>European Union</th>
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<th>Mexico</th>
<th>USA</th>
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<tbody>
<tr>
<td>Permitted list of food additives according to GMC 11/06. Food additives used according to GMP (GMC 34/10). Food additives used according to maximum levels (GMC 35/10)</td>
<td>Additives can only be added to food in order to achieve an identified technological function according to GMP. Some additives have specific permissions and maximum use levels allowed in food and other additives are limited to GMP. Specific flavouring agents, sweeteners, and colouring agents are regulated as food additives</td>
<td>Same as for Argentina*</td>
<td>Permitted list of food additives</td>
<td>Permitted list of food additives</td>
<td>Community list of food additives 129/2011 and 1130/2011</td>
<td>Approved food additives list containing 411 additives (under Article 10 of Food Sanitation Law)</td>
<td>Promised list of food additives</td>
<td></td>
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<tr>
<th>Approval process</th>
<th>Argentina</th>
<th>Australia/New Zealand</th>
<th>Brazil</th>
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<th>China</th>
<th>European Union</th>
<th>Japan</th>
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<th>USA</th>
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</thead>
<tbody>
<tr>
<td>Pre-market application is required. Submission to CONAL, who will forward petition to Mercosur’s Sub Work Group #3</td>
<td>Pre-market application is required</td>
<td>Pre-market application is required for a new substance, for an extension of the use of a permitted food additive, changing the maximum level of a permitted food additive, or adding a new organism to the list of permitted sources of enzymes used as a food additive</td>
<td>Pre-market application is required for new food additives; a new food additive is an additive that is not included in the national food safety standards, not included in the public announcement of permitted use issued by the MOH, and whose scope of use or dosage is increased</td>
<td>Pre-market application is required for new food additives; a new food additive is an additive that is not included in the national food safety standards, not included in the public announcement of permitted use issued by the MOH, and whose scope of use or dosage is increased</td>
<td>Pre-market application is required (new common authorisation procedure under Regulation (EC) No. 1331/2008. Details found in Regulation (EU) 234/2011 that implements Regulation (EC) No. 1331/2008 and in Practical Guidance for Applicants. New additives, or seeking to revise existing provisions regulating individual additives already authorised, or seeking confirmation that an approved additive made from a new source or method is acceptable, must submit an application</td>
<td>Pre-market application is required for a new substance intended to be used as a food additive and for applying for revision of standards for use of a food additive</td>
<td>Pre-market application is required</td>
<td>Pre-market application is required</td>
<td>Pre-market application in the form of a food or colour additive petition. Exempt from food additive petition if prior sanctioned or use determined to be GRAS</td>
</tr>
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</table>

Note: Regulations pertaining to Argentina follow Mercosur Standards. Brazil also follows Mercosur Standards.
<table>
<thead>
<tr>
<th></th>
<th>Argentina</th>
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<th>European Union</th>
<th>Japan</th>
<th>Mexico</th>
<th>USA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Definition</strong></td>
<td>No formal definition; however, novel foods are recognised in practice</td>
<td>A non-traditional food with no history of safe use and food requires an assessment of public health and safety considerations. Non-traditional food is defined as a food that does not have a history of human consumption in Australia/New Zealand; a substance derived from a food, where that substance does not have a history of human consumption in Australia/New Zealand other than as a component of that food; or any other substance, where that substance, or the source from which it is derived, does not have a history of human consumption as a food in Australia/New Zealand</td>
<td>Foods with no history of use in the country; foods containing novel ingredients, except those listed in Table 1; foods containing substances already consumed that may be added or used at levels much higher than those currently observed in the foods that constitute part of a regular diet; and food offered in the form of capsules, pills, tablets and the like</td>
<td>(1) Substance, including a microorganism, that does not have a history of safe use as a food; (2) a food that has been manufactured, prepared, preserved or packaged by a process that has not been previously applied to that food, and causes the food to undergo a major change; and (3) a food that is derived from a plant, animal or microorganism that has been genetically modified</td>
<td>Novel foods are referred to as new resource foods, which are defined as raw food materials or food ingredients, which do not have a significant history of consumption in China</td>
<td>Novel foods are foods and food ingredients that have not been used for human consumption to a significant degree within the Community before 15 May 1997</td>
<td>Not defined</td>
<td>Not defined</td>
<td>Not defined</td>
</tr>
</tbody>
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<tr>
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</thead>
<tbody>
<tr>
<td>Regulation</td>
<td>No authoritative statement found</td>
<td>Food Standard 1.5.1.</td>
<td>Resolution No. 16</td>
<td>Division B.28</td>
<td>Order No. 56 Administrative Measures on Novel Food</td>
<td>258/97/EC</td>
<td>No authoritative statement found</td>
<td>No authoritative statement found</td>
<td>Regulated as direct food additives or food contact substances depending on use</td>
</tr>
</tbody>
</table>

**Additional regulation details**

- This standard prohibits the sale of these foods unless they are listed in Food Standard 1.5.1 and comply with any special conditions of use.
- This provision prohibits the sale of these foods unless the manufacturer or importer of the novel food (1) has notified the Director in writing of their intention to sell or advertise for sale the novel food (which includes providing data to support the safety of the novel food); and (2) has received a written notice from the Director (called a Letter of No Objection).
- Foods commercialised in at least one member state before the entry into force of the Regulation on Novel Foods on 15 May 1997 are on the EU market under the “principle of mutual recognition”.
Table 13. Continued.

<table>
<thead>
<tr>
<th>Approval process</th>
<th>Argentina</th>
<th>Australia/New Zealand</th>
<th>Brazil</th>
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<th>European Union</th>
<th>Japan</th>
<th>Mexico</th>
<th>USA</th>
</tr>
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<tbody>
<tr>
<td>Requests for modification to follow guidance document GMC 26/03. If the product requested is considered a novel food in Europe its inclusion will be delayed until the novel foods chapter is written, approved and incorporated to the CAA</td>
<td>Pre-market application is required. FSANZ is reviewing the regulation of novel foods and nutritive substances. Criteria for “eligible foods” (i.e. foods in the wider sense including ingredients, additives and other substances added to food) is proposed. Any non-eligible foods would be prohibited and will require an appropriate safety assessment</td>
<td>Pre-market application is required</td>
<td>Pre-market application is required (safety assessment)</td>
<td>Pre-market application is required</td>
<td>Pre-market application is required (safety assessment)</td>
<td>No authoritative statement found</td>
<td>No authoritative statement found</td>
<td>Regulated as direct food additives or food contact substances depending on use</td>
<td></td>
</tr>
</tbody>
</table>
decisions. A recent review by Neltner et al. (2011) provides further discussion of the US food additive regulatory programme. For a summary of the regulations pertaining to direct food additives, see Table 12.

**Novel foods**

Novel foods (which usually encompass novel food ingredients) are generally foods or ingredients without a history of human consumption. Novel foods are specifically defined and regulated among Australia/New Zealand, Brazil, Canada, China and the EU. Although among these countries the definition of a novel food may differ (see definitions in Table 13), all novel foods marketed for sale in these countries require premarket approval or notification to their respective authoritative bodies. The safety assessment for novel foods that are whole foods cannot be conducted in the same manner as is used for individual ingredients. Whole foods are complex mixtures, often with considerable variation in composition depending on growing conditions. As they contribute calories, nutrients and bulk to the diet, they cannot be tested at high levels without altering the nutritional composition of the animal diet, and thus is not possible to achieve the same safety margins between animal dose levels and projected human intakes. Critical components include evaluation of the nutritional composition, presence of known toxins or anti-nutrients, and allergenicity of proteins as well as assessment of the potential nutritional impact of introducing the novel food into the human diet.

The majority of these target countries regulate novel foods and novel food ingredients based on a risk or safety assessment model to ensure safety following human consumption as outlined within the applicable regulations. In Argentina, Japan and Mexico, novel foods are neither defined nor regulated. In the US, novel foods are not defined but are regulated as direct food additives or food contact substances depending on their use (see “Direct food additives” above or “Food contact substances” below). For a summary of the regulations pertaining to novel foods, see Table 13.

**Food contact substances**

Food contact substances are generally any materials or articles intended to come into contact with food, including food containers, packaging, processing, etc. The basic safety principle of food contact materials is that they should generally be inert and not result in addition of compounds to the foods. Thus, the potential for migration of components of food contact materials into foods, and the assessment of exposure and toxicity of any migration is the basis for the safety determinations. In Argentina and Brazil, food contact substances are defined and regulated as food additives as per Mercosur standard GMC 32/07. In Canada and Japan, food contact substances are regulated separately from food additives and approval for use of food contact substances is not required (i.e. only voluntary submission of the food contact substance to the authoritative body).

In Australia/New Zealand, food contact substances are defined as any materials in contact with food and provided that such articles or materials, if taken into the mouth, are not capable of being swallowed or of obstructing any alimentary or respiratory passage and are not otherwise likely to cause bodily harm, distress or discomfort. Plastic materials for food contact use may voluntarily comply with the Australian Standard AS 2070-1999, a positive list of food contact substances. Discussions are in progress to include food packaging in the Food Standard Code, which would require compliance with US or EU regulations. At present, applications for food packaging materials are generally unnecessary provided there is approval in the US or the EU.

In contrast, pre-market notification or approval is required for use of food contact substances in China and the EU to confirm safety. In China, food contact substances are defined as materials in contact with the food, which include the food containers, packaging materials and anything in contact with the food in the course of manufacture, transport, sale and service. The EU has a more extensive definition for food contact substances (i.e. substances used to create all materials and articles intended to come into contact with foodstuffs, including packaging materials but also cutlery, dishes, processing machines, containers, etc.). The term also includes materials and articles that are in contact with water intended for human consumption.

In Mexico, food contact substances are not defined and no specific regulations are available pertaining to their use. The Regulation for the Sanitary Control of Products and Services establishes that packaging materials that contain substances that can migrate to the finished product, without endangering the health of consumers will be considered indirect additives. The classification of packaging materials and the physical, chemical and toxicological characteristic of each type of material will be established in specific norms. In general, the substances that are used to line packages used for foods, non-alcoholic beverages, alcoholic beverages, and health and beauty products will have to: remain perfectly adhered to the surface that is covered and do not crack, flake or become in any way a component of the food; be insoluble or inert in the food matrix, not be toxic; remain totally exempt of the volatile compounds that are used for their dilution and application; be free of heavy metals; avoid metal corrosion and not alter the pH of the product. Most major customers will require legal confirmation that a specific compound is allowed for use and should seek technical consultation. For certain products, a voluntary standard (Mexican Norm – NMX) may be available.

In the US, food contact substances are specifically defined and are also known as indirect additives. They include substances that are not intended to be added directly to food or to have a technical effect, but which
may reasonably be expected to become a component of food. These include substances used in packaging, transporting or the production of food.

Premarket approval is required for all food contact substance uses, unless exempted. A substance used in a food contact article may be exempted if the use in question has been shown to meet the requirements for a Threshold of Regulation exemption. The Threshold of Regulation is an application of the concept of the Threshold of Toxicological Concern (TTC). The TTC will be discussed in greater detail in the section on flavouring agents.

In 1995, the USFDA established a “threshold of regulation” of 0.5 ppb (equivalent to 1.5 μg/person/day) for indirect food additives that are not known to be carcinogens and do not contain structural alerts indicative of carcinogenicity (FDA 1995). A list the exemptions that have been issued under 21 CFR 170.39 Threshold of regulation for substances used in food-contact articles, is published on the USFDA website. Food contact substances uses that have been determined to be GRAS as also exempt from premarket approval. Similar to food additives, USFDA notification of the GRAS determination of the use of the food contact substances is voluntary.

In 1997, a food contact notification (FCN) process was established to allow for faster review of food contact substance uses that are not exempt from premarket approval. Unlike food additive regulations and threshold of regulation exemptions, approvals under the FCN process are proprietary and effective only for the manufacturer and substance identified in the notification.

An online inventory of effective premarket notifications for food contact substances that have been demonstrated to be safe for their intended use is maintained by the USFDA.

For a summary of the regulations pertaining to food contact substances, see Table 14.

**Flavouring agents**

Flavouring agents are substances or mixtures of substances intended for use in flavouring, intensifying or enhancing the aroma or taste of a food. Due to the very large number of flavouring agents and their use at very low levels in foods, regulations pertaining to this category represent a unique approach in many cases. Many flavouring agents have been approved based on long history of safe use (i.e. grandfathered) or through use of the TTC approach. The history, assumptions and databases used to develop the TTC were recently reviewed and evaluated by the EFSA Scientific Committee (2012) for consideration of potential application of the TTC to the safety assessment of other categories substances of present in foods and feed. In brief, the TTC is based on the concept that reasonable assurance of safety can be given, even in the absence of chemical-specific toxicity data, providing that the intake is sufficiently low, i.e. that an exposure level or threshold can be defined below which there is no significant risk to human health. The TTC requires that the chemical structure of the compound is known, and there is adequate information on the likely human exposure. Human threshold values or TTC values have been determined for structural classes of compounds using probabilistic approaches based on databases of toxicological testing for both cancer and non-cancer endpoints on a wide variety of chemical structures (EFSA 2012).

Flavouring agents, similar to food contact substances, do not require pre-market notification prior to use in Canada or the US. In the US, flavouring agents can be approved by the USFDA through a petition or though the GRAS determination process. The Flavor and Extract Manufacturers Association (FEMA) expert panel has served as the primary body for the safety evaluation of food flavourings since 1959, through GRAS assessment of flavouring substances, which often utilises the TTC approach. The panel safety decisions are provided to the USFDA and are published in the peer-reviewed literature (Smith et al. 2011) as well as on the FEMA webpage. As discussed below, the conclusions of the FEMA Expert Panel are often used as the basis for the acceptance of flavouring substances as safe food ingredients in many countries around the world.

In Australia/New Zealand, China, the EU, Japan and Mexico flavouring substances fall under the regulations for food additives. The permitted list of food additives generally includes specific flavouring substances. For a new flavouring substance (substance not listed on the permitted list of food additives for use), the approval process follows the same process as a food additive (see “Direct food additives” above). In Australia/New Zealand, FSANZ does not require a risk assessment to be done if the flavours are already listed in various publications such as the GRAS list published by the FEMA (Smith et al. 2011).

Recently in the EU, a new regulation was issued for flavourings under Regulation (EC) No. 1334/2008 and a new authorisation procedure under Regulation (EC) No. 1331/2008 was adopted (as discussed above in “Direct food additives”). EFSA and the JECFA also, when possible and feasible, apply the TTC approach for assessment of flavour substance.

In Argentina and Brazil, flavouring agents are regulated under Mercosur standards GMC 10/06. For new flavouring agents, applicants must submit to CONAL or ANVISA (for Argentina and Brazil, respectively), who will forward the application to Mercosur’s Sub Work Group #3. For a summary of the regulations pertaining to flavouring agents, see Table 15.
Table 14. Comparison of the regulations on food contact substances among the target countries.

<table>
<thead>
<tr>
<th></th>
<th>Argentina</th>
<th>Australia/New Zealand</th>
<th>Brazil</th>
<th>Canada</th>
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<th>European Union</th>
<th>Japan</th>
<th>Mexico</th>
<th>USA</th>
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<tbody>
<tr>
<td>Definition</td>
<td>Primary container or primary wrapping or container.</td>
<td>Any materials in contact with food, including packaging material, which may include materials such as moisture absorbers, mould inhibitors, oxygen absorbers, promotional materials, writing or other graphics.</td>
<td>Same as for Argentina.</td>
<td>Anything in which any food is wholly or partially contained, placed or packed.</td>
<td>All materials in contact with the food including the food containers, packaging materials and the things which contact the food in the course of manufacture, transport, sale and serve.</td>
<td>Food contact materials and articles are those which in their finished state are intended to be brought into contact with food, or are already brought into contact with food and intended for that purpose or can reasonably be expected to be brought into contact with food or to transfer their constituents to food under normal and foreseeable conditions of use. Includes packaging materials cutlery, dishes, processing machines, containers, etc. The term also includes materials and articles in contact with water intended for human consumption. Fixed water installations are excluded, however</td>
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Table 14. Continued.

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<th>Argentina</th>
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<th>Mexico</th>
<th>USA</th>
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</table>

Additional regulation details

There is a positive list of substances that are added to plastics to achieve a technical effect in the final product. Food contact substances may be placed in contact with food, provided such articles or materials, if taken into the mouth, are not capable of being swallowed or of obstructing any alimentary or respiratory passage and are not otherwise likely to cause bodily harm, distress or discomfort.

(EU) 10/2011 is the specific Regulation for plastic FCM with a positive list of substances. (EC) 450/2009 is the specific Regulation for A&I FCM. Regulation (EC) 282/2008 is specific for Recycling of plastics. Directive 2007/42/EC is specific for Regenerate cellulose film. Directive 84/500/EEC is specific for ceramics. For all those materials for which no specific measure is adopted at the EU level, national legislation in member states may exist.

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Table 14. Continued.

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<th>Argentina</th>
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<th>European Union</th>
<th>Japan</th>
<th>Mexico</th>
<th>USA</th>
<th>Notes: *Regulations pertaining to Argentina follow Mercosur Standards. Brazil also follows Mercosur Standards. JHOSOA, Japan Hygienic Orefin and Styrene Plastics Association.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Approval process</td>
<td>Same process as direct food additives</td>
<td>Applications for food packaging materials are generally unnecessary if there is approval in the EU or US. Plastic materials for food contact use may comply with the voluntary Australian Standard AS 2070-1999</td>
<td>Same as for Argentina*</td>
<td>Voluntary submission to the Health Products and Food Branch (HPFB) for a pre-market assessment of their chemical safety</td>
<td>Pre-market application is required</td>
<td>Pre-market application is required only for substances for which a positive listing is not set out specifically. Regulation at the EU includes plastics, A&amp;I FCM and Regenerated cellulose film. For the other materials the general provisions of the framework Regulation 1935/2004 apply and specific measures may exist at the national level</td>
<td>JHOSPA established the industry’s voluntary standards composed of a positive list of raw materials that can be used safely for food utensils, containers, packaging materials, and Standard Methods of Analysis with specifications for each resin</td>
<td>Although no specific process, most major customers require legal confirmation that the compound is allowed. Any approval should be obtained through technical consultation</td>
<td>Pre-market request for review in the form of a food contact notification, a threshold of regulation exemption request, or a GRAS determination. Notification to the USFDA of GRAS determinations is voluntary. The USFDA review of GRAS notifications is not considered a formal approval</td>
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</table>
Table 15. Comparison of the regulations on flavouring agents among the target countries.

<table>
<thead>
<tr>
<th>Country</th>
<th>Argentina</th>
<th>Australia/New Zealand</th>
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<th>Mexico</th>
<th>USA</th>
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<tbody>
<tr>
<td><strong>Definition</strong></td>
<td>Substances or mixtures of substances with odoriferous and/or flavour properties that are able to confer or enhance the aroma and/or taste of food</td>
<td>Flavourings are defined as intense preparations that are added to foods to impart taste and/or odour, which are used in small amounts and are not intended to be consumed alone, but do not include herbs, spices and substances which have an exclusively sweet, sour or salt taste</td>
<td>Same as for Argentina</td>
<td>Flavourings are defined as intense preparations that are used in small amounts and are not intended to be consumed as such, but which are added to food in order to impart or modify odour and/or taste of food. Flavouring substances are chemically defined substances obtained by chemical synthesis or isolated using chemical processes, and natural flavouring substances</td>
<td>Food additive and food ingredient necessary for producing, preserving and applying the flavourings. The additive added to food (except flavour enhancer) does not play the role for final aromatic products</td>
<td>Flavourings are products that are not intended to be consumed as such, but which are added to food in order to impart or modify odour and/or taste of food. Flavouring substances are chemically defined substances obtained by chemical synthesis or isolated using chemical processes, and natural flavouring substances</td>
<td>Natural flavouring agents are defined as food additives intended for use in flavouring food and are substances obtained from animals or plants, or mixtures thereof</td>
<td>Substance or blend of substances of natural origin, identical to natural or synthetic with or without solvents and with or without the addition of other additives that are used to give or intensify flavours or aromas to products</td>
<td>Not defined; regulated as substances added directly to food, specifically as direct food additives and GRAS substances</td>
</tr>
<tr>
<td><strong>Additional regulation details</strong></td>
<td>Same as for Argentina</td>
<td>Certain prohibitions exist in Section B.01.046. Definition of food additive excludes flavouring preparations</td>
<td></td>
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<th>Mexico</th>
<th>USA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Approval process</td>
<td>Application submission to CONAL and subsequent submission to Mercosur</td>
<td>Flavouring agents are regarded as food additives. An application is generally not required for a flavouring agent</td>
<td>Application submission to ANVISA and subsequent submission to Mercosur</td>
<td>Voluntary request a letter of opinion on a flavouring agent (see “approval process for processing aids”)</td>
<td>Same process as direct food additives</td>
<td>New common authorisation procedure under Regulation (EC) No. 1331/2008, data requirements provided in Regulation (EC) No. 234/2011. For smoke flavourings Regulation (EC) 2065/2003 specifies the procedure</td>
<td>Same process as direct food additives</td>
<td>Same process as direct food additives</td>
<td>Same process as substances added directly to food. See “direct food additives” above. Most flavouring agents in the US are reviewed by the Flavor and Extract Manufacturers Association (FEMA) expert panel to determine if their uses are GRAS</td>
</tr>
</tbody>
</table>

Note: *Regulations pertaining to Argentina follow Mercosur Standards. Brazil also follows Mercosur Standards.*
Enzymes

In general, enzymes are substances or products extracted from animals, plants or microbes that act by either promoting a desirable chemical reaction or are used in processing. The definition of an enzyme according to each target country is presented in Table 17. In Canada, China, the EU, Japan, Mexico and the US enzymes are regulated as food additives. Likewise, the approval process for new enzymes also follows the same approval process for direct food additives (see “Direct food additives” above). However, depending on the context of use, Canada may consider enzymes to be processing aids. In Australia/New Zealand, enzymes as such are not specifically defined in the Food Standards Code; however, specified enzymes are permitted for use as processing aids (see “Processing aids” below). Recently in the EU, a new regulation was issued for enzymes under Regulation (EC) No. 1332/2008 and a new authorisation procedure under Regulation (EC) No. 1331/2008 was adopted (as discussed above). In Argentina and Brazil, enzyme regulations are not harmonised in Mercosur; thus, enzyme regulations are regulated differently for these two countries. In Argentina, enzymes are regulated under the CAA, Chapter XVI (Articles 1261, 1262 and 1263). The approval processes for new enzymes require an application submitted to CONAL. In Brazil, enzymes are regulated under Resolution No. 26 and the use of new enzymes requires an application submitted to ANVISA.

JECFA (2001) has also provided specific comments for consideration of the safety assessment of enzymes derived from recombinant sources. These include: (1) characterisation of genetic materials introduced into the organism producing the enzyme to demonstrate that no unexpected genetic materials are introduced into the host; (2) consideration of potential of proteins from the micro-organism leading to antibiotic resistance; and (3) evaluation of the allergenic potential of the gene products.

For a summary of the regulations pertaining to enzymes, see Table 16.

Processing aids

The definition of processing aid varies across the target countries; however, in general, processing aids are substances not consumed as food ingredients by themselves and are used intentionally in processing or in the production of raw materials, ingredients or foods to achieve a technological purpose. As illustrated in Codex inventory of processing aids, with the exception of enzymes and solvents, most processing aids have not been evaluated by JECFA. Furthermore, this inventory is not considered to be complete. Development of a comprehensive Codex database of processing aids is under discussion.

In Canada, similar to flavouring agents and food contact substances, processing aids are permitted for use without pre-market notification providing they meet the specific criteria for the definition of a processing aid (i.e. results in no or negligible residues in the food). If they do not meet these criteria, the processing aid is considered to be a food additive and the approval process for direct food additives would be applicable. Similarly in the EU, no regulations pertain to processing aids specifically; however, if a processing aid does not meet the criteria for the definition of a processing aid, it is classified as a food additive, and Regulation (EC) No. 1333/2008 (regulation for food additives) would be applicable. Processing aids are regulated as food additives for the following countries: China, Japan and Mexico; therefore, all regulations pertaining to direct food additives are applicable (see “Direct food additives” above). In Mexico, publication in 2012 of the new updated food additive positive lists processing aids separate from food additives. In Argentina and Brazil, processing aids are not harmonised in Mercosur. The only harmonised regulation is 84/93, which establishes the definitions of the functions of processing aids. In Argentina, certain processing aids are listed under Chapter XVI of the CAA; however, this list is not comprehensive. In Australia/New Zealand, processing aids are regulated under Food Standard 1.3.3, a general standard for processing aids. New processing aids or existing processing aids with new food uses require an application to modify the Standard Food Code. It should be noted that processing aids are regulated into a specific horizontal standard (i.e. across the whole food supply) in Australia, unlike the systems used by other countries, as well as for standards promulgated by the CAC. In the US, processing aids would be known as “secondary direct food additives” and regulated as direct food additives. In some cases, they may also be regulated as food contact substances (see “Direct food additives” and “Food contact substances” above).

For a summary of the regulations pertaining to processing aids, see Table 17.

Nanoscale materials

The regulation of products of nanotechnology is a dynamic and evolving activity, due largely to the wide spectrum of nanomaterials, nano-enabled products, and applications that are being developed and the uncertainties that are associated with defining, characterising, and appropriately testing them for efficacy and safety (PEN 2011). Nanotechnology is a term that has been defined in a few countries (Australia/New Zealand, Canada, China and the EU), but not all. In Canada, nanotechnology is described as the application of nanoscience to develop new materials and products, and involves the manipulation of matter at the nanometre scale. The development of the definition of nanomaterials has begun in several countries, including Canada, the EU and the US. For example, the European Commission (EC) proposed definition was “a material that
Table 16. Comparison of the regulations on enzymes among the target countries.

<table>
<thead>
<tr>
<th>Country</th>
<th>Definition</th>
<th>Argentina</th>
<th>Australia/New Zealand</th>
<th>Brazil</th>
<th>Canada</th>
<th>China</th>
<th>European Union</th>
<th>Japan</th>
<th>Mexico</th>
<th>USA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enzymes or enzyme preparations are substances of animal, plant or microbial origin that act by promoting the desirable chemical reactions</td>
<td>Not specifically defined in the Food Standards Code</td>
<td></td>
<td>Enzymes or enzyme preparations are substances of animal, plant or microbial origin that act by promoting the desirable chemical reactions</td>
<td>Not specifically defined in the Food and Drug Regulations</td>
<td>Biological products directly extracted from edible or non-edible parts of a plant or animal or fermented and extracted from traditional or genetically modified microorganisms (including but not limited to bacteria, actinomycetes, and fungi) that are used in food processing and have a special catalytic function</td>
<td>A food enzyme is defined as a product obtained from plants, animals or microorganisms, or products thereof including a product obtained by a fermentation process using a microorganism containing one or more enzymes capable of catalysing a specific biochemical reaction and added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods</td>
<td>Not defined</td>
<td>Not defined</td>
<td>Not defined</td>
</tr>
</tbody>
</table>

(continued)
Table 16. Continued.

<table>
<thead>
<tr>
<th>Country</th>
<th>Regulations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argentina</td>
<td>CAA, Chapter XVI, Articles 1261, 1262 and 1263. Enzymes regulations are not harmonised in Mercosur. Enzymes may be used in the course of manufacture of any food, provided the enzyme is derived from the corresponding source or sources specified in the tables of Sections 15-17 of Food Standard 1.3.3 (standard for processing aids).</td>
</tr>
<tr>
<td>Australia/New Zealand</td>
<td>Resolução RDC #26 dated 27 May 2009 (on the ANVISA site). Enzymes regulations are not harmonised in Mercosur. Enzymes are regulated as food additives or may be considered processing aids depending on their context of use.</td>
</tr>
<tr>
<td>Brazil</td>
<td>Regulation (EC) No. 1332/2008 (Regulation (EC) No. 1332/2008 does not include food enzymes used in the production of food additives within the scope of Regulation (EC) No. 1333/2008 or processing aids). The scope of this regulation does not extend to enzymes that are not added to food to perform a technological function but are intended for human consumption such as enzymes for nutritional or digestive purposes.</td>
</tr>
<tr>
<td>Canada</td>
<td>Enzymes are listed as a permitted food additive can be used in food under the conditions indicated.</td>
</tr>
<tr>
<td>China</td>
<td>Enzymes are regulated as food additives.</td>
</tr>
<tr>
<td>European Union</td>
<td>Enzymes are regulated as food additives.</td>
</tr>
<tr>
<td>Japan</td>
<td>Enzymes are regulated as food additives.</td>
</tr>
<tr>
<td>Mexico</td>
<td>Enzymes are regulated as substances added directly to food, specifically as direct food additives, secondary direct food additives, or GRAS substances.</td>
</tr>
<tr>
<td>USA</td>
<td>Enzymes are regulated as food additives.</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Approval process</th>
<th>Application submission to CONAL. Same process as processing aids.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Application submission to ANVISA</td>
<td>Same process as direct food additives and processing aids.</td>
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<td>Same process as direct food additives and processing aids</td>
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<td>Same process as direct food additives</td>
<td>Same process as direct food additives.</td>
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<tr>
<td>Same process as direct food additives</td>
<td>Same process as substances added directly to food. See “direct food additives” above.</td>
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<tr>
<td></td>
<td>Argentina</td>
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</tr>
<tr>
<td><strong>Definition</strong></td>
<td>Any substance, excluding equipment and utensils that is not consumed by itself as a food ingredient and which is intentionally used in the processing of raw materials, foods or ingredients, for a technological purpose during treatment or processing. It must be removed from the food or inactivated; the presence of traces of the substances or their derivatives may be admitted in the final product</td>
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</table>

(continued)
Table 17. Continued.

<table>
<thead>
<tr>
<th>Regulation</th>
<th>Argentina</th>
<th>Australia/New Zealand</th>
<th>Brazil</th>
<th>Canada</th>
<th>China</th>
<th>European Union</th>
<th>Japan</th>
<th>Mexico</th>
<th>USA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processing aids are not harmonised in Mercosur, the only harmonised</td>
<td>Food Standard 1.3.3, a general standard for processing aids</td>
<td>Processing aids</td>
<td>No specific regulations on most processing aids. If a substance does</td>
<td>Processing aids are regulated as food additives</td>
<td>Processing aids are regulated as food additives</td>
<td>Regulated as food additives or in some cases as food contact substances.</td>
<td>Regulated as food additives or in some cases as food contact substances.</td>
<td>Regulated as food additives or in some cases as food contact substances.</td>
<td>Regulated as food additives or in some cases as food contact substances.</td>
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<tr>
<td>regulation is GMC 84/93 which establishes the definitions of the functions</td>
<td></td>
<td>are not harmonised in</td>
<td>does not meet the specific criteria for the definition of a processing</td>
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<td></td>
<td>When approved in the regulations, they are known as “secondary</td>
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<td>of processing aids. Certain processing aids are listed under Chapter XVI</td>
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<td>Mercosur, the only</td>
<td>aid as outlined above, it may be considered to be a food additive</td>
<td></td>
<td></td>
<td>direct additives”</td>
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<td>of the CAA, but this list is not comprehensive</td>
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<td>harmonised regulation</td>
<td>and the regulations pertaining to food additives would be applicable</td>
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<td>is GMC 84/93 which</td>
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<td>definitions of the</td>
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<td>functions of</td>
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<td></td>
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<td>processing aids</td>
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</tr>
</tbody>
</table>

| Approval process | Application submission to CONAL | Pre-market application is required | Application submission to ANVISA | May seek a so-called “Letter of Opinion” which confirms that, under its conditions of use, the substance is considered a processing aid and use is acceptable | Same process as direct food additives | As they fall out of the scope of EU legislation, there is no process for their approval. Nevertheless, member states or the COM may address the issue of their safety to EFSA | Same process as direct food additives | Same process as direct food additives | Same process as direct food additives |

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consists of particles with one or more external dimensions in the size range 1–100 nm for more than 1% of their number; and/or “has internal or surface structures in one or more dimensions in the size range 1 nm–100 nm”; and/or “has a specific surface area by volume greater than 60 m$^2$ cm$^{-3}$, excluding materials consisting of particles with a size lower than 1 nm”.

The development of engineered nanomaterials with new beneficial properties has potential food applications including improved nutrient and bioactive delivery systems, improved texture and flavour encapsulation; improved microbiological control, food processing, packaging, and package biodegradability; and highly sensitive biosensors for detecting pathogens, allergens, contaminants and degradants (reviewed in Chaudhry et al. 2008; Augustin & Sanguansri 2009; Magnuson et al. 2011). The regulation of use of engineered nanomaterials in food and feed production represents many new challenges. However, it should be noted that nanomaterials and nanostructures also occur naturally in all plant and animal products that are consumed as food. Examples of naturally occurring nanomaterials include DNA molecules and proteins such as casein micelles and whey proteins. Examples of nanostructures include the muscle structure of meats and fish, and pectin nanostructure in fruits.

The challenges regarding safety assessment of engineered nanomaterials that may be present in foods were discussed in a FAO/WHO Expert meeting on the application of nanotechnologies in the food and agriculture sectors (FAO/WHO 2010). As numerous studies have demonstrated that the biological properties of materials can change substantially when reduced to the nano-size range, the toxicological properties of nanomaterials cannot be assumed to be the same as their non-nano counterparts. A critical review of the published literature on the toxicity of nanomaterials with potential use or occurrence in food, food-related materials or dietary supplements demonstrated that there are very few published studies with adequate characterisation and repeated-dose exposures, which are necessary to assess safety of food-related exposures (Card et al. 2011). Thus, considerable additional research is needed to understand the potential effects of oral exposure to engineered nanomaterials. The FAO/WHO Expert meeting report also discussed gaps in knowledge and a need for sharing of existing data on the characterisation, toxicological and exposure data as well as appropriate methodologies to facilitate risk assessment (FAO/WHO 2010).

Currently, none of the listed “target countries” has established specific regulations on nanoscale materials for food-related uses; however, several have issued comments or opinions which are briefly discussed below. Many of the countries are involved in international organisations working toward developing approaches to risk assessment of nanomaterials. For further details, see the summary tables (Tables 1–9) for each target country.

**Australia and New Zealand**

In Australia and New Zealand, any new substances intended to be added to food that are manufactured using nanotechnologies that may present safety concerns will have to undergo a comprehensive scientific safety assessment under the appropriate standard (e.g. as a novel food or food additive) before they can be legally sold in those countries. FSANZ has developed a factsheet for nanotechnology and foods, outlining the presence of naturally occurring nanomaterials in foods and the environment (FSANZ 2011b).

**Canada**

Health Canada is using existing legislative and regulatory frameworks to regulate applications of nanotechnology but recognises that new approaches may be necessary in future to keep pace with the advances in this area, particularly given that there currently is inadequate information on risks associated with nanomaterials (Health Canada 2010). Various acts (and regulations contained therein) are envisioned by Health Canada to be relevant to nanomaterials, including the Food and Drugs Act, the Canadian Environmental Protection Act 1999, the Hazardous Products Act, and the Pest Control Products Act.

Health Canada has indicated that in order to identify and assess potential risks and benefits (where applicable) of nanomaterials, the following types of information may be required to be submitted for review:

- Intended use of the nanomaterial, including any end product in which it will be used.
- Characterisation of the nanomaterial, including manufacturing methods, identity and purity.
- Physicochemical properties and toxicological, ecotoxicological, metabolism and environmental fate data that may be both generic and specific to the nanomaterial if applicable.
- Risk assessment and risk management strategies, if considered or implemented.

Health Canada has noted that future guidance specific to different programme areas and legislative and regulatory authorities will be developed in a manner that promotes a consistent set of approaches (Health Canada 2010).

**European Union**

Although there are no specific provisions in the EU legislation on nanoscale materials, existing legislation covers in principle the potential health, safety and environmental risks in relation to nanomaterials. A close examination of the definition for food additives already incorporates nanoscale materials (i.e. food additives that are prepared
through nanotechnology would be considered under novel foods). In Article 12 of 1333/2008/EC:

when a food additive is already included in a Community list and there is a significant change in its production methods or in the starting materials used, or there is a change in particle size, for example through nanotechnology, the food additive prepared by those new methods or materials shall be considered as a different additive and a new entry in the Community lists or a change in the specifications shall be required before it can be placed on the market.

EFSA has published guidance for assessing the risks of nanoscience and nanotechnologies in food and feed (EFSA 2011). It stressed the importance of adequate physicochemical characterisation of the forms of engineered nanomaterials in food/feed products and under testing conditions. The physicochemical parameters that should be characterised regardless of the route of exposure include agglomeration and/or aggregation, chemical composition, crystal structure/crystallinity, particle size/size distribution, purity, shape, surface area, surface charge, and surface chemistry including composition and reactivity. The following characteristics are considered to be indicators of increased probability of toxicity: a high level of reactivity (e.g. catalytic, chemical); complex morphology (e.g. long fibre, crystal); interaction with biomolecules (e.g. proteins, DNA); complex transformations (e.g. loss of coating); the presence of antimicrobial activity; and evidence of persistence and/or bioaccumulation (EFSA 2011).

United States of America

The USFDA issued draft guidance in April 2012 regarding nanotechnology. At this time, there is no formal definition for “nanotechnology” or “nanoscale”, however the USFDA indicated that, “in the absence of a formal definition, when considering whether a USFDA-regulated product contains nanomaterials or otherwise involves the application of nanotechnology, the USFDA will ask: (1) whether an engineered material or end product has at least one dimension in the nanoscale range (approximately 1–100 nm); or (2) whether an engineered material or end product exhibits properties or phenomena, including physical or chemical properties or biological effects, that are attributable to its dimension(s), even if these dimensions fall outside the nanoscale range, up to 1 μm”. New substances, whether direct food additives or food contact substances, cannot be GRAS per this draft guidance. Manufacturers seeking approval for a nano-engineered substance must submit a food additive petition or a food contact substance notification.

No specific documents on the regulation of nanomaterials for food-related uses were found for Argentina, Brazil, China, Japan and Mexico, although work is in progress. Argentina, Brazil, China and Japan have established or are participating in various groups for the development of nanotechnology standards in the areas of health, safety and environment. A summary of the developing approaches to risk assessment of nanomaterials among the target countries is listed in Table 18.

Conclusions

JECFA, whose genesis occurred at the FAO/WHO Conference on Food Additives in 1955, continues to be of fundamental importance to the activities of the CAC and especially to the Codex Committee on Food Additives and the Codex Committee on Contaminants in Foods. While the outcome of JECFA’s evaluations does not have any direct bearing on the regulatory approval of a food additive in any specific country, JECFA’s scientific evaluations and reassessments are widely recognised and may affect an application for approval for a new food additive in a particular country. Similar to JECFA, the CAC has no regulatory authority and its standards are not enforceable unless they have been adopted into the regulatory framework for a nation; however, its standards for food additives continue to serve as guidelines to many nations.

In addition to their guiding international influence, the early work of JECFA and CAC and the principles outlined by those committees laid the foundation for how substances added to food are regulated in many individual countries today, including the countries examined: Argentina, Australia, Brazil, Canada, China, the EU, Japan, Mexico, New Zealand, and the US. The regulatory authority for each target jurisdiction/country utilises its own regulatory framework and although the definitions, regulations and approval processes may vary among all target countries, in general there are many similarities across all target countries. In all cases, the main purpose of each regulatory authority is to establish a framework and maintain/enforce regulations to ensure the safety of food consumed and sold within its respective countries. Although the path for approval of different categories of food additives varies from jurisdiction to jurisdiction, there are many commonalities in terms of the data requirements and considerations for assessment of the safety of use of substances added to food, including the use of positive lists of approved substances, pre-market approval, and a separation between science and policy decisions. There is also a move toward harmonisation of food regulations, as illustrated by Australia and New Zealand, by Mercosur and by the EU. International collaboration is occurring to address the challenge of developing regulatory guidance and safety assessment for use of nanomaterials in foods. Harmonisation of global food regulations is envisioned to promote use of all available foods through free trade, to support farmers, and to reduce hunger and poverty globally.
Table 18. Summary of the developing approaches to risk assessment of nanomaterials among the target countries.

<table>
<thead>
<tr>
<th>Argentina</th>
<th>Australia/New Zealand</th>
<th>Brazil</th>
<th>Canada</th>
<th>China</th>
<th>European Union</th>
<th>Japan</th>
<th>Mexico</th>
<th>USA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argentina has established or is participating in various groups for the development of standards in the area of nanomaterials (see nanoscale materials under Table 1 for further details)</td>
<td>Any new food substances manufactured using nanotechnologies that may present safety concerns will have to undergo a comprehensive scientific safety assessment under the appropriate standard before they can be legally sold in those countries (see nanoscale materials under Table 2 for further details)</td>
<td>Brazil has established or is participating in groups for the development of standards in the area of nanomaterials (see nanoscale materials under Table 3 for further details)</td>
<td>Health Canada is using existing legislative and regulatory frameworks to regulate applications of nanotechnology, but it recognises that new approaches may be necessary in future to keep pace with the advances in this area, particularly given that there currently is inadequate information on risks associated with nanomaterials. Various acts (and regulations contained therein) are envisioned by Health Canada to be relevant to nanomaterials. Health Canada has indicated that in order to identify and assess potential risks and benefits (where applicable) of nanomaterials, a list of documents is required. For further details, see nanoscale materials under Table 4.</td>
<td>China has established or is participating in groups for the development of standards in the area of nanomaterials (see nanoscale materials under Table 5 for further details)</td>
<td>EFSA published an opinion on nanotechnologies and a guidance document on risk assessment was published in May 2011. Existing legislation covers in principle the potential health, safety and environmental risks in relation to nanomaterials. Recently, specific provisions on risk assessment of nanomaterials were introduced in EU legislation on food additives and food contact materials. A definition of &quot;engineered nanomaterial&quot; and a mandatory labelling requirement for food ingredients containing such nanomaterials were introduced. Examination of the definition for food additives already incorporates nanoscale materials (i.e. food additives prepared through nanotechnology would be considered new food additives). EFSA considers the risk assessment paradigm applicable to nanomaterials, but suggests that a risk assessment of nanomaterials in food and feed take into consideration specific properties of nanomaterials in addition to those common to the equivalent non-nano-forms. For further details, see nanoscale materials under Table 6.</td>
<td>Japan has established or is participating in groups for the development of standards in the area of nanomaterials (see nanoscale materials under Table 7 for further details)</td>
<td>No specific documents on the regulation of nanomaterials for food-related uses were identified</td>
<td>The USFDA issued draft guidance on nanotechnology and food in April 2012. There is no formal definition for &quot;nanotechnology&quot; or &quot;nanoscale&quot; at this time, however the guidance contains considerations the USFDA takes into account when evaluating nanomaterials. New nano-engineered substances, whether direct food additives or food contact substances, cannot be GRAS per the USFDA's recently issued draft guidance. Manufacturers seeking approval for a nano-engineered substance must submit a food additive petition or a food contact substance notification. See &quot;food contact substances&quot; and &quot;food additives&quot; above. For further details see nanoscale materials under Table 9.</td>
</tr>
</tbody>
</table>
Acknowledgements

Dr Ian Munro was instrumental in the development of this review, and wrote the description of the history and accomplishments of JECFA. Sadly, he passed away before this work was completed. He is greatly missed. This work was supported by funding from The Pew Charitable Trusts. The authors gratefully acknowledge the following individuals for reviewing the manuscript and providing comments during the preparation of this review: Thomas G. Neltner and Neesha R. Kulkarni with The Pew Charitable Trusts; the International Food Additives Council; Dr Jordi Serratos and Dr Alexandre Feigenbaum with the European Food Safety Authority; Annette Schaefer and Sirkku Heinimaa with the European Commission; Josée Bouchard, Luc Bourbonnière, Mark Perry and Anastase Rulibikiye with Health Canada; Mr John van den Beuken with New Zealand Ministry for Primary Industries (MPI); Dr Paul Brent with FSANZ; Dr Inocencio Higuera, Centro de Investigacion Cientifica de Yucatan; and Dr Tomás González Estrada, Consejo de Ciencia y Tecnologia del Estrado de Yucatan.

References


[EFSA] European Food Safety Authority. 2009. Guidance of EFSA prepared by the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids on the


Food and Drug Administration. 2012. Draft Guidance for Industry: Assessing the safety of significant new manufacturing process changes, including emerging technologies, on the safety and regulatory status of food ingredients and food contact substances, including food ingredients that are color additives [Internet]; [cited 2012 Apr 25]. Available from: http://www.regulations.gov/contentStreamer?objectID=0900006480aff8&disposition=inline&contentType=html.


Mercosur. 2011. [Página Brasileira do Mercosul]. Brasilia: Mercosur [An economic and political agreement between
Argentina, Brazil, Paraguay and Uruguay [Internet]. Available from: http://www.mercosul.gov.br/


Chinese Approach on Regulating Food Additives, Novel Foods, Functional Foods and Dietary Supplements

Ashley Roberts and Rebecca Rogerson
Cantox Health Sciences International, Mississauga, ON, Canada

Abstract

As a result of increasing interest within the Chinese market among food and supplement industries, the Chinese Authorities have the responsibility of ensuring that any new food or food ingredient imported into or produced in China meets the safety standards established by the government. By adhering to these standards, the government establishes a decreased risk of food contamination and safety issues within their food supply. In China, the Food Hygiene Law (1995) establishes the general principles for food hygiene regarding foods, food additives, containers, packages, utensils and equipment used for foods. As the Food Hygiene Law (1995) establishes only the basic requirements for food hygiene, supplementary regulations regarding food additives, novel foods, functional foods and dietary supplements provide specific safety requirements. This chapter discusses the regulations of the above-mentioned products, providing the requirements for regulatory approval within the Chinese market.

Introduction

The Chinese marketplace, having over a billion potential customers, is becoming of increasing interest to the multinational food and food ingredient industry wishing to
market their products in this rapidly developing economy. The food and supplement industries are looking at China as a major business expansion opportunity for their products. Further interest has resulted because the changing economic climate of China has led to a change in the diet of many urban Chinese. The diet of the Chinese people is becoming increasingly ‘Westernized’ with the outcome that there is increasing evidence of rising obesity rates in children and adults. As a result of these dietary changes, the Chinese government has the responsibility of ensuring that any new food or food ingredient imported into China or produced in China meets the established safety standards and that there is a decreased risk of food contamination. The latest round of changes to the Chinese regulatory system provides the Chinese public with potential increased food choices.

The Chinese regulatory system for food products could be classified as being unique within the world of fully established systems such as those developed in the USA, Europe and Australia/New Zealand. The purpose of this chapter is therefore to provide an overview of the regulations currently governing food additives, novel foods, functional foods and dietary supplements in China and how regulatory clearance is obtained for these products.

History of Food Regulations in China

Food inspection and execution of food laws were established by the People’s Central Government Ministry of Public Health or Ministry of Health (MOH) soon after the commencement of the People’s Republic of China on October 1, 1949 (1). Subsequently, the State Council, which comprises various ministries including the MOH, was established in 1954. By 1965, the State Council had promulgated its prototype for food safety laws, named the Regulation for Food Sanitation Control for Trial Implementation, however, due to the Cultural Revolution from 1966 to 1976, this trial Regulation was not implemented owing to the collapse of the legal system (1,2). It was not until 1979 that new food hygiene laws were established under the Regulations on the Administration of Food Hygiene. As the Chinese economy grew, the laws regarding food hygiene were adapted to reflect the need to ensure food safety for the Chinese public. In 1982, regulations were formulated to establish the requirements for the sanitization of imported foods and food hygiene supervision and, on July 1 1983, these regulations were developed into the Food Hygiene Law of the People’s Republic of China (trial implementation). A finalized version of the Food Hygiene Law of the People’s Republic of China (Food Hygiene Law) was promulgated and implemented on October 30 1995, which provided revisions to the 1983 trial implementation to reflect the current economic position of the People’s Republic of China and remains today the most current version of the Food Hygiene Law in China.

Food Hygiene Law of the People’s Republic of China

The purpose of the Food Hygiene Law (1995) is to ensure food hygiene, prevent food contamination or harmful substances from injuring human health, safeguard
the health of the people, and improve physical fitness. This law consists of 9 chapters and 57 articles that provide the standards of hygiene for all foods, food additives, containers, packages, utensils and equipment used for foods. Standards for detergents, disinfectants, premises, facilities and establishments associated with food production or marketing are likewise established within this law, however, while general standards are established, the Food Hygiene Law (1995) does not establish specific limits for food production or marketing, sensory properties or safety. Instead, specific limits are established within supplementary regulations to the Food Hygiene Law (1995).

While the majority of the hygiene standards for food and anything associated with food are controlled by the State Council and associated national agencies, the Food Hygiene Law (1995) allows provincial and municipal governments and interested parties to set hygiene standards where national standards are lacking. This law allows the responsibility of food hygiene control to be shared throughout all levels of government within the People’s Republic of China, with the overall control existing with the national authorities.

The Food Hygiene Law (1995) provides definitions for foods, food additives, food fortifiers, containers and packages used for food, food production or marketing and producers or marketers of food, however, the control of farming industries, such as the crop and extensive livestock industry, is excluded from this law. This law also provides a rudimentary outline of administrative procedures for hygiene, outlining the basic approval process for domestic and imported or exported foods, as well as providing general labeling requirements. Since the Food Hygiene Law (1995) establishes general standards for food hygiene, this law is intended to be supported through the use of regulations that specifically outline the requirements for foods, food additives and all things associated with food. For example, the basic regulatory requirements for food additives, novel foods, functional foods and dietary supplements are established within this law, but greater detail regarding the regulatory procedure of these products is provided in the regulations discussed below.

**Food Additive Regulations**

Food additives are regulated and approved by the Ministry of Health of the People’s Republic of China (MOH). This is the department of the State Council that is directly responsible for all health related issues within China. Health administrative departments within the provinces, autonomous regions and municipalities also are involved in the regulation of food additives by reviewing domestic food additive applications before they are forwarded to the MOH.

The China Food Additive Association (CFAA), established in 1993, assists the Chinese government in making regulations and policies regarding industrial standards, quality supervision and market entry by collecting domestic and foreign opinions on behalf of the industries and presenting the opinions to the MOH. The CFAA also expands the application of any newly developed technology and products and is the only nationwide food additive and ingredients industry organization approved
by the government. The purpose of the CFAA is to contribute to the sound development of the food and food additive industries, as well as to the safety and health of consumers.

Food additives, as defined by the Food Hygiene Law (1995), are synthetic chemicals or natural materials added to food for the purpose of improving quality, color, flavor or taste. Food additives also may be added to food for the purposes of preservation from decay and for processing technology. While the Food Hygiene Law (1995) does set hygiene standards for food additives, the regulatory requirements for food additives are established in two documents promulgated by the MOH. The Administrative Measures for Food Additive Hygiene (Ministry of Health Decree No. 26) defines the regulatory process for obtaining approval from the MOH for new food additives or changes in uses and use levels of previously approved additives, whereas the Food Additive Hygiene Standard (GB 2760-2007) provides a list of approved food additives.

Administrative Measures for Food Additive Hygiene
(Ministry of Health Decree No. 26)

On July 1 2002, the Administrative Measures for Food Additive Hygiene (Ministry of Health Decree No. 26) became effective, replacing the previous version, dated March 15 1993. The 2002 version of the Administrative Measures (Decree No. 26) provided updates to the 1993 version so as to reflect the changing Chinese economy and to accelerate administration regarding the hygiene of food additives, prevention of food contamination and to protect the health of consumers.

Gaining regulatory approval for food additives is a two-step process. The first step is to obtain approval from the MOH for the food additive by proving safety, whereas the second step is for the manufacturer to obtain a hygiene license before producing, marketing or using the food additive. Both of these steps are explained in further detail below.

Approval from the Ministry of Health

All food additives permitted for use in food at defined use levels are published within the Food Additive Hygiene Standard. Any new food additives or previously approved food additives with an increase in the application scope or use level are required to obtain regulatory approval from the MOH before it can be imported, produced or marketed.

Food additives are evaluated on the basis of their safety. The standard of safety for food additives is established by providing documents that explain the production techniques, the source of the material along with the physical and chemical properties, the testing methodologies, the product quality standards and the regulatory status of the food additive in foreign markets. Safety of the additive is further supported through toxicity studies. A prior favorable Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluation also is taken into consideration and provides further supporting evidence of the safety of the material. In the absence of a JECFA
safety opinion or listing by Codex Alimentarius (Codex), a food additive normally requires approval in two or more major countries before acceptance is granted by the MOH.

Before a new food additive application is made to the provincial health administrative departments or the MOH, a sample of the food additive should be sent to a testing agency accredited by the provincial health administrative department. The testing agency will perform toxicity tests, if required, as well as hygiene tests on three consecutive batches of the product. After the completion of the tests, the testing agency will provide a report to the manufacturer or importer that must be included in the application documents to the provincial health administrative department or the MOH. Also, if microorganisms are used to produce the food additive, the approved testing agency will provide appraisal reports on the bacteria strain and safety assessments.

**Domestic Food Additive Application**

Should a manufacturer wish to produce, market or use a new food additive variety or increase the application scope or use level of a previously approved food additive, an application for approval must be made to the local health administrative department at the provincial level. The provincial health administrative department will conduct a preliminary review of the food additive application, to verify if the application is complete, legitimate and consistent. Should the province accept the application, they will submit a preliminary opinion to the MOH. The MOH convenes an expert panel of toxicological, nutritional, medical, pharmaceutical and other technical personnel once every 2 months to undertake a technical review of all food additive applications collected during that time period and prepare opinions on the acceptability of the food additives.

**Imported Food Additive Application**

An importer wishing to market or use a new food additive variety or increase the application scope or use level of a previously approved food additive must submit an application for approval to the MOH. The MOH convenes an expert panel of toxicological, nutritional, medical, pharmaceutical, and other technical personnel once every 2 months to undertake a technical review of all food additive applications collected during that time period and prepare opinions on the acceptability of the food additives. If the food additive is manufactured overseas and is intended to be sold in China, the application also must include certificates from the government of the exporting country demonstrating that the food additive has been approved for production and marketing. Accreditation documents of the manufacturer produced by the regulated agency or organization from the country of origin must be provided. It is recommended by the Chinese Authorities that overseas applicants have the registration of new food additives handled by a representative officer or authorized agent in China.

**Hygiene License**

Once the applicant has obtained approval from the MOH regarding the safety of the food additive, the manufacturer must obtain a hygiene license before producing,
marketing or using the food additive. To obtain a hygiene license, the manufacturer must submit an application and documents to support the hygiene of the manufacturing facility, which should include information regarding production conditions and techniques, equipment, quality guarantee system and quality standards.

The Food Additive Hygiene Standard

The *Food Additive Hygiene Standard* lists permitted food additives for use in food in China. The original version of this *Standard* (GB 2760-1996) published December 29 1996 and implemented February 1 1997. Since its implementation, many new food additives or alterations in food additive uses have been approved by the MOH and subsequently published in circular reports from the MOH. On August 22, 2007, a new version of the *Food Additive Hygiene Standard* (GB 2760-2007) was issued and became effective June 1, 2008. The new *Standard* references the format and provisions of CODEX STAN 192 *General Standard for Food Additives* of codex Alimentarius Commission. Furthermore, this *Standard* replaces GB 2760-1996 as well as GB/T 12493-1990 (*Classification and Coding of Food Additives*).

The *Food Additive Hygiene Standard* (GB 2760-2007) provides categorized lists of food additives based on their use in food and further explains the scope of the application of the food additive and the maximum allowable levels to be used in food. The *Standard* also includes a list of permitted natural and synthetic flavorings, tentatively permitted flavorings, gum-based substances and ingredients permitted in chewing gum and processing aids recommended for food industry use.

Novel Food Regulations

In China, novel foods are known as new resource foods. On July 28, 1990, the *Hygiene Administration Regulation for New Resource Foods* became effective, establishing the regulatory process and supporting documents required for the regulatory approval and labeling of the new resource foods. In 2007, these *Regulations* underwent a substantial review and new regulations for new resource foods were drafted February 21, 2007. The *Regulations*, now named *Administrative Measures on New Resource Foods* (Order of the Ministry of Health No. 56) defined new resource foods as raw food materials or food ingredients which do not have a significant history of consumption in China, meaning that the regulatory process will apply to novel food ingredients and not final novel food products. Furthermore, new resource foods are separated into the following 4 categories: (1) animals, plants and microorganisms that are not traditionally consumed in China; (2) raw food materials that are derived from animals, plants, and microorganisms and are not traditionally consumed in China; (3) new varieties of microorganisms that are used during food processing; or (4) raw food materials, the original composition or structures of which are significantly changed by the adoption of new techniques during production. This definition, however, does not include transgenic foods, which are regulated by the Ministry of Agriculture and are outside the scope of this discussion.
Administrative Measures on New Resource Foods

On December 1 2007, the Administrative Measures for New Resource Foods became effective, establishing the regulatory process and supporting documents required for the regulatory approval and labeling of new resource foods. Under these Measures, applicants wishing to obtain regulatory approval for a new resource food must submit an application for registration to the MOH along with documentation supporting the safety of the new resource food. As the Administrative Measures for New Resource Foods (Order No. 56) establishes the regulatory approval process for new resource foods based on the safety of the food and not efficacy, new resource foods are not permitted to make health claims. The regulatory process for approval of new resource foods is described in detail below.

Approval from the Ministry of Health

New resource foods are evaluated on the basis of their safety or, if applicable, the basis of substantial equivalence to other approved new resource foods. Materials required for the new resource food application and assessment include the name of the new resource food, source of the material, production techniques and processes, testing methodologies, product quality standards, food hygiene quality analysis data, composition analysis data, estimated intake, toxicological data, regulatory status of the new resource food in foreign markets, new resource food labels, purpose and scope of use of the new resource food biological traits of strains, genetic stability, pathogenicity, virulence, etc. of microorganism products, and a sample of the new resource food.

Should a manufacturer or importer wish to produce or market a new resource food, an application for approval must be made to the MOH by sending the New Resource Food Registration Application Form along with all required materials listed within the Measures. A preliminary review of the new resource food application package will be conducted by an Assessment Committee consisting of toxicological, nutritional, medical, pharmaceutical, and other technical personnel to determine if the application is complete, legitimate and consistent. After the preliminary technical review, the Assessment Committee will determine the required safety testing, test sample batches, test methods, and test institution approved by the MOH. The cost of the testing conducted by the MOH approved institution will be the responsibility of the applicant. The Assessment Committee also will determine whether to conduct on-site examinations and collections of sealed samples and will inform the applicant. If it is determined that on-site examinations and collections of sealed samples are required following the preliminary technical assessment, the provincial health administration authority will organize the examinations and collections. Based on the Assessment Committee's conclusions following the technical review and the results of the testing agency and on-site examination, the MOH will conduct an administrative review to decide whether to accept the registration of the new resource food and inform the applicant of the decision. After the MOH has decided to accept the registration of the new resource food, a list of approved new resource foods will be published on the MOH website, which will include the
name, source, specification, usage scope, and purpose of the approved new resource food. Furthermore, any new resource food substantially equivalent to resource foods previously approved by the MOH can be directly sold in the Chinese market without approval by the MOH. It is recommended by the Chinese Authorities that overseas applicants have the registration of new resource foods handled by a representative officer or authorized agent in China.

Functional Food and Dietary Supplement Regulations

As a result of government restructuring in 1998, the MOH's Department of Drug Administration merged with the State Pharmaceutical Administration of China to become the State Drug Administration (SDA). The SDA was responsible for all drug manufacturing, trade and registration, whereas the responsibility of food manufacturing, trade and registration remained with the MOH. In 2003, however, the SDA was reorganized to become the State Food and Drug Administration (SFDA). The SFDA is based on the US Food and Drug Administration (US FDA), providing a centralized governmental organization that controls and regulates the safety management of foods, health foods and medicines. Interestingly, however, the regulations guiding food additives and new resource foods remained under the control of the MOH. As a result of the formation of the SFDA, new interim regulations regarding health foods were drafted and accepted by the State Council in 2005.

The Administration Regulation for Health Food, promulgated June 1 1996 by the MOH, was revised in 2005 by the SFDA and became the Interim Administrative Measures for Health Food Registration (Decree of the State Food and Drug Administration No. 19). The Interim Measures (2005) expand on the original definition of a health food as defined by the MOH. While the basic definition of a health food remained the same, such as regulating bodily functions for a specific population and not being permitted for the treatment of diseases, the SFDA expanded the definition to include vitamin or mineral fortified or enriched foods and further established that a health food is not to produce any harmful effects. The SFDA also published 27 accepted functions of health foods, such as regulating the immune system, delaying senility, memory enhancement, growth enhancement, anti-mutation and tumor inhibition, blood lipid regulation, libido enhancement, blood sugar regulation, etc. As before, the new definition did not provide any distinction between functional foods and dietary supplements. The definition retained that functional foods and dietary supplements are to be considered under the same regulation.

The Interim Measures (2005) also expand on the requirements for approval, providing outlines for the application process for new or changes in the composition of approved health foods and for transferring the manufacturing of the health food from one manufacturer to another. Furthermore, the measures differentiate between application processes for health foods that are manufactured in China (domestic health foods) and those that are manufactured overseas (imported health foods).
These measures further explain and define raw materials and supplementary materials used in the manufacture of health foods and the required standards that they must meet, as well as specifications for labels, testing, re-registration, re-examination, legal liabilities and supplementary provisions for health foods. While the Interim Measures (2005) provide much greater detail regarding application requirements in comparison to the former, the Interim Measures (2005) are not meant to be used alone. The SFDA has published seven other Interim documents stating specific requirements beyond those established in the Interim Measures (2005) for the application of health foods containing fungi, probiotic microflora, nucleic acids, wild plants, animal products and amino acid chelates, as well as nutritional supplements and health foods applying macro reticular resin separation and purification techniques. Furthermore, five other health food-related documents have been published by the SFDA outlining the supplementary requirements, requirements for application items and formats for applications, license and registration related notifications.

The basic approval process for health foods did not change between the MOH version of the Administrative Measures and the SFDA's version, with the exception that the SFDA now assumes the responsibility for health foods rather than the MOH.

Approval of Health Foods

The process for domestic and imported health food regulatory approval is similar, with the exception of where the application is originally sent. In the case of imported health foods, the use of a local Chinese agent to help with the application process is recommended by the SFDA as the agent will have access to notarized translators to translate any foreign documents into Chinese and will be able to advise the applicant of any requirements beyond the basic requirements presented in this chapter.

Before the application for a health food is sent to the local food and drug administration department or the SFDA, the applicant must send samples of the health food to testing agencies approved by the SFDA for testing and examination. Along with the health food sample, the applicant should prepare a product research and development report if the functions of the health food are within the specified functions of health foods published by the SFDA. A product research and development report should include information on the basic concept of the research and development of the health food, function screening process and expected effects. If the functions of the health food are outside the 27 functions recognized and published by the SFDA, the applicant should conduct animal and human trials as well as provide a function research and development report to the testing agency. The function research and development report includes information on the name of the function, the reason for application, function testing, evaluation methods and test results. If the applicant is unable to conduct animal and human trials, a reason should be supplied in writing with the sample and the function research and development report to the testing agency. The testing agency will conduct the animal and/or human trial at a fee to the applicant. The testing agency will conduct safety toxicology tests, function tests on the effective or main ingredients, hygiene tests and stability tests.
on the health food and provide a report of the results to the applicant that is to be included with the application to the SFDA or local food and drug administrative departments.

**Domestic Health Food Application**

A health food that is manufactured and sold within China must gain regulatory approval though the SFDA. Applicants must send the Domestic Health Food Registration Application Form along with all required materials listed in the *Interim Administrative Measures for Health Food Registration* (2005), as well as any other relevant SFDA published requirements and samples of health food, to the food and drug administrative departments in the provinces, autonomous regions or municipalities. The food and drug administrative departments will review the application to see if the application documents are standard and complete. If the application is accepted, the food and drug administrative departments also are responsible for checking the testing and sample production sites for compliance with good manufacturing practices. During the application process, the approved testing agency is to carry out the examination of the sample and re-examination provided from the food and drug administrative department within 50 days and produces a test report. The test report is to be sent to the SFDA as well as to the food and drug administrative departments of the province, autonomous region and municipality and the applicant. Upon receipt of the test and examination report from the testing agency and samples from the food and drug administrative departments, the SFDA will form an expert panel of food, nutritional, medical, pharmaceutical and other technical personnel to review the application dossiers and form an opinion accepting or rejecting the registration of the health food. This expert panel meets once a month to discuss the registration of new health food products. As stipulated within the *Interim Administrative Measures for Health Food Registration* (2005), the application process should take about 150 days from time of application to receipt of approval or rejection notification; however, depending on the quality of the submitted dossier and product, this process could take more than a year. Should the SFDA decide to accept the registration of the health food, the applicants will be issued a Domestic Health Food Registration Certificate.

**Imported Health Food Application**

An imported health food refers to a health food that is manufactured and sold overseas for more than a year that is intended to be sold within the territory of China. The registration process for an imported health food follows a similar examination process as a domestic health food, with a few exceptions.

The applicant must submit an Imported Health Food Registration application form along with application materials and product samples to the SFDA directly, rather than through the food and drug administrative departments of the provinces, autonomous regions or municipalities. As a result, the SFDA will examine the application materials for compliance with the standard format and completeness. If the application is accepted for further examination, the SFDA will send a test notification and product samples to the approved testing agencies, which will carry out the examination
of the sample and produce a test report. The SFDA may decide to examine the manufacturing site and experimental site to ensure compliance with good manufacturing practices. Upon receipt of the test report from the approved testing agency, the SFDA will assemble an expert panel of similar members as in the Domestic Health Food Registration process once a month to review the application dossiers and decide on approval or rejection of the health food registration. The entire application process should take approximately 140 days as stipulated within the Interim Administrative Measure for Health Food Registration (2005). This amount of time is less than the domestic registration as the application is sent directly to the SFDA and does not have to go through the food and drug administrative departments of the provinces, autonomous regions or municipalitie, however, depending on the quality of the dossier and product submitted for approval, this process could take longer than a year. If the health food is approved for registration, the SFDA will send notification along with an Imported Health Food Approval Certificate, which is valid for 5 years, effective after issuance.

Conclusions

In China, food additives are considered materials that are added to foods in an effort to improve quality, color, flavor, taste and for the purpose of preservation or the required processing techniques. Food additives not listed in the Food Additive Hygiene Standard or other official published sources must be approved by the Ministry of Health (MOH) before import and marketing. An application should be made to the local health administrative department at the provincial level or directly to the MOH. Prior to making an application, a sample of the food additive should be sent to a testing agency accredited by the provincial health administrative department. The testing agency will perform toxicity tests, if required, as well as hygiene tests. After the completion of the tests, the testing agency will provide a report to the manufacturer or importer that must be included in the application documents to the provincial health administration or MOH. The MOH shall arrange an expert meeting once every 2 months to decide if the applications for food additives gathered during that period shall be approved. Once approval has been granted, the manufacturer must obtain a hygiene license before producing or marketing the food additive. Overseas applicants are requested to have the registration of new food additives handled by a representative officer or authorized agent in China.

Novel foods in China are called new resource foods and are currently defined as raw food materials or food ingredients which do not have a significant history of consumption in China, meaning that the regulatory process will apply to novel food ingredients and not final novel food products. Furthermore, new resource foods are separated into 4 distinct categories. Similar to new food additives, new resource foods require regulatory approval from the MOH prior to being available for sale within the Chinese market. The approval process likewise includes the submission of an application to the MOH who will convene an Assessment Committee to decide the necessary product testing to be conducted by a testing agency approved
by the MOH and to decide whether on-site examinations and sample collections are required. Based on the conclusions of the Assessment Committee, the MOH will conduct an administrative review of the new resource food. The MOH will publish a list of approved new resource foods on the MOH website, which will include the name, source, specification, usage scope, and purpose of the approved new resource food. Furthermore, any new resource food substantially equivalent to resource foods previously approved by the MOH can be directly sold in the Chinese market without approval by the MOH. Overseas applicants also are requested to have the registration of new resource foods handled by a representative officer or authorized agent in China.

In contrast, functional foods and dietary supplements in China are considered a 'health food' and require registration with the State Food and Drug Administration (SFDA) in order to be permitted for import and sale. Health foods are permitted to be marketed with a health claim. However, before the application for registration will be accepted, testing of samples, including toxicity, efficacy, active ingredient, hygiene and stability tests, must be conducted by designated testing agencies. Health foods are permitted to make reference to health benefits or altered bodily functions, but may not make reference to the treatment or cure of disease. Imported health foods are required to be manufactured overseas for more than one year. An application for approval of a health food requires a list of health claims, concentrations and testing methods for the functional ingredients, manufacturing process information, product quality specifications, test certificates from a suitable test facility for toxicology, efficacy, formulation, contaminants, stability, sample product packaging, documents demonstrating the product is approved for sale in the country of origin, a sample of the product, evidence the product has been produced and marketed in the country of origin for more than 1 year, and standards from the country of origin. Application materials are required to be prepared in Chinese and translations should be notarized by a domestic notary to ensure consistency with the original. To facilitate the Chinese regulatory process, foreign applicants are advised to have the registration of health foods handled by a representative officer or authorized agent in China. Once the application for health food registration is received, the SFDA will provide a letter of acceptance or rejection, which is dependent upon receipt of the required application materials. If registration is granted, an Imported Health Food Approval Certificate is issued to the applicant.

The unique regulatory system established by the Chinese Authorities and presented in this chapter allows interested manufacturers and importers of this greatly expanding economy to easily comply with the regulations, while clearly establishing a safe food supply for the Chinese population.

References

Nutraceutical and Functional Food Regulations in the United States and Around the World

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POISON CENTRES

An analysis of energy-drink toxicity in the National Poison Data System

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Context. Small studies have associated energy drinks—beverages that typically contain high concentrations of caffeine and other stimulants—with serious adverse health events. Objective. To assess the incidence and outcomes of toxic exposures to caffeine-containing energy drinks, including caffeinated alcoholic energy drinks, and to evaluate the effect of regulatory actions and educational initiatives on the rates of energy drink exposures. Methods. We analyzed all unique cases of energy drink exposures reported to the US National Poison Data System (NPDS) between October 1, 2010 and September 30, 2011. We analyzed only exposures to caffeine-containing energy drinks consumed as a single product ingestion and categorized them as caffeine-containing non-alcoholic, alcoholic, or “unknown” for those with unknown formulations. Non-alcoholic energy drinks were further classified as those containing caffeine from a single source and those containing multiple stimulant additives, such as guarana or yerba mate. The data were analyzed for the demographics and outcomes of exposures (unknown data were not included in the denominator for percentages). The rates of change of energy drink-related calls to poison centers were analyzed before and after major regulatory events. Results. Of 2.3 million calls to the NPDS, 4854 (0.2%) were energy drink-related. The 3192 (65.8%) cases involving energy drinks with unknown additives were excluded. Of 1480 non-alcoholic energy drink cases, 50.7% were children < 6 years old; 76.7% were unintentional; and 60.8% were males. The incidence of moderate to major adverse effects of energy drink-related toxicity was 15.2% and 39.3% for non-alcoholic and alcoholic energy drinks, respectively. Major adverse effects consisted of three cases of seizure, two of non-ventricular dysrhythmia, one ventricular dysrhythmia, and one tachypnea. Of the 182 caffeinated alcoholic energy drink cases, 68.2% were < 20 years old; 76.7% were referred to a health care facility. Educational and legislative initiatives to enhance understanding of the health consequences of energy drink consumption were significantly associated with a decreased rate of energy drink-related cases (p = 0.036). Conclusions. About half the cases of energy drink-related toxicity involved unintentional exposures by children < 6 years old. Educational campaigns and legal restrictions on the sale of energy drinks were associated with decreasing calls to poison centers for energy drink toxicity and are encouraged.

Keywords energy drink; caffeine; poison control; overdose

Introduction

Energy drinks are beverages that typically contain high concentrations of caffeine, as well as vitamins, herbal supplements, and sweeteners. Most are marketed for their stimulant properties, likely linked to their caffeine content, with claims of increasing energy, weight loss, stamina, athletic performance, and concentration. Common additives, however, such as guarana, kola nut, yerba mate, and cocoa, also have stimulant, cardiac, and hematologic activity. These ingredients are often non-standardized in their caffeine content, sometimes considered as masked caffeine. Hence, the actual amount of caffeine content in energy drinks may be higher than that reported in their labels (Table 1).

The threshold of caffeine toxicity is 400 mg/day in healthy adults (≥ 19 years old), 100 mg/day in healthy adolescents (12–18 years old), and 2.5 mg/kg/day in healthy children (< 12 years old). One 8-ounce can of a popular energy drink contains approximately 240 mg of caffeine, well above the threshold for toxicity. These high caffeine contents point to the potential for caffeine toxicity in energy drink ingestions.
Energy drink toxicity

Energy drink provides 77 mg of caffeine (or 1.1 mg/kg) for a 70-kg male and twice that, 2.2 mg/kg, for a 35-kg pre-teen.5

Caffeine intoxication is a clinical syndrome of nervousness, irritability, anxiety, insomnia, tremor, tachycardia, palpitations, and gastrointestinal upset.2,6,7 Additional adverse effects include vomiting and abdominal pain, hypokalemia, hallucinations, increased intracranial pressure, cerebral edema, stroke, paralysis, altered consciousness, rigidity, seizures, arrhythmias, and death.1–3,7–9 Some case reports, scientific studies, and surveys have associated many of these serious adverse effects with energy drink ingestion.6,7,8,10–18 The American Academy of Pediatrics holds that “Caffeine and other stimulants contained in energy drinks have no place in the diet of children and adolescents.”19

Prior to January 2011, pre-packaged alcoholic energy drinks were available for purchase. Due to concerns regarding their safety the Food and Drug Administration (FDA) removed these co-formulated beverages from the market.20 The use and effects of energy drinks are not well-understood, but several adverse effects have been linked with energy drink consumption.6,7,8,13,16 Emergency department visits for energy drink ingestion increased more than 10-fold between 2005 and 2009, from 1128 to 13,114,21 and continued to substantially increase since 2009 to 20,783 in 2011.22

Despite the claim by the American Beverage Association (ABA) that “children and teens are not large consumers of energy drinks” and that “total caffeine consumption from energy drinks among pre-teens is nearly zero,”23 self-report surveys indicate that approximately 30–50% of children, adolescents, and young adults reported consuming more than one energy drink a month.1,24,25 The Center for Disease Control and Prevention estimates that daily consumption of energy drinks among high school students is slightly less than soda consumption (5% vs. 7.1%) and about one-third that of daily coffee or tea consumption (14.8%).26

In 2010, the US National Poison Data System (NPDS) added generic category codes (energy drinks with a single-source of caffeine, with caffeine from multiple sources, non-caffeinated, alcohol-containing, and unknown formulations) for caffeine toxicity so cases specifically related to energy drink calls can now be tracked.27 Here, we report the first year of data from these category codes and identify the demographic characteristics of individuals experiencing energy drink-related toxicities and the associated clinical effects and outcomes. We also evaluate the impact of regulatory actions and educational initiatives in the rate of energy drink exposures reported to NPDS.

Methods

The Human Subjects Research Office of the University of Miami Miller School of Medicine determined that this study does not constitute Human Subjects research. This was based on the fact that the data analyzed in this study do not contain any of the HIPAA identifiers. As such, it was not subject to Institutional Review Board review under 45 CFR 46.

We queried all closed, unique cases coded as energy drink exposures in the NPDS between October 1, 2010 and September 30, 2011. We analyzed only exposures to caffeine-containing energy drinks consumed as a single product ingestion. Energy drinks were categorized as caffeine-containing

Table 1. Examples of caffeinated energy drinks and caffeinated soft drinks sold in the US.

<table>
<thead>
<tr>
<th>Top-selling energy drinks*</th>
<th>Ounces per bottle or can</th>
<th>Caffeine concentration, mg/oz</th>
<th>Total caffeine, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Bull</td>
<td>8.3</td>
<td>9.6</td>
<td>80</td>
</tr>
<tr>
<td>Monster</td>
<td>16.0</td>
<td>10.0</td>
<td>160</td>
</tr>
<tr>
<td>No Fear</td>
<td>16.0</td>
<td>10.9</td>
<td>174</td>
</tr>
<tr>
<td>Higher caffeine energy drinks†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wired X505</td>
<td>24.0</td>
<td>21.0</td>
<td>505</td>
</tr>
<tr>
<td>Wired X344</td>
<td>16.0</td>
<td>21.5</td>
<td>344</td>
</tr>
<tr>
<td>Jolt cola</td>
<td>23.5</td>
<td>11.9</td>
<td>280</td>
</tr>
<tr>
<td>Lower caffeine energy drinks‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bomba Energy</td>
<td>8.4</td>
<td>8.9</td>
<td>75</td>
</tr>
<tr>
<td>HiBall Energy</td>
<td>10.0</td>
<td>7.5</td>
<td>75</td>
</tr>
<tr>
<td>Vitamin Water (energy citrus)</td>
<td>20.0</td>
<td>2.5</td>
<td>50</td>
</tr>
<tr>
<td>High concentration energy drinks†</td>
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<td></td>
</tr>
<tr>
<td>RedLine Power Rush</td>
<td>2.5</td>
<td>140.0</td>
<td>350</td>
</tr>
<tr>
<td>Powershott</td>
<td>1.0</td>
<td>100.0</td>
<td>100</td>
</tr>
<tr>
<td>Fuel Cell</td>
<td>2.0</td>
<td>90.0</td>
<td>180</td>
</tr>
<tr>
<td>Classic soft drinks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coca-Cola Classic</td>
<td>12.0</td>
<td>2.9</td>
<td>34.5</td>
</tr>
<tr>
<td>Dr Pepper</td>
<td>12.0</td>
<td>3.4</td>
<td>41</td>
</tr>
<tr>
<td>Mountain Dew</td>
<td>12.0</td>
<td>4.5</td>
<td>54</td>
</tr>
</tbody>
</table>

*Top selling energy drinks in the US in 2006, listed sequentially as a percentage of market share (packaged facts, 2007).
†Examples of energy drinks selected from the hundreds of energy drink products marketed in the US.
‡Adapted from Reissig et al. 20095
nonalcoholic, alcoholic, or “unknown” for those with unknown formulations. Non-alcoholic energy drinks were further classified as those containing caffeine from a single source with no additional stimulant additives and those containing multiple stimulant additives, such as guarana or yerba mate. Because comparisons among substances in energy drinks could not be made when the formulation was unknown, energy drink exposures to substances with unknown ingredients were excluded from analysis. Energy drinks taken in conjunction with other substances (co-ingestants), such as other medications in overdose, illegal drugs or toxic chemicals, or those categorized as “unknown”, were also excluded from this analysis. The NPDS classifies the severity of adverse effects in five ordinal categories: no effect (no signs or symptoms resulting from the exposure); minor (“minimally bothersome signs and symptoms that resolved rapidly”); moderate (“more pronounced, prolonged, or systemic signs and symptoms requiring treatment but not life-threatening”); major (“life-threatening signs or symptoms or marked residual disability”); and death (as a direct result or complication of the poison exposure).

We also analyzed the demographics, clinical effects, therapies, outcomes of the included exposures, and the rates of change of NPDS reports of energy drink exposures in relation to the FDA warnings on alcoholic energy drinks reported on November 17, 2010, the release of the American Academy of Pediatrics’ nutrition recommendations published on May 29, 2011, and increased media coverage surrounding February 2011 publications related to the health consequences of energy drinks.19,20,28–30

**Statistical analyses**

Data are reported as frequencies and percentages. Unknown data are reported but not included in the denominator for percentages. Chi-square tests were used for primary comparisons among age categories, sex, intentional or unintentional exposure, the presence or absence of alcohol in the drink involved, and the severity of adverse effects. The Spearman rank-sum test was used to analyze actual age differences. The effects of educational and federal legislative actions on reported exposure incidence trends in the NPDS over time were analyzed with a general linear model. The data conformed to the assumptions of the test used to analyze them. Alpha was set at 0.05, and all tests were two-tailed. The SAS 9.3 statistical software program was used in the analysis.

**Results**

Of the 2.4 million calls received during the study period, 4854 (0.2%) were for energy drink exposure cases. The 3192 (65.8%) cases involving energy drinks categorized as “unknown” or confounded with co-ingestants were excluded. Of the remaining energy drink-related cases, 1662 (34%)—1480 nonalcoholic and 182 alcoholic—were included in the analysis (Fig. 1). Overall, 46% of cases (734 of 1588 cases with reported age) were children less than 6 years old. The median (inner quartiles) ages for non-alcoholic and alcoholic energy drink cases were 5 (2 – 18) and 17 (15 – 21) years old.

**Fig. 1.** Sample selection and data availability of energy drink-related cases to the US National Poison Data System, October 1, 2010 through September 30, 2011.
Exposures to caffeinated non-alcoholic energy drinks

When non-alcoholic energy drink exposures are viewed as a whole, of 1480 cases, 717 (50.7% of 1,425 who reported age) were children less than 6 years old; 896 (60.8% of 1475 who reported gender) were males; 969 (76.0% of 1,251 who reported intentionality) were unintentional; and 210 (15.2% of 789 who had available referral data) were coded as “moderate” or “major” adverse effects (Table 2). This is a similar age distribution for other types of poisonings where in Table 2 we show that 49.6% (of 2,330,538 reporting) of all human exposure cases in the NPDS involve children less than 6 years old. Compared with the overall database, pre-adolescents and adolescents are more frequently represented in these exposures. Compared with all of the other age categories, children less than 6 years old had the highest proportion of unintentional exposures to non-alcoholic energy drinks, whereas teenagers had the highest proportions of intentional exposures in non-alcoholic energy drinks (Fig. 2a). When comparing only the teenagers with the ≥ 20 category, teenagers had a higher proportion of intentional exposures to non-alcoholic energy drinks (57.1% vs. 42.9%; Fig. 2b). Minor or moderate adverse effects were reported in 28% (103 of 372 reporting) of children less than 6 years old. No major effects were reported. Adolescents represented the largest proportion of cases of minor to moderate, with 1 major adverse effect (Fig. 3a). Other major adverse effects were reported among the 6–12 year old (n = 1) and the 20 years and older age group (n = 5). Overall, major effects reported consisted of three cases of seizures, two of non-ventricular dysrhythmias, one of ventricular dysrhythmia, and one of tachypnea.

Of the 1480 cases of non-alcoholic energy drinks, 946 were caffeine-only and 534 had additives. The two groups differed significantly in age, intentional or unintentional exposure, referrals to a health care facility, and severity of effects. Compared with energy drinks with additives, caffeine-only cases had a significantly greater proportion of cases less than 6 years old and unintentional exposures. A significantly greater proportion of cases involving additives was referred to a healthcare facility and experienced more minor, moderate, or major toxic effects (Table 2). Among those in the caffeine-only group who were referred to a healthcare facility, 34 cases resulted in hospital admissions (13 to a critical care unit, 18 to a non-critical care unit, and 3 to a psychiatric facility); the remaining 192 cases (84.9% of 233 reporting) were treated and released. There were no deaths.

Table 2. Characteristics of energy drink-related toxicity cases in the US National Poison Data System (NPDS).*  

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All cases in the NPDS (n = 2,343,834)</th>
<th>Caffeine only (n = 946)</th>
<th>Caffeine + additives (n = 534)</th>
<th>Non-alcoholic caffeinated (caffeine ≥ additives) (n = 1,480)</th>
<th>Alcoholic caffeinated (n = 182)</th>
<th>p †</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age categories, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–5 years</td>
<td>1,156,383 (49.6)</td>
<td>501 (55.6)</td>
<td>216 (42.0)</td>
<td>717 (50.7)</td>
<td>17 (9.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>6–12 years</td>
<td>145,674 (6.3)</td>
<td>82 (9.1)</td>
<td>67 (13.0)</td>
<td>149 (10.5)</td>
<td>7 (4.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>13–19 years</td>
<td>158,565 (6.8)</td>
<td>127 (14.1)</td>
<td>122 (23.8)</td>
<td>249 (17.6)</td>
<td>94 (54.3)</td>
<td></td>
</tr>
<tr>
<td>≥ 20 years</td>
<td>869,916 (37.3)</td>
<td>191 (21.2)</td>
<td>109 (21.2)</td>
<td>300 (21.2)</td>
<td>55 (31.8)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>13,296</td>
<td>45</td>
<td>20</td>
<td>65</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1,136,598 (48.7)</td>
<td>568 (60.4)</td>
<td>328 (62.4)</td>
<td>896 (60.8)</td>
<td>91 (50.3)</td>
<td>0.688</td>
</tr>
<tr>
<td>Female</td>
<td>1,196,680 (51.3)</td>
<td>373 (39.6)</td>
<td>206 (38.6)</td>
<td>579 (39.2)</td>
<td>90 (49.7)</td>
<td>0.001</td>
</tr>
<tr>
<td>Unknown</td>
<td>10,556</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Intentional exposure, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unintentional</td>
<td>1,754,061 (83.3)</td>
<td>643 (78.8)</td>
<td>317 (72.9)</td>
<td>960 (76.7)</td>
<td>36 (21.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intentional</td>
<td>350,883 (16.7)</td>
<td>173 (21.2)</td>
<td>118 (27.1)</td>
<td>291 (23.3)</td>
<td>131 (78.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Unknown</td>
<td>238,890</td>
<td>130</td>
<td>99</td>
<td>229</td>
<td>15</td>
<td></td>
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<tr>
<td>Referrals, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-healthcare facility</td>
<td>1,644,861 (71.5)</td>
<td>693 (75.4)</td>
<td>365 (70.5)</td>
<td>1058 (73.6)</td>
<td>41 (23.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Healthcare facility</td>
<td>654,655 (28.5)</td>
<td>226 (24.6)</td>
<td>153 (29.5)</td>
<td>379 (26.4)</td>
<td>135 (76.7)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>44,318</td>
<td>27</td>
<td>16</td>
<td>43</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Severity of toxicity, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No effect</td>
<td>450,605 (46.2)</td>
<td>232 (47.2)</td>
<td>127 (42.8)</td>
<td>359 (45.5)</td>
<td>13 (11.1)</td>
<td>0.039</td>
</tr>
<tr>
<td>Minor effect</td>
<td>354,546 (36.4)</td>
<td>196 (39.8)</td>
<td>114 (38.4)</td>
<td>310 (39.3)</td>
<td>58 (49.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Moderate effect</td>
<td>147,517 (15.1)</td>
<td>58 (11.8)</td>
<td>55 (18.5)</td>
<td>113 (14.3)</td>
<td>42 (35.9)</td>
<td></td>
</tr>
<tr>
<td>Major effect</td>
<td>22,049 (2.3)</td>
<td>6 (1.2)</td>
<td>1 (0.3)</td>
<td>7 (0.9)</td>
<td>4 (3.4)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>1,369,117</td>
<td>454</td>
<td>237</td>
<td>691</td>
<td>65</td>
<td></td>
</tr>
</tbody>
</table>

*Numbers and percentages are based on the number of cases for which data are provided (see Fig. 1).

†p value for differences between cases of caffeine-only and caffeine + additives energy drinks.

‡p value for differences between all non-alcoholic cases (caffeine-only plus caffeine + additives) and alcohol-containing energy drink cases.

§“No effect” = no signs or symptoms resulting from the exposure; “Minor” = minimally bothersome signs and symptoms that resolved rapidly; “Moderate” = more pronounced, prolonged, or systemic signs and symptoms requiring treatment but not life-threatening; “Major” = life-threatening signs or symptoms or marked residual disability; “Death” = as a direct result or complication of the poison exposure.
In the 182 cases (11% of 1662 reported single-substance energy drink cases) involving alcoholic energy drinks, males and females were equally involved, whereas most non-alcoholic cases involved males. Although more than half of these ingestions were among adolescents, 17 (9.8% of 173 reporting) were among children (Table 2). Overall, the proportion of intentional exposures was significantly higher in alcoholic than in non-alcoholic cases. However, 16 children less than 6 years old (44.1% of 36 reporting) had the highest proportion of unintentional exposures to alcoholic energy drinks, whereas 82 teenagers 13–19 years old (65.6% of 125 reporting) had the highest proportion of intentional exposures (Fig. 2c). Furthermore, the proportion of teenagers who intentionally consumed alcoholic energy drinks was twice that of those 20 years or older (68.3 vs. 31.7; Fig. 2b).

Exposures to caffeinated alcoholic energy drinks

In the 182 cases (11% of 1662 reported single-substance energy drink cases) involving alcoholic energy drinks, males and females were equally involved, whereas most non-alcoholic cases involved males. Although more than half of these ingestions were among adolescents, 17 (9.8% of 173 reporting) were among children (Table 2). Overall, the proportion of intentional exposures was significantly higher in alcoholic than in non-alcoholic cases. However, 16 children less than 6 years old (44.1% of 36 reporting) had the highest proportion of unintentional exposures to alcoholic energy drinks, whereas 82 teenagers 13–19 years old (65.6% of 125 reporting) had the highest proportion of intentional exposures (Fig. 2c). Furthermore, the proportion of teenagers who intentionally consumed alcoholic energy drinks was twice that of those 20 years or older (68.3 vs. 31.7; Fig. 2b).

A greater proportion of alcoholic energy-drink cases, compared with non-alcoholic were advised to seek treatment in a health-care facility (Table 2). Of 23 reported admissions 9 were to a critical care unit, 12 to a non-critical care unit, and 2 to a psychiatric facility; 71 (75.5% of 94 reporting) were treated and released. The recommended care did not differ significantly from that for cases of non-alcoholic energy drinks.

Changes in the frequency of cases for energy drink-related toxicities

In the winter and spring of 2010–2011, consumer awareness efforts increased. Most notable were the FDA statement regarding the health risks of alcohol-containing energy drinks and their removal from the market; publications with extraordinarily large global media coverage in February 2011;1,31 the American Academy of Pediatrics’ statement that energy drinks have no nutritional value for children and adolescents; and extensive media coverage of these events, related research findings, and consumer concern about possible health risks.1,19,20,28–31,32 The number of cases involving energy drinks during that time, between

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Fig. 2. Number of cases reported to US Poison Control Centers for (a) non-alcoholic energy drinks by age and intentionality (n = 1212); (b) non-alcoholic and alcoholic energy drinks by intentionality comparing only the 13–19 and ≥ 20 age groups; and (c) alcoholic energy drinks by age and intentionality (n = 162). Diagonal line-filled bars, cases reported as “intentional”; Dot-filled bars, cases reported as “unintentional” (colour version of this figure can be found in the online version at www.informahealthcare.com/ctx).
October 2010 and March 2011, increased at a significant rate (slope = 17.7 ± 4.33; *p* = 0.004), and was then followed by a significant downward trend (slope = −6.54; *p* = 0.036; Fig. 4). Further, the trends were significantly different before and after regulatory and media effects (*R*² = 0.83; *p* = 0.002; Fig. 4). The trends in cases involving alcohol-containing energy drinks did not reach statistical significance, however, the intercepts before and after the FDA statement were each significant and were significantly different from each other.

**Discussion**

The true incidence of energy drink consumption is unknown but probably under-reported. Recent surveys in the US and elsewhere have produced findings similar to ours. A 2009 cross-sectional study of a convenience sample of 2158 respondents at least 18 years old from two San Diego emergency departments found that 1298 (60.1%) reported having consumed energy drinks, and most were male. Respondents 18–29 years old were most likely to use energy drinks, and more than 6% of all respondents used energy drinks with alcohol. In a retrospective study of 297 energy drink cases reported to an Australian poison information center between 2004 and 2010, the predominance of cases involving males increased annually (to 57%), and 21% involved accidental exposure of children (mean age, 38 months; range, 7 months–10 years). Of the recreational users, 50% simultaneously consumed alcohol, and 44% also consumed other caffeinated products. Forty-three percent (19% with no co-ingestants) required hospitalization, and 7% had adverse effects that included...
Children less than 6 years old were the largest proportion of the energy drink–related poison center calls (non-alcoholic and alcoholic combined). This is roughly equal to the proportion of exposures to other substances in this age group in NPDS, whereas the proportion of older children and adolescents exposed to energy drinks is much higher than to other substances in the database.

Given what is known about other unintentional childhood toxic exposures, these results suggest that energy drinks are easily within reach of children and that packaging is appealing and looks like other beverages children consume.\textsuperscript{3,4} If so, similar preventive measures should be used: childproof containers, unappealing packaging, and keeping products out of the reach of children. Adult consumers of energy drinks must be alerted to the potential adverse effects of energy drinks in children, which may occur in as many as 70% after only one serving.\textsuperscript{37}

Some energy drink containers are labeled with a warning that they may not be safe for children, those who are sensitive to caffeine, or for pregnant or nursing women. The labeling is voluntary and is contrary to marketing strategies that appear to be aimed at youth.\textsuperscript{38–40} Marketing campaigns often target extreme sporting events, sponsor athletes, associate products with illegal stimulant drugs, and advertise products in media (including social media websites and video games) oriented to children, adolescents, and young adults.\textsuperscript{26,41} Such strategies may explain the male and youth predominance of cases involving energy drink ingestion.\textsuperscript{39} However, lawmakers question these marketing strategies, inquiring if any studies were done or underwritten by these marketers that have examined the effects of energy drink use on this younger population, given that the limited studies have taken place among adults.\textsuperscript{42}

Although the clinical effects of caffeine have been well studied, and caffeine is generally recognized as safe by the FDA,\textsuperscript{43} the effects of caffeine in energy drinks, where it may be combined with a number of other substances, is still relatively under-studied in vivo, and remain a concern, especially for children and people with certain medical conditions carrying increased risk for energy drink toxicity, such as cardiovascular, renal, or liver disease; seizures; diabetes; mood and behavioral disorders; hyperthyroidism; or those who take certain medications.\textsuperscript{1,6,7,8,14,15,44} Data on existing health conditions are routinely collected by regional poison centers, but these data are not included in the NPDS dataset and so were not analyzed here.

Nevertheless, the risk of energy drink toxicity to young children is apparent and likely underrecognized by caretakers. Poison prevention materials routinely alert parents to the dangers of household cleaning agents, medications, toxic plants, make-up, and art supplies,\textsuperscript{45–47} and the addition of the dangers of energy drinks may also have an impact on these exposures.

The NPDS has several limitations for determining the frequency and severity of product-related adverse effects. First, the NPDS is based on self-reported calls to poison control centers. Thus, NPDS exposures are only a portion of the total number of incidents that actually occur and likely under-estimate the incidence of energy drink–related toxicity. There may be reporting bias, for example, in which parents of infants and children are more likely to call a poison center than for adolescents or for themselves. In fact,
the Institute of Medicine estimates that NPDS captures only about 50% of the actual poisonings that occur each year.\(^4\) Limitations in the data collection methods inherent in the database required excluding more than 3000 cases of energy drink ingestion for insufficient information. The NPDS database does not include detailed case information, association of management or temporal relationships between clinical effects and treatments. The presence or absence of substances in the body are usually not confirmed by laboratory testing and, although we have attempted to limit the analysis to single-substance exposures, we cannot confirm exposures and co-ingestants that may have been present. Lastly, data are collected by telephone, so subtle clinical effects, such as electrocardiographic changes, could easily be missed or omitted during poison center follow-up, and in most cases, it is not possible to confirm exposure data through blood or other tests. If a portion of cases represents mistaken exposures, or if there is reporting bias in favor of exposures with greater toxicity, the true danger associated with energy drinks reported here would be overstated. On the other hand, under-reporting of clinical effects has been documented in some poison-control center data.\(^49\)\(^50\)

**Conclusion**

The NPDS provides the first comprehensive means to collect standardized information about potentially harmful exposures from energy drinks. Although it is limited to self-initiated calls, the database provides another method for tracking these types of exposures. We now have our first look into energy drink use and outcomes among children, adolescents, and adults across the US, which are consistent with those of poison centers in other countries.\(^16\)\(^34\)\(^37\)\(^51\) Most calls for alcoholic energy drink ingestion resulted in advice to seek medical care and resulted in critical care unit admissions for almost 10% of cases. Although some of the major effects could be secondary to the alcohol content and/or the interaction of alcohol with other energy drink constituents, nevertheless the findings here underscore the importance of the FDA ban on pre-mixed drinks.

The incidence of moderate to major adverse effects of energy drink-related toxicity was 15.2% and 39.3% for non-alcoholic and alcoholic energy drinks, respectively, although the incidence may be under-reported. About half the cases of energy drink-related toxicity involve unintentional exposures by children less than 6 years old. Educational campaigns and legal restrictions on the sale of energy drinks are associated with decreasing calls to poison centers for energy drink consumption. If a cause and effect relationship can be shown for regulatory and/or educational initiatives and a reduction in such exposures and for a correlation with the NPDS database, this national surveillance data may assist in guiding policy change decisions in an evidence-based way.\(^52\) The recent report from the American Medical Association stating their support to ban the marketing of energy drinks to children under 18\(^18\) together with statements from the American Academy of Pediatrics claiming energy drinks have no place in the diet of children and adolescents\(^19\) shows a growing support from physicians to protect children from the potentially adverse effects of caffeinated energy drinks by decreasing the likelihood of their exposure.

**Declaration of interest**

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

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**References**


Speaker Recommended References

DAY 1, SESSION 3: CAFFEINE EFFECTS ON THE CARDIOVASCULAR SYSTEM


Long-term alcohol and caffeine intake and risk of sudden cardiac death in women

Monica L Bertoia, Elizabeth W Triche, Dominique S Michaud, Ana Baylin, Joseph W Hogan, Marian L Neuhausser, Matthew S Freiberg, Matthew A Allison, Monika M Safford, Wenjun Li, Yasmin Mossavar-Rahmani, Milagros C Rosal, and Charles B Eaton

ABSTRACT

Background: Alcohol and caffeine intakes may play a role in the development of sudden cardiac death (SCD) because of their effects on cholesterol, blood pressure, heart rate variability, and inflammation.

Objective: Our objective was to examine the association between long-term alcohol and caffeine intakes and risk of SCD in women.

Design: We examined 93,676 postmenopausal women who participated in the Women’s Health Initiative Observational Study. Women were enrolled between 1993 and 1998 and were followed until August 2009. Women completed a food-frequency questionnaire at baseline and again at year 3. We modeled exposure to alcohol 3 ways: by using baseline intake only, a cumulative average of baseline and year 3 intake, and the most recent reported intake (a simple time-varying analysis).

Results: Intake of 5–15 g alcohol/d (about one drink) was associated with a nonsignificantly reduced risk of SCD compared with 0.1–5 g/d of baseline intake (HR: 0.64; 95% CI: 0.40, 1.02), of cumulative average intake (HR: 0.69; 95% CI: 0.43, 1.11), and of most recent intake (HR: 0.58; 95% CI: 0.35, 0.96), with adjustment for age, race, income, smoking, body mass index, physical activity, hormone use, and total energy. No association was found between SCD and total caffeine intake (mg/d) or cups of caffeinated coffee, decaffeinated coffee, and decaffeinated tea.

Conclusions: Our results suggest that about one drink per day (or 5.1–15 g/d) may be associated with a reduced risk of SCD compared with a baseline intake only, a cumulative average of baseline and year 3 intake, and the most recent reported intake (a simple time-varying analysis).

INTRODUCTION

Sudden cardiac death (SCD)4 accounts for approximately one-half of all cardiac deaths (1), and, although coronary artery disease (CAD) underlies most SCD events, SCD may be the first manifestation of CAD in many individuals, especially women (2). Furthermore, women experience fewer SCD events than do men and are underrepresented in most studies of CAD, which makes the etiology of SCD in women less clear. Current efforts aimed at the primary prevention of SCD have focused on the placement of implantable defibrillators in patients with left ventricular dysfunction; however, only 30% of SCD events occur in this high-risk group (3), and prevention strategies applicable to the entire population are needed. Dietary habits, such as alcohol and caffeine intake, are associated with risk of CAD, are changeable, and therefore may provide a potential focus of preventative efforts.

Much previous research regarding alcohol and caffeine intakes and SCD has examined the acute effects of heavy consumption. For example, binge drinking can create a hyperadrenergic state that may induce arrhythmias (4). Long-term rather than acute intake of alcohol and caffeine may also influence the risk of SCD. For example, moderate alcohol intake has a beneficial effect on lipids, plaque rupture, and inflammation. Three large cohort studies confirm the adverse effect of heavy drinking and suggest a protective effect of moderate drinking on risk of SCD in men (5–7); however, other studies in men found no association (8–10) or an adverse effect (11). To date, only one study has examined the effect of long-term alcohol intake in women; this study suggests that light-to-moderate drinking is associated with a reduced risk of SCD (12).

Like alcohol, coffee increases HDL cholesterol (13) which may protect against the development of atherosclerosis and SCD. On the other hand, coffee negatively effects subclinical inflammation (13) and plasma homocysteine concentrations (14).

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4Abbreviations used: CAD, coronary artery disease; FFQ, food-frequency questionnaire; MI, myocardial infarction; SCD, sudden cardiac death; WHI, Women’s Health Initiative.

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The association between SCD and caffeine is less studied compared with alcohol (15, 16). Some studies show that large amounts of caffeine are associated with an increased risk of acute cardiac events (17–19). Case-control studies tend to show a negative effect of higher coffee intake, but cohort studies show either no effect or a protective effect on cardiovascular disease risk (16). Only one study has examined the association of long-term caffeine intake with risk of SCD; no relation between coffee and risk of SCD was found in men and women with recent nonfatal myocardial infarction (MI) (15).

Our objective was to investigate the relation between long-term alcohol and caffeine intakes and risk of SCD. Because heavy intake of alcohol and caffeine is associated with an increased risk of cardiovascular disease events, and because both may be linked to SCD through similar mechanisms, we chose to examine them simultaneously. We examined this association in postmenopausal women who now have the greatest population burden of cardiovascular disease, including SCD (20). We additionally analyzed the association of alcohol and caffeine with risk of non-SCD to explore potential differential associations.

SUBJECTS AND METHODS

Study participants

The Women’s Health Initiative (WHI) Observational Study included 93,676 study participants at 40 study sites across the United States (21). All participants were female, postmenopausal, and aged 50–79 y at baseline (1993–1998). Women were excluded if they did not provide written informed consent, did not plan to reside in the study recruitment area for ≥3 y, had medical conditions predictive of a survival time of <3 y, had characteristics inconsistent with study compliance (alcoholism, drug dependency, mental illness, dementia), or were actively participating in another controlled trial. Other exclusion criteria were used for each of the trials, as described previously (22).

Exposure measurement

Dietary intake (total alcohol, wine, beer, liquor, and total caffeine) was measured in the WHI by using a validated (23) semiquantitative food-frequency questionnaire (FFQ) designed specifically for this postmenopausal population. Women completed the FFQ at baseline and year 3. The WHI FFQ asked participants to recall diet over the past 3 mo and included 122 items. Women reported their intake of beer, wine, and liquor using the following frequency categories for numbers of servings: never or <1/mo, 1–3/mo, 1/wk, 2–4/wk, 5–6/wk, 1/d, 2–3/d, 4–5/d, and ≥6/d. Medium servings were defined as a 12-oz (355-mL) can or bottle of beer, a 6-oz (177-mL) glass of wine, or 1 shot (1.5 oz, or 44 mL) of liquor. In a separate questionnaire, women reported whether they had ever consumed 12 drinks of any kind of alcohol over their lifetime and whether they still drank alcohol.

Total caffeine intake (in g/d) was calculated from the FFQ based on intake of the following beverages: soda (all types), coffee, and tea. Intake of these beverages was reported according to the same frequency categories as alcohol, and medium servings were defined as 12 oz (355 mL) or 1 can of soda and an 8-oz (237-mL) cup of coffee or tea. The FFQ did not differentiate between caffeinated and decaffeinated soda, coffee, or tea. The FFQ also did not ask about chocolate intake, so we could not consider this source of caffeine in the diet. The caffeine content of medications was not available in this data set; therefore, we were unable to consider this source of caffeine intake. The WHI FFQ nutrient database was derived from the Nutrition Data Systems for Research food and nutrient database (Nutrition Coordinating Center, University of Minnesota) (23).

Although the FFQ did not distinguish between caffeinated and decaffeinated coffee and tea, a separate questionnaire administered at baseline asked women about their usual consumption of regular (caffeinated) coffee, decaffeinated coffee, and regular tea. The exact questions were as follows: “How many cups of regular coffee (not decaf) do you usually drink each day? [count tall (12 oz. or more) cups and espresso drinks made with double shots as 2 cups],” “How many cups of decaf coffee do you usually drink each day? [count tall (12 oz. or more) cups and espresso drinks made with double shots of espresso as 2 cups],” and “How many cups of tea do you usually drink each day? (do not include decaf or herbal tea).”

Outcome measurement

Our primary endpoint, incident SCD, was defined as death from fatal MI, fatal definite CAD, or fatal possible CAD, and this cardiac death must have occurred within 1 h of symptom onset. Trained physician adjudicators reviewed medical records of potential CAD death cases, including death certificates, autopsy reports, circumstances of death recorded by next of kin, and all hospital records, including electrocardiograms, laboratory test results, and reports from all relevant cardiac procedures (hospitalized and nonhospitalized) (24). The medical record or interview of witnesses had to document that patient collapse was directly observed, as by hospital notes and cardiopulmonary resuscitation records or by a relative or observer clearly reporting that the patient was found unresponsive within <60 min from previous direct observation of stable clinical status. As a secondary analysis, we also examined non-SCD, defined as death >24 h after symptom onset. We excluded rapid deaths (1–24 h; n = 157) from all analyses (SCD and non-SCD) because of potential misclassification bias.

Covariates

Sociodemographic variables were measured by interview or by self-report at baseline with the use of standardized questionnaires (age, race, income, and education). Traditional CAD risk factors were also measured by self-report at baseline with the use of questionnaires [smoking status and physical activity (metabolic equivalents) per week from recreational activity] and by trained, certified staff at the baseline exam [height, weight, BMI (in kg/m²), and waist-to-hip ratio]. Height was measured with a stadiometer, weight was measured while the participants were wearing light clothing, and BMI was calculated as weight (in kg) divided by height (in m) squared. Waist circumference was measured at the natural waist or narrowest part of the torso and hip circumference at the maximal circumference, both to the nearest 0.1 cm. Waist-to-hip ratio was calculated as the ratio of these 2 measures. Trained certified staff also measured pulse, and participants additionally reported their diet (with a validated FFQ) and comorbidities/disease history at baseline. We additionally
measured the following potential confounders (not included in Table 1): marital status, family history of MI, multivitamin use, medication use including drugs that prolong the QT-interval, and white blood cell count.

With the exception of hypertension, participants were considered to have a disease at baseline if they self-reported a physician diagnosis and were also using drugs for that disease. For example, high cholesterol was defined as self-report of physician diagnosis of

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Selected baseline characteristics stratified by sudden cardiac death status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sudden cardiac death</td>
</tr>
<tr>
<td></td>
<td>((n = 239))</td>
</tr>
<tr>
<td>Sociodemographic</td>
<td></td>
</tr>
<tr>
<td>Age at screening (y)</td>
<td>69.0 ± 6.4²</td>
</tr>
<tr>
<td>Race [n (%)]</td>
<td></td>
</tr>
<tr>
<td>American Indian/Alaska Native</td>
<td>3 (1.3)</td>
</tr>
<tr>
<td>Asian/Pacific Islander</td>
<td>6 (2.5)</td>
</tr>
<tr>
<td>African American</td>
<td>31 (13)</td>
</tr>
<tr>
<td>Hispanic/Latino</td>
<td>2 (0.8)</td>
</tr>
<tr>
<td>White, non-Hispanic</td>
<td>195 (82)</td>
</tr>
<tr>
<td>None of the above</td>
<td>2 (0.8)</td>
</tr>
<tr>
<td>Family income [n (%)]</td>
<td></td>
</tr>
<tr>
<td>&lt;$20,000</td>
<td>73 (32)</td>
</tr>
<tr>
<td>$20,000–$74,999</td>
<td>129 (57)</td>
</tr>
<tr>
<td>≥$75,000</td>
<td>23 (10)</td>
</tr>
<tr>
<td>Smoking status [n (%)]</td>
<td></td>
</tr>
<tr>
<td>Never-smoker</td>
<td>105 (45)</td>
</tr>
<tr>
<td>Former smoker</td>
<td>104 (44)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>27 (11)</td>
</tr>
<tr>
<td>Completed high school [n (%)]</td>
<td>220 (92)</td>
</tr>
<tr>
<td>Physiologic</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.5 ± 6.2</td>
</tr>
<tr>
<td>Waist-to-hip ratio [n (%)]</td>
<td></td>
</tr>
<tr>
<td>Quartile 1: 0.28–0.76</td>
<td>37 (16)</td>
</tr>
<tr>
<td>Quartile 2: 0.77–0.80</td>
<td>38 (16)</td>
</tr>
<tr>
<td>Quartile 3: 0.81–0.86</td>
<td>55 (23)</td>
</tr>
<tr>
<td>Quartile 4: 0.87–2.88</td>
<td>109 (46)</td>
</tr>
<tr>
<td>Systolic blood pressure [n (%)]</td>
<td></td>
</tr>
<tr>
<td>≤120 mm Hg</td>
<td>57 (24)</td>
</tr>
<tr>
<td>&gt;120 to 140 mm Hg</td>
<td>100 (42)</td>
</tr>
<tr>
<td>&gt;140 mm Hg</td>
<td>81 (34)</td>
</tr>
<tr>
<td>Diastolic blood pressure [n (%)]</td>
<td></td>
</tr>
<tr>
<td>≤90 mm Hg</td>
<td>222 (93)</td>
</tr>
<tr>
<td>≥90 mm Hg</td>
<td>16 (7)</td>
</tr>
<tr>
<td>Behavioral</td>
<td></td>
</tr>
<tr>
<td>Dietary total energy (kcal/d)</td>
<td>1650 ± 1586</td>
</tr>
<tr>
<td>Dietary total alcohol (g/d)</td>
<td>5.72 ± 15</td>
</tr>
<tr>
<td>Dietary total caffeine (mg/d)</td>
<td>155 ± 133</td>
</tr>
<tr>
<td>Physical activity ((\text{MET-h}^3/\text{wk}))</td>
<td>10.4 ± 12.7</td>
</tr>
<tr>
<td>Postmenopausal hormone use [n (%)]</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>105 (45)</td>
</tr>
<tr>
<td>Past</td>
<td>59 (25)</td>
</tr>
<tr>
<td>Current</td>
<td>71 (30)</td>
</tr>
<tr>
<td>Comorbidity, history at baseline [n (%)]</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>43 (18)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>146 (61)</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>35 (15)</td>
</tr>
<tr>
<td>Prior coronary artery disease⁴</td>
<td>64 (27)</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>16 (6.7)</td>
</tr>
<tr>
<td>Coronary bypass surgery</td>
<td>17 (7.3)</td>
</tr>
<tr>
<td>Angioplasty of coronary arteries</td>
<td>15 (6.4)</td>
</tr>
<tr>
<td>Carotid endarterectomy/angioplasty</td>
<td>10 (4.3)</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>32 (14)</td>
</tr>
<tr>
<td>Angina</td>
<td>49 (21)</td>
</tr>
</tbody>
</table>

¹Calculated by using \( t \) tests for continuous variables and chi-square tests for categorical variables.
²Mean ± SD (all such values).
³MET-h, metabolic equivalent task hours.
⁴Includes myocardial infarction.
high cholesterol and taking lipid-lowering medication. Hypertension was defined as high measured blood pressure or the use of antihypertensive medication. Prior CAD at baseline includes a history of the following: MI, cardiac arrest, coronary bypass surgery (angioplasty of coronary arteries, angina (doctor said you had angina “chest pains from a heart problem”), or revascularization.

**Statistical analysis**

Less than 6% of participants were missing data on the variables of interest and were excluded from our analysis, which resulted in a final sample size of 92,847. Person-years of follow up were calculated from the date of return of the baseline FFQ to the first of the following: SCD, death, or August 2009. HRs for SCD according to quintile or category of nutrient intake were computed with the use of Cox proportional hazards models, with control for confounders. Because alcohol and caffeine intakes did not remain constant between baseline and year 3, we used Cox proportional hazards regression with time-dependent exposure and covariates (diseases) using previously described methods (25). This approach uses information from both the FFQ completed at baseline and the FFQ completed at year 3.

Potential confounders were identified based on previous knowledge and existing literature. Potential confounders were included as covariates in multivariable models if the variable was (1) associated with both SCD and the exposure of interest using a P value ≤0.20 and (2) not on the causal pathway. Diseases such as diabetes and hypertension may be on the causal pathway because they can be caused in part by poor diet, and they are risk factors for SCD. To control for this, and to explore effect modification, we additionally ran our analysis stratified by CAD status at baseline. Furthermore, we present a fully adjusted model (model 2) and a model that includes the following potential mediators: atrial fibrillation, CAD, heart failure, diabetes, high cholesterol, and hypertension (model 3). We adjusted for energy intake by including total energy (kcal/d) in our multivariable models. We checked for multicollinearity among covariates, and none had a variance inflation factor >2.0.

To calculate tests for linear trend, the quintile median (ordinal variable) was assigned to each participant and modeled in separate proportional hazards models that included all other covariates. Because we used a priori hypotheses, we do not believe a correction for multiple comparisons was necessary (26, 27). We additionally ran our analyses using quartiles instead of quintiles, using the cumulative average method, and using baseline nutrient intake only to check the sensitivity of our results. The cumulative average method uses baseline diet information for all SCD events that happened before year 3 and the average of baseline and year 3 diet for all subsequent events. Models using most recent intake use baseline diet for all SCD events that happened before year 3 and diet reported at year 3 for all subsequent events. Baseline quantile categories were used to categorize year 3 and average total alcohol and total caffeine intake.

**TABLE 2**

Baseline characteristics according to quintile of total alcohol

<table>
<thead>
<tr>
<th></th>
<th>Total alcohol quintile (g/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 (n = 20,466)</td>
</tr>
<tr>
<td>Mean alcohol (g/d)</td>
<td>0.00</td>
</tr>
<tr>
<td>Age (y)</td>
<td>64.1 ± 7.2</td>
</tr>
<tr>
<td>Ethnicity (%)</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>72</td>
</tr>
<tr>
<td>Black</td>
<td>16</td>
</tr>
<tr>
<td>Latino</td>
<td>6</td>
</tr>
<tr>
<td>Income (%)</td>
<td></td>
</tr>
<tr>
<td>&lt;$20,000</td>
<td>25</td>
</tr>
<tr>
<td>$20,000–$74,999</td>
<td>61</td>
</tr>
<tr>
<td>≥$75,000</td>
<td>14</td>
</tr>
<tr>
<td>Smoking status (%)</td>
<td></td>
</tr>
<tr>
<td>Never-smoker</td>
<td>59</td>
</tr>
<tr>
<td>Past smoker</td>
<td>36</td>
</tr>
<tr>
<td>Current smoker</td>
<td>5</td>
</tr>
<tr>
<td>Waist-to-hip ratio (%)</td>
<td></td>
</tr>
<tr>
<td>Quartile 1</td>
<td>25</td>
</tr>
<tr>
<td>Quartile 2</td>
<td>24</td>
</tr>
<tr>
<td>Quartile 3</td>
<td>24</td>
</tr>
<tr>
<td>Quartile 4</td>
<td>27</td>
</tr>
<tr>
<td>Pulse in 60 s (beats/min)</td>
<td>69.6 ± 12</td>
</tr>
<tr>
<td>Total physical activity (MET-h³/wk)</td>
<td>12.8 ± 15</td>
</tr>
<tr>
<td>Total energy (kcal/d)</td>
<td>1269 ± 585</td>
</tr>
<tr>
<td>Atrial fibrillation (%)</td>
<td>6</td>
</tr>
<tr>
<td>Congestive heart failure (%)</td>
<td>2</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>7</td>
</tr>
<tr>
<td>Carotid artery disease (%)</td>
<td>0.5</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>71</td>
</tr>
</tbody>
</table>

1 Mean ± SD (all such values).

2 MET-h, metabolic equivalent task hours.
Because we found no correlation between alcohol intake and caffeine intake ($r = 0.08$), we did not adjust for caffeine in our alcohol analyses and vice-versa and did not examine alcohol-caffeine interactions. All data analyses were conducted by using SAS version 9.2 (SAS Institute Inc). This study was approved by the institutional review boards of all collaborating institutions, and all participants gave informed consent.

RESULTS

A total of 239 women experienced SCD after an average of 11 y of follow-up in the observational study. We found that the following were independent risk factors for SCD in this cohort of postmenopausal women: older age, African American race, smoking, higher resting pulse, higher waist-to-hip ratio, and a history of heart failure, diabetes, MI, carotid artery disease, and hypertension (28). A history of atrial fibrillation was also associated with an increased risk of SCD; however, this association was not statistically significant (Table 1).

Women in higher quintiles of alcohol and caffeine intakes were more likely to be white, to be a current or former smoker, and to have a lower waist-to-hip ratio (Tables 2 and 3). Women in higher quintiles of alcohol and caffeine intakes were less likely to have a history of atrial fibrillation, CAD, congestive heart failure, diabetes, and hypertension. Unlike women in higher quintiles of caffeine intake, women in higher quintiles of alcohol intake had higher incomes and were more active. Although alcohol and caffeine were associated with a similar set of risk factors, they were not correlated ($r = 0.08$).

Compared with very light alcohol intake (0.1–5 g/d), light alcohol intake (one drink or 5.1–15 g/d) was associated with a reduced risk of SCD by using baseline intake (HR: 0.64; 95% CI: 0.40, 1.02), the cumulative average intake (HR: 0.69; 95% CI: 0.43, 1.11), and a simple time-varying exposure analysis (HR: 0.58; 95% CI: 0.35, 0.96) (Table 4). This association was only statistically significant for the model using most recent alcohol intake. Compared with very light alcohol intake (0.1–5 g/d), no alcohol intake, moderate alcohol intake (15–30 g/d), and heavy alcohol intake (>30 g/d) were not associated with risk of SCD. Beer and liquor intake was low in this population; therefore, we had limited power to explore different types of alcohol, including wine, liquor, and beer. When we examined beer, wine, and liquor intake, none was individually associated with risk of SCD after adjustment for the others. Results for non-SCD were very similar (see Supplemental Tables 1 and 2 under “Supplemental data” in the online issue); however, the protective effect of alcohol appeared to extend to 30 g alcohol/d for non-SCD.

When we stratified our SCD analysis according to history of CAD at baseline, alcohol appeared to have a larger magnitude of association among women without a history of CAD, and HRs were attenuated among women with a history of CAD; however,

TABLE 3
Baseline characteristics according to quintile of total caffeine

<table>
<thead>
<tr>
<th>Total caffeine quintile (mg/d)</th>
<th>1 ($n = 20,235$)</th>
<th>2 ($n = 19,405$)</th>
<th>3 ($n = 19,661$)</th>
<th>4 ($n = 17,128$)</th>
<th>5 ($n = 16,886$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean caffeine (mg/d)</td>
<td>19</td>
<td>75</td>
<td>148</td>
<td>182</td>
<td>368</td>
</tr>
<tr>
<td>Age (y)</td>
<td>63.8 ± 6</td>
<td>64.4 ± 7</td>
<td>63.6 ± 7</td>
<td>63.4 ± 7</td>
<td>62.9 ± 7</td>
</tr>
<tr>
<td>Ethnicity (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>70</td>
<td>81</td>
<td>88</td>
<td>90</td>
<td>91</td>
</tr>
<tr>
<td>Black</td>
<td>17</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Latino</td>
<td>6</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Income (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;$20,000</td>
<td>21</td>
<td>17</td>
<td>14</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>$20,000–$74,999</td>
<td>61</td>
<td>63</td>
<td>63</td>
<td>66</td>
<td>65</td>
</tr>
<tr>
<td>≥$75,000</td>
<td>18</td>
<td>20</td>
<td>22</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>Smoking status (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never-smoker</td>
<td>62</td>
<td>53</td>
<td>46</td>
<td>49</td>
<td>42</td>
</tr>
<tr>
<td>Past smoker</td>
<td>34</td>
<td>42</td>
<td>48</td>
<td>44</td>
<td>47</td>
</tr>
<tr>
<td>Current smoker</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>Waist-to-hip ratio (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 1</td>
<td>26</td>
<td>27</td>
<td>29</td>
<td>28</td>
<td>29</td>
</tr>
<tr>
<td>Quartile 2</td>
<td>24</td>
<td>26</td>
<td>26</td>
<td>26</td>
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<tr>
<td>Quartile 3</td>
<td>25</td>
<td>24</td>
<td>23</td>
<td>24</td>
<td>23</td>
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<tr>
<td>Quartile 4</td>
<td>25</td>
<td>23</td>
<td>21</td>
<td>21</td>
<td>22</td>
</tr>
<tr>
<td>Pulse in 60 s (beats/min)</td>
<td>69 ± 12</td>
<td>69 ± 12</td>
<td>69 ± 12</td>
<td>70 ± 12</td>
<td>69 ± 12</td>
</tr>
<tr>
<td>Total physical activity (MET-h$^2$/wk)</td>
<td>13.6 ± 15</td>
<td>13.8 ± 14</td>
<td>14.9 ± 15</td>
<td>12.7 ± 13</td>
<td>13.3 ± 14</td>
</tr>
<tr>
<td>Total energy (kcal/d)</td>
<td>1363 ± 661</td>
<td>1434 ± 610</td>
<td>1493 ± 619</td>
<td>1725 ± 666</td>
<td>1795 ± 829</td>
</tr>
<tr>
<td>Atrial fibrillation (%)</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Coronary artery disease (%)</td>
<td>9</td>
<td>8</td>
<td>7</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Congestive heart failure (%)</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Carotid artery disease (%)</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>71</td>
<td>70</td>
<td>66</td>
<td>66</td>
<td>63</td>
</tr>
</tbody>
</table>

1 Mean ± SD (all such values).
2 MET-h, metabolic equivalent task hours.
Exposure model 1: baseline alcohol intake

<table>
<thead>
<tr>
<th>Total alcohol (g/d)</th>
<th>No. of cases</th>
<th>Patient-years</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never-drinker</td>
<td>7</td>
<td>12,371</td>
<td>1.84 (0.86, 3.93)</td>
<td>1.86 (0.86, 4.00)</td>
<td>1.54 (0.71, 3.33)</td>
</tr>
<tr>
<td>Former drinker</td>
<td>9</td>
<td>29,764</td>
<td>1.09 (0.56, 2.15)</td>
<td>0.97 (0.49, 1.91)</td>
<td>0.84 (0.42, 1.65)</td>
</tr>
<tr>
<td>0.1–5 g/d</td>
<td>142</td>
<td>522,934</td>
<td>1.0 (Reference)</td>
<td>1.0 (Reference)</td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td>5.1–15 g/d</td>
<td>21</td>
<td>147,098</td>
<td>0.54 (0.34, 0.85)</td>
<td>0.64 (0.40, 1.02)</td>
<td>0.72 (0.45, 1.15)</td>
</tr>
<tr>
<td>15.1–30 g/d</td>
<td>17</td>
<td>67,933</td>
<td>0.90 (0.54, 1.49)</td>
<td>1.11 (0.66, 1.86)</td>
<td>1.24 (0.74, 2.10)</td>
</tr>
<tr>
<td>&gt;30 g/d</td>
<td>9</td>
<td>36,728</td>
<td>0.89 (0.45, 1.75)</td>
<td>0.91 (0.46, 1.81)</td>
<td>1.04 (0.52, 2.08)</td>
</tr>
</tbody>
</table>

Exposure model 2: cumulative average alcohol intake

<table>
<thead>
<tr>
<th>Total alcohol (g/d)</th>
<th>No. of cases</th>
<th>Patient-years</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never-drinker</td>
<td>3</td>
<td>4983</td>
<td>1.55 (0.49, 4.88)</td>
<td>1.59 (0.50, 5.05)</td>
<td>1.26 (0.39, 4.00)</td>
</tr>
<tr>
<td>Former drinker</td>
<td>0</td>
<td>11,702</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>0.1–5 g/d</td>
<td>137</td>
<td>509,954</td>
<td>1.0 (Reference)</td>
<td>1.0 (Reference)</td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td>5.1–15 g/d</td>
<td>21</td>
<td>138,714</td>
<td>0.58 (0.37, 0.92)</td>
<td>0.69 (0.43, 1.11)</td>
<td>0.78 (0.49, 1.26)</td>
</tr>
<tr>
<td>15.1–30 g/d</td>
<td>12</td>
<td>64,271</td>
<td>0.60 (0.38, 1.24)</td>
<td>0.82 (0.45, 1.51)</td>
<td>0.94 (0.51, 1.74)</td>
</tr>
<tr>
<td>&gt;30 g/d</td>
<td>9</td>
<td>30,495</td>
<td>1.09 (0.55, 2.13)</td>
<td>1.11 (0.55, 2.21)</td>
<td>1.29 (0.65, 2.59)</td>
</tr>
</tbody>
</table>

Exposure model 3: most recent alcohol intake

<table>
<thead>
<tr>
<th>Total alcohol (g/d)</th>
<th>No. of cases</th>
<th>Patient-years</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never-drinker</td>
<td>3</td>
<td>13,660</td>
<td>0.75 (0.24, 2.34)</td>
<td>0.72 (0.23, 2.27)</td>
<td>0.62 (0.20, 1.97)</td>
</tr>
<tr>
<td>Former drinker</td>
<td>0</td>
<td>11,702</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>0.1–5 g/d</td>
<td>137</td>
<td>509,954</td>
<td>1.0 (Reference)</td>
<td>1.0 (Reference)</td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td>5.1–15 g/d</td>
<td>21</td>
<td>138,714</td>
<td>0.58 (0.37, 0.92)</td>
<td>0.69 (0.43, 1.11)</td>
<td>0.78 (0.49, 1.26)</td>
</tr>
<tr>
<td>15.1–30 g/d</td>
<td>12</td>
<td>64,271</td>
<td>0.60 (0.38, 1.24)</td>
<td>0.82 (0.45, 1.51)</td>
<td>0.94 (0.51, 1.74)</td>
</tr>
<tr>
<td>&gt;30 g/d</td>
<td>9</td>
<td>30,495</td>
<td>1.09 (0.55, 2.13)</td>
<td>1.11 (0.55, 2.21)</td>
<td>1.29 (0.65, 2.59)</td>
</tr>
</tbody>
</table>

The number of events among this population was small and our estimates were highly variable. We found no association between total caffeine, decaffeinated coffee, decaffeinated coffee, or decaffeinated tea and risk of SCD (Table 5).

DISCUSSION

In a large multiethnic cohort of postmenopausal women, we found that light alcohol intake (one drink a day or 0.1–5 g/d) was associated with a reduced risk of SCD compared with 0.1–5 g/d. This association was only statistically significant for a model using most recent alcohol intake. Three previous studies found an increased risk of SCD associated with heavy drinking and a protective effect of light-to-moderate drinking on risk of SCD in men (5–7); however, only one study has examined this association in women. This study (12), which used the Nurses’ Health Study cohort, confirmed the potentially protective effects of light-to-moderate alcohol intake in women. They found a U-shaped relation between alcohol intake and SCD, with a nadir at 5–14.9 g/d. We found no differential associations by type of alcohol, including wine, beer, or hard liquor.

Our results were similar when we used a simple time-varying exposure analysis (which uses most recent diet) and using the cumulative average method. Because we only had 2 measures of diet over time, at baseline and at year 3, both methods theoretically examined the effect of chronic, long-term alcohol intake, assuming that intake remains relatively constant after year 3.

We found that total caffeine, regular coffee, decaffeinated coffee, and regular tea intakes were not associated with risk of SCD in this population of postmenopausal women. Only one previous study has examined the association between coffee intake and SCD. This study, the Gruppo Italiano per lo Studio della Sopravvivenza nell’Infarto miocardico (GISSI-Prevenzione), found no relation between coffee and risk of SCD in men and women with recent nonfatal MI (15). Our study examined total caffeine intake, regular coffee, decaffeinated coffee, and regular tea and also found no association in women.

The strengths of our study included its prospective design, the inclusion of women from multiple ethnic groups, and geographic sites across the United States, physician adjudicated SCD, and comprehensive data on confounders. In addition, we used a validated FFQ designed specifically for postmenopausal women, which was administered twice during follow-up. The correlation coefficients between the FFQ and 8 d of dietary intake (four 24-h recalls and a 4-d food record) collected from 113 women participating in the WHI in 1995 was 0.89 for alcohol (23), which suggests good agreement.

Our study had several potential weaknesses, including residual confounding because of its observational nature, limited generalizability to men and premenopausal women, wide CIs, limited...
under “Supplemental data” in the online issue).

message.

recommending light-to-moderate drinking as a public health

ditions such as cancer; therefore, caution should be taken before

alcohol may negatively influence other common chronic con-

to-moderate alcohol intake on cardiovascular health; however,

in this cohort of postmenopausal women. Previous research

dietary caffeine intake was not associated with risk of SCD

15 g alcohol).

w

alcohol/d drank 1 drink/d or 7 drinks in one day (1 drink is typically

intake, such as whether an individual with an average of 15 g

be useful to have more information about pattern of alcohol

assumption that the FFQ represents average diet. Finally, it would

null. FFQs in general have several limitations, including the

nondifferential and would therefore bias our estimates toward the

example if there was inadequate documentation of the timing of

death. We believe that this potential bias, if present, would be

power as a result of the small number of SCD events, and

tential misclassification of both the exposure and outcome. De-

spite uniform data collection procedures and central adjudication,

few of those who experienced SCDs were autopsied, and some

udden deaths because of cerebral hemorrhage, acute pulmonary

embolism, or aortic rupture may have been incorrectly classified

as SCDs. In addition, some SCDs may have been missed, for

spite uniform data collection procedures and central adjudication,

potential misclassification of both the exposure and outcome. De-

Model 2 was adjusted for age, total energy intake, race, income, smoking status, physical activity, waist-to-hip ratio, BMI, atrial fibrillation, coronary artery disease, heart failure, diabetes, high cholesterol, and hypertension.

Betrie HRs (95% CIs) were calculated by using multivariable Cox proportional hazard regression.

REFERENCES


JN, Stamper MJ, Manson JE. Prospective study of sudden cardiac


McAnulty JH, Gunson K, Jui J, Chugh SS. Population-based analysis of

sudden cardiac death with and without left ventricular systolic
dysfunction: two-year findings from the Oregon Sudden Unexpected

4. Kozarevic D, Demirovic J, Gordon T, Kaelber CT, McGee D, Zukel

WJ. Drinking habits and coronary heart disease: the Yugoslavia car-


We acknowledge the WHI investigators (see Supplemental Appendix A under “Supplemental data” in the online issue).

The authors’ responsibilities were as follows—CBE: conducted the re-

search; MLB: performed the statistical analyses; MLB, EWT, DSM, AB,

JWH, MLN, MSF, MAA, MMS, WL, YM-R, MCR, and CBE: wrote the

manuscript; and MLB and CBE: had primary responsibility for the final

content. All authors read and approved the final manuscript. No conflicts

of interest were declared.

TABLE 5

HRs (and 95% CIs) for sudden cardiac death according to intake of caffeine, coffee, and tea

<table>
<thead>
<tr>
<th>Caffeine quintile</th>
<th>No. of cases</th>
<th>HR (95% CI)</th>
<th>P-trend</th>
<th>HR (95% CI)</th>
<th>P-trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (0–58 mg/d)</td>
<td>47</td>
<td>1.0 (Reference)</td>
<td></td>
<td>1.0 (Reference)</td>
<td></td>
</tr>
<tr>
<td>2 (59–92 mg/d)</td>
<td>42</td>
<td>0.60 (0.40, 0.92)</td>
<td>0.17</td>
<td>0.65 (0.42, 0.99)</td>
<td>0.52</td>
</tr>
<tr>
<td>3 (93–178 mg/d)</td>
<td>34</td>
<td>0.71 (0.46, 1.09)</td>
<td></td>
<td>0.79 (0.51, 1.23)</td>
<td></td>
</tr>
<tr>
<td>4 (179–266 mg/d)</td>
<td>35</td>
<td>0.66 (0.42, 1.04)</td>
<td></td>
<td>0.80 (0.50, 1.27)</td>
<td></td>
</tr>
<tr>
<td>5 (267–855 mg/d)</td>
<td>47</td>
<td>0.81 (0.52, 1.28)</td>
<td></td>
<td>0.90 (0.56, 1.42)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Regular coffee</th>
<th>No. of cases</th>
<th>HR (95% CI)</th>
<th>P-trend</th>
<th>HR (95% CI)</th>
<th>P-trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>97</td>
<td>1.0 (Reference)</td>
<td></td>
<td>1.0 (Reference)</td>
<td></td>
</tr>
<tr>
<td>1 cup/d</td>
<td>34</td>
<td>0.86 (0.58, 1.26)</td>
<td>0.51</td>
<td>0.90 (0.61, 1.33)</td>
<td>0.84</td>
</tr>
<tr>
<td>2–3 cups/d</td>
<td>55</td>
<td>0.86 (0.62, 1.20)</td>
<td></td>
<td>0.94 (0.67, 1.31)</td>
<td></td>
</tr>
<tr>
<td>≥4 cups/d</td>
<td>19</td>
<td>0.97 (0.59, 1.59)</td>
<td></td>
<td>1.02 (0.61, 1.70)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Decaffeinated coffee</th>
<th>No. of cases</th>
<th>HR (95% CI)</th>
<th>P-trend</th>
<th>HR (95% CI)</th>
<th>P-trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>134</td>
<td>1.0 (Reference)</td>
<td></td>
<td>1.0 (Reference)</td>
<td></td>
</tr>
<tr>
<td>1 cup/d</td>
<td>36</td>
<td>1.17 (0.81, 1.69)</td>
<td>0.52</td>
<td>1.23 (0.85, 1.78)</td>
<td>0.37</td>
</tr>
<tr>
<td>2–3 cups/d</td>
<td>26</td>
<td>0.97 (0.63, 1.47)</td>
<td></td>
<td>1.00 (0.66, 1.53)</td>
<td></td>
</tr>
<tr>
<td>≥4 cups/d</td>
<td>9</td>
<td>1.43 (0.73, 2.81)</td>
<td></td>
<td>1.51 (0.76, 2.97)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Regular tea</th>
<th>No. of cases</th>
<th>HR (95% CI)</th>
<th>P-trend</th>
<th>HR (95% CI)</th>
<th>P-trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>144</td>
<td>1.0 (Reference)</td>
<td></td>
<td>1.0 (Reference)</td>
<td></td>
</tr>
<tr>
<td>1 cup/d</td>
<td>32</td>
<td>1.23 (0.84, 1.80)</td>
<td>0.32</td>
<td>1.27 (0.86, 1.86)</td>
<td>0.30</td>
</tr>
<tr>
<td>2–3 cups/d</td>
<td>20</td>
<td>0.93 (0.58, 1.48)</td>
<td></td>
<td>0.93 (0.58, 1.49)</td>
<td></td>
</tr>
<tr>
<td>≥4 cups/d</td>
<td>9</td>
<td>1.78 (0.91, 3.49)</td>
<td></td>
<td>1.77 (0.90, 3.48)</td>
<td></td>
</tr>
</tbody>
</table>

1 Total caffeine intake was measured at baseline and year 3; the most recent caffeine intake was used. Coffee and tea were measured at baseline only.

2 Model 1 was adjusted for age and total energy intake.

3 Model 2 was adjusted for age, total energy intake, race, income, smoking status, physical activity, waist-to-hip ratio, BMI, atrial fibrillation, coronary artery disease, heart failure, diabetes, high cholesterol, and hypertension.

4 HRs (95% CIs) were calculated by using multivariable Cox proportional hazard regression.
Coffee, CYP1A2 Genotype, and Risk of Myocardial Infarction

Marilyn C. Cornelis, BSc
Ahmed El-Sohemy, PhD
Edmond K. Kabagambe, PhD
Hannia Campos, PhD

Context  The association between coffee intake and risk of myocardial infarction (MI) remains controversial. Coffee is a major source of caffeine, which is metabolized by the polymorphic cytochrome P450 1A2 (CYP1A2) enzyme. Individuals who are homozygous for the CYP1A2*1A allele are “rapid” caffeine metabolizers, whereas carriers of the variant CYP1A2*1F are “slow” caffeine metabolizers.

Objective  To determine whether CYP1A2 genotype modifies the association between coffee consumption and risk of acute nonfatal MI.

Design, Setting, and Participants  Cases (n=2014) with a first acute nonfatal MI and population-based controls (n=2014) living in Costa Rica between 1994 and 2004, matched for age, sex, and area of residence, were genotyped by restriction fragment-length polymorphism polymerase chain reaction. A food frequency questionnaire was used to assess the intake of caffeinated coffee.

Main Outcome Measure  Relative risk of nonfatal MI associated with coffee intake, calculated using unconditional logistic regression.

Results  Fifty-five percent of cases (n=1114) and 54% of controls (n=1082) were carriers of the slow *1F allele. For carriers of the slow *1F allele, the multivariate-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) of nonfatal MI associated with consuming less than 1, 1, 2 to 3, and 4 or more cups of coffee per day were 1.00 (reference), 0.99 (0.69-1.44), 1.36 (1.01-1.83), and 1.64 (1.14-2.34), respectively. Corresponding ORs (95% CIs) for individuals with the rapid *1A/*1A genotype were 1.00, 0.75 (0.51-1.12), 0.78 (0.56-1.09), and 0.99 (0.66-1.48) (P=.04 for gene × coffee interaction). For individuals younger than the median age of 59 years, the ORs (95% CIs) associated with consuming less than 1, 1, 2 to 3, or 4 or more cups of coffee per day were 1.00, 1.24 (0.71-2.18), 1.67 (1.08-2.60), and 2.33 (1.39-3.89), respectively, among carriers of the *1F allele. The corresponding ORs (95% CIs) for those with the *1A/*1A genotype were 1.00, 1.24 (0.71-2.18), 1.67 (1.08-2.60), and 2.33 (1.39-3.89), respectively, among carriers of the *1F allele. The corresponding ORs (95% CIs) for those with the *1A/*1A genotype were 1.00, 0.48 (0.26-0.87), 0.57 (0.35-0.95), and 0.83 (0.46-1.51).

Conclusion  Intake of coffee was associated with an increased risk of nonfatal MI only among individuals with slow caffeine metabolism, suggesting that caffeine plays a role in this association.

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Corresponding Author: Ahmed El-Sohemy, PhD, Department of Nutritional Sciences, University of Toronto, 150 College St, Toronto, Ontario, Canada M5S 3E2 (a.el.sohemy@utoronto.ca).
well as urban, periurban, and rural lifestyles. Medical services in this area were covered by 6 large hospitals, which are part of the National Social Security System. Eligible case participants were men and women who were survivors of a first acute MI as diagnosed by a cardiologist at any of the 6 recruiting hospitals in the catchment area between 1994 and 2004. To achieve 100% ascertainment, the hospitals were visited daily by the study fieldworkers. All cases were confirmed by 2 independent cardiologists according to the World Health Organization criteria for MI, which require typical symptoms plus either elevation in cardiac enzyme levels or diagnostic change in electrocardiogram tracings.28 Enrollment was carried out while cases were in the hospital’s step-down unit. Case participants were ineligible if they died during hospitalization, were 75 years or older on the day of their first MI, were physically or mentally unable to answer the questionnaire, or had a previous hospital admission related to cardiovascular disease. One control participant for each case, matched for age (±5 years), sex, and area of residence (county), was randomly selected using information available at the National Census and Statistics Bureau of Costa Rica. Eligible controls were identified within 1 week of the case selection. On average, it took 27 days to complete data collection for cases and 31 days for controls. Because of the comprehensive social services provided in Costa Rica, all persons living in the catchment areas had access to medical care without regard to income. Therefore, control participants came from the source population that gave rise to the cases and are not likely to have had cardiovascular disease that was not diagnosed because of poor access to medical care. Controls were ineligible if they were physically or mentally unable to answer the questionnaires or if they had a previous hospital admission related to MI or other cardiovascular disease.

Participation for eligible cases and controls was 98% and 88%, respectively. All participants were visited at their homes for collection of biological specimens and information on diet, medical history, and anthropometric measurements. Cases and controls gave written informed consent and the study was approved by the ethics committees of the Harvard School of Public Health and the University of Costa Rica, the Office for the Protection from Research Risks at the National Institutes of Health, and the ethics review committee at the University of Toronto.

All data were collected by trained fieldworkers during an interview using 2 questionnaires consisting of closed-ended questions regarding smoking, sociodemographic characteristics, socioeconomic status, physical activity, diet, and medical history including use of medication and personal history of diabetes and hypertension. Information on dietary intake was collected using a 135-item semiquantitative food frequency questionnaire (FFQ) specifically developed and validated to assess dietary intake during the past year in the Costa Rican population.29 For cases, average intake represented the year preceding their MI. Intakes of nutrients were calculated using the US Department of Agriculture food composition data file. Included in the FFQ were questions related to the consumption of caffeinated coffee, tea, cola beverages, and chocolate. The standard portion size for coffee in the FFQ was fixed as 1 cup equivalent to 250 mL, based on the habitual portion size for coffee-drinking habits established in this population during methods development.29 Participants were asked to specify 1 of 9 categories of coffee intake: none or less than 1 cup/day, 1 to 3 cups/day, 1 cup/wk, 2 to 4 cups/wk, 5 to 6 cups/wk, 1 cup/d, 2 to 3 cups/d, 4 to 5 cups/d, or 6 cups/d or more. The correlation coefficient for caffeine intake between seven 24-hour recalls and the average of 2 FFQ interviews was 0.83, and the correlation coefficient between both FFQs was 0.77.29 These results indicate high validity and reliability for the usual intake of coffee. Method of coffee preparation was ascertained for coffee drinkers. Approximately 90% of coffee consumed in Costa Rica is filtered. Participants were categorized into 4 groups with reported coffee intakes of less than 1, 1, 2 to 3, or 4 or more 250-mL cups per day.

CYP1A2 Genotyping

Blood samples were collected in the morning at the participant’s home after an overnight fast and were centrifuged to separate the plasma and leukocytes for DNA isolation by standard procedures. The CYP1A2*1F (rs762551) polymorphism was detected by restriction fragment–length polymorphism polymerase chain reaction as previously described,30 without knowledge of case-control status. The genotype distribution among controls did not deviate from Hardy-Weinberg equilibrium according to a Pearson χ² test with 1 df.

Statistical Analyses

All data were analyzed using SAS version 8.2 (SAS Institute Inc, Cary, NC); P<.05 was considered statistically significant. DNA was available from 4369 participants (2113 cases and 2256 controls). A total of 341 participants were excluded because they were missing data on confounders (33 cases, 26 controls), could not be genotyped (63 cases, 73 controls), or became unmatched because of missing data (3 cases, 143 controls), leaving 2014 matched case-control pairs for the final analysis. Individual nutrient intakes were adjusted for total energy as described elsewhere.28,31 Because of the matched design, significant differences in the distribution of variables between cases and controls were tested using the McNemar test (categorical variables) and either paired t tests or Wilcoxon signed rank tests (continuous variables). Categorical and continuous non-diary and energy-adjusted dietary variables were assessed for potential confounding by measuring their effect on the model parameter estimates using the likelihood ratio test. Odds ratios (ORs) and Wald 95% confidence intervals (95% CIs) were estimated by conditional logistic regression to de-
Table 1. Demographic and Risk Factor Characteristics by Case-Control Status and by Coffee Intake Among Controls

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases (n = 2014)</th>
<th>Controls (n = 2014)</th>
<th>P Value, Cases vs Controls</th>
<th>&lt;1 Cup/d (n = 269)</th>
<th>1 Cup/d (n = 338)</th>
<th>2-3 Cups/d (n = 1133)</th>
<th>≥4 Cups/d (n = 274)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2*1A/1F, No. (%)</td>
<td>1114 (55)</td>
<td>1082 (54)</td>
<td>.31</td>
<td>156 (58)</td>
<td>180 (53)</td>
<td>596 (53)</td>
<td>151 (55)</td>
</tr>
<tr>
<td>Age, mean (SD), y*</td>
<td>58.4 (11.0)</td>
<td>58.1 (11.3)</td>
<td>.001</td>
<td>58.7 (12.4)</td>
<td>58.4 (11.9)</td>
<td>58.7 (11.1)</td>
<td>56.7 (10.2)</td>
</tr>
<tr>
<td>Men, No. (%)</td>
<td>1488 (74)</td>
<td>1488 (74)</td>
<td>.01</td>
<td>190 (71)</td>
<td>243 (72)</td>
<td>618 (72)</td>
<td>237 (86)</td>
</tr>
<tr>
<td>Urban residence, No. (%)</td>
<td>1482 (74)</td>
<td>1482 (74)</td>
<td>.001</td>
<td>209 (78)</td>
<td>273 (81)</td>
<td>801 (71)</td>
<td>198 (72)</td>
</tr>
<tr>
<td>Secondary education or higher, No.</td>
<td>733 (36)</td>
<td>806 (40)</td>
<td>.007</td>
<td>148 (55)</td>
<td>163 (48)</td>
<td>392 (35)</td>
<td>103 (38)</td>
</tr>
<tr>
<td>Household income, mean (SD), US $/mo</td>
<td>499 (388)</td>
<td>571 (425)</td>
<td>&lt;.001</td>
<td>735 (492)</td>
<td>632 (456)</td>
<td>522 (381)</td>
<td>543 (441)</td>
</tr>
<tr>
<td>Waist-hip ratio, mean (SD)</td>
<td>0.97 (0.07)</td>
<td>0.95 (0.07)</td>
<td>&lt;.001</td>
<td>0.94 (0.08)</td>
<td>0.95 (0.08)</td>
<td>0.95 (0.08)</td>
<td>0.96 (0.06)</td>
</tr>
<tr>
<td>Physical activity, mean (SD), METs</td>
<td>1.51 (0.69)</td>
<td>1.57 (0.70)</td>
<td>.01</td>
<td>1.46 (0.48)</td>
<td>1.50 (0.68)</td>
<td>1.56 (0.69)</td>
<td>1.76 (0.87)</td>
</tr>
<tr>
<td>Medical history, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of hypertension†</td>
<td>779 (39)</td>
<td>596 (30)</td>
<td>&lt;.001</td>
<td>78 (29)</td>
<td>113 (33)</td>
<td>341 (30)</td>
<td>64 (23)</td>
</tr>
<tr>
<td>History of diabetes†</td>
<td>492 (24)</td>
<td>285 (14)</td>
<td>&lt;.001</td>
<td>29 (11)</td>
<td>48 (14)</td>
<td>179 (16)</td>
<td>29 (11)</td>
</tr>
<tr>
<td>Current smoking‡</td>
<td>805 (40)</td>
<td>425 (21)</td>
<td>&lt;.001</td>
<td>32 (12)</td>
<td>45 (13)</td>
<td>230 (20)</td>
<td>118 (43)</td>
</tr>
<tr>
<td>Current alcohol consumption‡</td>
<td>984 (49)</td>
<td>1059 (53)</td>
<td>&lt;.001</td>
<td>138 (51)</td>
<td>200 (59)</td>
<td>576 (51)</td>
<td>145 (53)</td>
</tr>
<tr>
<td>Nutrient intakes, mean (SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total energy, kcal</td>
<td>2714 (946)</td>
<td>2457 (764)</td>
<td>&lt;.001</td>
<td>2461 (759)</td>
<td>2340 (674)</td>
<td>2446 (748)</td>
<td>2648 (894)</td>
</tr>
<tr>
<td>Carbohydrate, % energy</td>
<td>54.3 (7.6)</td>
<td>55.4 (7.3)</td>
<td>&lt;.001</td>
<td>54.9 (7.9)</td>
<td>53.7 (7.5)</td>
<td>55.9 (6.8)</td>
<td>55.8 (8.1)</td>
</tr>
<tr>
<td>Protein, % energy</td>
<td>13.2 (2.2)</td>
<td>12.9 (2.1)</td>
<td>&lt;.001</td>
<td>12.9 (2.3)</td>
<td>13.2 (2.2)</td>
<td>13.0 (2.0)</td>
<td>12.7 (2.2)</td>
</tr>
<tr>
<td>Fat, % energy</td>
<td>32.4 (5.9)</td>
<td>31.9 (5.5)</td>
<td>.008</td>
<td>32.6 (6.6)</td>
<td>32.9 (6.4)</td>
<td>31.6 (5.3)</td>
<td>31.3 (6.4)</td>
</tr>
<tr>
<td>Saturated fat, % energy</td>
<td>12.4 (3.1)</td>
<td>11.7 (2.9)</td>
<td>&lt;.001</td>
<td>11.4 (3.0)</td>
<td>11.8 (2.9)</td>
<td>11.7 (2.8)</td>
<td>12.0 (3.2)</td>
</tr>
<tr>
<td>Polyunsaturated fat, % energy</td>
<td>6.9 (2.3)</td>
<td>7.1 (2.3)</td>
<td>&lt;.001</td>
<td>7.2 (2.0)</td>
<td>7.3 (2.4)</td>
<td>7.1 (2.4)</td>
<td>6.6 (2.4)</td>
</tr>
<tr>
<td>Monounsaturated fat, % energy</td>
<td>11.1 (3.5)</td>
<td>11.2 (4.1)</td>
<td>.70</td>
<td>12.2 (5.2)</td>
<td>11.9 (4.8)</td>
<td>10.9 (3.4)</td>
<td>11.0 (4.1)</td>
</tr>
<tr>
<td>Trans fat, % energy</td>
<td>1.3 (0.6)</td>
<td>1.3 (0.6)</td>
<td>.06</td>
<td>1.2 (0.6)</td>
<td>1.2 (0.6)</td>
<td>1.3 (0.7)</td>
<td>1.3 (0.6)</td>
</tr>
<tr>
<td>Cholesterol, mg/1000 kcal</td>
<td>127 (59)</td>
<td>118 (52)</td>
<td>&lt;.001</td>
<td>120 (48)</td>
<td>113 (50)</td>
<td>119 (53)</td>
<td>117 (57)</td>
</tr>
<tr>
<td>Sucrose, g/d</td>
<td>80.1 (50.8)</td>
<td>75.2 (43.2)</td>
<td>&lt;.001</td>
<td>74.3 (40.1)</td>
<td>63.6 (33.0)</td>
<td>73.9 (39.0)</td>
<td>95.8 (62.7)</td>
</tr>
<tr>
<td>Fiber, g/1000 kcal</td>
<td>9.5 (2.4)</td>
<td>10.0 (2.5)</td>
<td>&lt;.001</td>
<td>10.1 (2.7)</td>
<td>9.9 (2.5)</td>
<td>10.1 (2.4)</td>
<td>9.3 (2.3)</td>
</tr>
<tr>
<td>Folate, µg/1000 kcal</td>
<td>170 (46)</td>
<td>175 (47)</td>
<td>&lt;.001</td>
<td>183 (50)</td>
<td>174 (46)</td>
<td>177 (46)</td>
<td>162 (43)</td>
</tr>
</tbody>
</table>

Abbreviation: METs, metabolic equivalent tasks.
*Matching variable.
†See “Methods” for definition.
‡One or more cigarettes per day.
genotype, the increased risk of MI associated with coffee intake was observed only among carriers of the slow *1F allele (P = .04 for gene × coffee interaction). In this group, the OR (95% CI) of MI was 1.64 (1.14-2.34) for 4 cups/d or more, compared with less than 1 cup/d. The corresponding OR (95% CI) among those who were homozygous for the rapid *1A allele was 0.99 (0.66-1.48). Similar results were observed when men and women were examined separately. Compared with less than 1 cup/d, the ORs (95% CIs) of MI for 4 cups/d or more among individuals with the *1A/*1A genotype were 0.86 (0.53-1.36) for men and 1.43 (0.54-3.72) for women. Corresponding ORs (95% CIs) among carriers of the *1F allele were 1.54 (1.03-2.32) for men and 2.83 (1.15-6.99) for women.

Because smoking is associated with coffee consumption and is also a strong inducer of CYP1A2, we performed analyses separately for current smokers and nonsmokers (never, past). Although the gene × coffee interaction did not reach significance in either group, the modifying effect of CYP1A2 genotype on risk of MI associated with coffee consumption was similar for both smokers and nonsmokers (Table 3).

It has previously been suggested that coffee may be associated with an increased risk of MI only among younger individuals. To investigate whether age modified the interaction between CYP1A2 and coffee on risk of MI, we assessed risk separately for participants above and below the median age (59 years). A significant gene × coffee interaction (P = .003) was observed only among the younger participants (Table 3). For those individuals who were carriers of the *1F allele, the ORs (95% CIs) of MI associated with consuming less than 1, 1, 2 to 3, or 4 or more cups of coffee per day were 1.00 (reference), 1.24 (0.71-2.18), 1.67 (1.08-2.60), and 2.33 (1.39-3.89), respectively. Corresponding ORs (95% CIs) for those with the *1A/*1A genotype were 1.00, 0.48 (0.26-0.87), 0.57 (0.35-0.95), and 0.83 (0.46-1.51). Because of the observed interaction with participants younger than the median age of 59 years, we also analyzed those younger than 50 years (448 cases, 478 controls), as has been previously done. For carriers of the *1F allele, the ORs (95% CIs) of MI associated with consuming less than 1, 1, 2 to 3, or 4 or more cups of coffee per day were 1.00, 2.12 (0.86-5.24), 2.43 (1.22-4.82), and 4.07 (1.89-8.74), respectively. Corresponding ORs (95% CIs) for those with the *1A/*1A genotype were 1.00, 0.39 (0.15-0.97), 0.35 (0.17-0.76), and 0.81 (0.32-2.05) (P < .001 for gene × coffee interaction).

**COMMENT**

Coffee is a major source of caffeine, which has multiple physiological effects that could increase the risk of MI. Numerous studies have examined the association between coffee consumption and risk of MI, but the findings have been inconclusive. Caffeine is detoxified primarily through an initial N3-demethylation that is catalyzed by CYP1A2, an enzyme that displays wide interindividual variability in activity. We investigated whether a common genetic polymorphism (CYP1A2*1F) that results in a "slow" metabolizer phenotype modifies the association between intake of caffeinated coffee and risk of nonfatal MI. Our findings show that coffee consumption increases the risk of MI only among individuals with a slow metabolizer genotype.

Meta-analyses examining the relationship between coffee intake and risk of coronary heart disease have observed a positive association among case-control studies but not among prospective cohort studies. According to the most recent meta-analysis, the pooled case-control data show a 60% increased risk for drinking 5 cups/d. It has been suggested that the positive associations reported in case-control studies may have resulted from recall bias or confounding by factors such as smoking. However, because we observed an association between coffee and risk of MI among carriers of the *1F allele, and not among those homozygous for the *1A allele, the associations between coffee and MI are unlikely due to recall bias or residual confounding. Moreover, when we stratified our population by smoking...
status, the results were similar for nonsmokers and current smokers (Table 3).

A more likely explanation for the discrepancies between case-control and prospective cohort studies is that coffee drinking has mainly acute effects, which would be misclassified in prospective studies with a long follow-up and no updating of coffee intake. \(^1\) In a study by LaCroix et al, \(^4\) the relative risk of coronary heart disease for 5 or more cups per day compared with none increased from 1.89 when intake was assessed 10 or more years previously to 2.49 when intake within the past 5 years was used. Similarly, a strong association between coffee consumption and mortality from coronary heart disease, reported after 6 years of follow-up, \(^5\) was weakened by 6 more years of follow-up. \(^6\) The decreased effect of coffee after longer follow-up could also be a result of caffeine having a weaker effect in an older population. Indeed, the CYP1A2 × coffee interaction we observed among individuals younger than the median age suggests that caffeine has a greater relative effect on younger individuals. Among the slow metabolizers, the risk associated with drinking 4 cups/d or more compared with less than 1 cup/d increased from 2-fold for individuals younger than 59 years to more than 4-fold for those younger than 50 years. A similar effect of age was observed by Palmer et al, \(^7\) who found a greater risk of MI with caffeinated coffee consumption among women 45 through 59 years of age but not among women 60 years or older.

The absence of an association between coffee and risk of MI in some case-control studies may have been due to a lower frequency of \(*IF\) carriers in the populations that were examined. In the present study, the frequency of carriers of the \(*IF\) allele was 54%, but frequencies have been reported to vary by population. \(^35\)-\(^38\) Because cases in the present study experienced nonfatal MI, we cannot exclude the possibility that the observed interaction may affect survival after an acute MI.

Although smokers metabolize caffeine more rapidly than nonsmokers

<table>
<thead>
<tr>
<th>Coffee Intake, Cups/d</th>
<th>No. (%)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
</tr>
<tr>
<td>Nonsmokers</td>
<td>Model 1</td>
<td>Model 2</td>
</tr>
<tr>
<td>*1A/*1A</td>
<td>n = 532</td>
<td>n = 745</td>
</tr>
<tr>
<td>&lt;1</td>
<td>75 (14)</td>
<td>101 (14)</td>
</tr>
<tr>
<td>1</td>
<td>84 (16)</td>
<td>135 (18)</td>
</tr>
<tr>
<td>2-3</td>
<td>312 (59)</td>
<td>436 (58)</td>
</tr>
<tr>
<td>≥4</td>
<td>61 (11)</td>
<td>73 (10)</td>
</tr>
<tr>
<td>*1A/*1F + *1F/*1F</td>
<td>n = 677</td>
<td>n = 844</td>
</tr>
<tr>
<td>&lt;1</td>
<td>85 (13)</td>
<td>136 (16)</td>
</tr>
<tr>
<td>1</td>
<td>97 (14)</td>
<td>158 (18)</td>
</tr>
<tr>
<td>2-3</td>
<td>399 (59)</td>
<td>467 (55)</td>
</tr>
<tr>
<td>≥4</td>
<td>98 (14)</td>
<td>83 (10)</td>
</tr>
</tbody>
</table>

Smokers

| *1A/*1A              | n = 368 | n = 187     |
| <1                   | 19 (5)  | 12 (6)      | 1.00 | 1.00   |
| 1                    | 33 (9)  | 23 (12)     | 0.90 (0.36-2.23) | 0.87 (0.30-2.51) |
| 2-3                  | 198 (54)| 102 (55)    | 1.23 (0.57-2.64) | 1.11 (0.45-2.76) |
| ≥4                   | 118 (32)| 50 (27)     | 1.59 (0.71-3.54) | 1.22 (0.47-3.18) |
| *1A/*1F + *1F/*1F    | n = 437 | n = 238     |
| <1                   | 25 (6)  | 20 (8)      | 1.00 | 1.00   |
| 1                    | 20 (5)  | 22 (9)      | 0.65 (0.27-1.54) | 0.90 (0.33-2.50) |
| 2-3                  | 237 (54)| 128 (54)    | 1.42 (0.75-2.70) | 1.77 (0.83-3.76) |
| ≥4                   | 155 (35)| 68 (29)     | 1.83 (0.94-3.56) | 1.79 (0.80-3.98) |

**Table 3.** Coffee Intake and Relative Risk of Myocardial Infarction by CYP1A2 Genotype, Smoking Status, and Age Category

<table>
<thead>
<tr>
<th>Age Category†</th>
<th>No. (%)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &lt;59 y</td>
<td>Model 1</td>
<td>Model 2</td>
</tr>
<tr>
<td>*1A/*1A</td>
<td>n = 451</td>
<td>n = 446</td>
</tr>
<tr>
<td>&lt;1</td>
<td>51 (11)</td>
<td>50 (11)</td>
</tr>
<tr>
<td>1</td>
<td>53 (12)</td>
<td>78 (17)</td>
</tr>
<tr>
<td>2-3</td>
<td>228 (51)</td>
<td>252 (57)</td>
</tr>
<tr>
<td>≥4</td>
<td>119 (26)</td>
<td>66 (15)</td>
</tr>
<tr>
<td>*1A/*1F + *1F/*1F</td>
<td>n = 505</td>
<td>n = 552</td>
</tr>
<tr>
<td>&lt;1</td>
<td>49 (10)</td>
<td>98 (18)</td>
</tr>
<tr>
<td>1</td>
<td>46 (9)</td>
<td>89 (16)</td>
</tr>
<tr>
<td>2-3</td>
<td>266 (53)</td>
<td>282 (51)</td>
</tr>
<tr>
<td>≥4</td>
<td>144 (29)</td>
<td>83 (15)</td>
</tr>
</tbody>
</table>

Age ≥59 y

| *1A/*1A       | n = 449 | n = 486     |
| <1            | 43 (10) | 63 (13)     | 1.00 | 1.00   |
| 1             | 64 (14) | 80 (16)     | 1.17 (0.70-1.94) | 1.10 (0.63-1.92) |
| 2-3           | 282 (63)| 286 (59)    | 1.44 (0.94-2.20) | 1.06 (0.67-1.69) |
| ≥4            | 60 (13) | 57 (12)     | 1.56 (0.92-2.66) | 1.06 (0.58-1.94) |

| *1A/*1F + *1F/*1F | n = 609 | n = 530     |
| <1                | 59 (10) | 58 (11)     | 1.00 | 1.00   |
| 1                 | 71 (12) | 91 (17)     | 0.78 (0.48-1.25) | 0.79 (0.47-1.32) |
| 2-3               | 370 (61)| 313 (59)    | 1.18 (0.79-1.74) | 1.06 (0.69-1.63) |
| ≥4                | 109 (18)| 68 (13)     | 1.54 (0.95-2.48) | 1.09 (0.64-1.86) |

Abbreviations: CI, confidence interval; OR, odds ratio.

*Model 1: unconditional logistic regression model adjusted for age, sex, and area of residence. Model 2: unconditional logistic regression model adjusted for age, sex, area of residence; waist-hip ratio; income; physical activity; history of diabetes; history of hypertension; and intakes of alcohol, total energy, and energy-adjusted saturated fat, polyunsaturated fat, trans fat, folate, and sucrose (see “Methods”). Nonsmokers were further adjusted for never and past smoking, and smokers adjusted for cigarettes smoked per day.

†Model 1: unconditional logistic regression model adjusted for age, sex, area of residence. Model 2: unconditional logistic regression model adjusted for age, sex, area of residence; smoking (never, past, 1-19 cigarettes/d, ≥20 cigarettes/d); waist-hip ratio; income; physical activity; history of diabetes; history of hypertension; and intakes of alcohol, total energy, and energy-adjusted saturated fat, polyunsaturated fat, trans fat, folate, and sucrose (see “Methods”).

\(P = .003\) for gene × coffee interaction.
due to the well-known CYP1A2-inducing effect of smoking,\textsuperscript{23} the extent of CYP1A2 induction among smokers is lower for carriers of the *1F allele.\textsuperscript{25,26} Thus, smokers with the slow metabolizer genotype may still have an increased risk of MI with increasing coffee consumption. Indeed, for carriers of the *1F allele, a similar pattern of risk associated with coffee was observed among smokers and nonsmokers (Table 3).

Among younger individuals who were rapid caffeine metabolizers, coffee intakes of either 1 cup/d or 2 to 3 cups/d were associated with a lower risk of MI compared with intakes of less than 1 cup/d. This finding is consistent with a number of previous reports of J- or U-shaped associations between coffee and MI,\textsuperscript{11,14} suggesting a protective effect of moderate coffee consumption. It has been proposed that the higher risk of heart disease among the group with the lowest intake might be due to individuals with underlying diseases who are limiting their coffee intake.\textsuperscript{12,14} However, the absence of an elevated risk in the lowest category of coffee intake among the slow metabolizers in the present study indicates that this is unlikely.

Coffee contains other chemicals that may have adverse effects on the cardiovascular system.\textsuperscript{18} Distinguishing between the effects of caffeine and those of these other compounds has been difficult, given the strong collinearity between caffeine and coffee intake in many populations. Diterpenoids that are present in the lipid fraction of boiled coffee have been shown to increase levels of serum cholesterol\textsuperscript{20-22} and may increase the risk of MI.\textsuperscript{9} However, the levels of diterpenoids are greatly reduced in filtered coffee.\textsuperscript{42} About 10% of coffee drinkers in the current population did not report consuming filtered coffee, and excluding them from our analyses did not materially alter the results (data not shown). Besides caffeine, no other major compound found in filtered coffee is known to be detoxified by CYP1A2. Thus, our findings suggest that caffeine is the major component of filtered coffee that increases risk of nonfatal MI. Although the mechanism by which caffeine increases risk of MI remains unclear, it is known to block the A\textsubscript{1} and A\textsubscript{2A} adenosine receptors.\textsuperscript{43,44}

Adenosine is a potent coronary and systemic vasodilator that may play an important role in the reactivity of inflammatory cells and platelets during periods of myocardial ischemia.\textsuperscript{45,46}

Although the CYP1A2*1F polymorphism is located in a noncoding region of the gene, the polymorphism may result in differential binding of regulatory proteins to the surrounding sequence that may affect CYP1A2 expression levels.\textsuperscript{23} The regulatory mechanisms of intrinsic polymorphisms on transcriptional activity have been described for several genes.\textsuperscript{47} Alternatively, the polymorphism may be in linkage disequilibrium with other single nucleotide polymorphisms influencing CYP1A2 inducibility.\textsuperscript{23} Nevertheless, in vivo studies clearly show marked differences in CYP1A2 activity between carriers of the different CYP1A2 alleles.\textsuperscript{24,26}

In summary, consistent with most case-control studies, we found that increased coffee intake is associated with an increased risk of nonfatal MI. The association between coffee and MI was found only among individuals with the slow CYP1A2*1F allele, which impairs caffeine metabolism, suggesting that caffeine plays a role in the association.

Author Contributions: Dr El-Sohemy had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: El-Sohemy, Campos. Acquisition of data: Cornilis, El-Sohemy, Campos. Analysis and interpretation of data: Cornilis, El-Sohemy, Kabagambe, Campos. Drafting of the manuscript: Cornilis, El-Sohemy. Critical revision of the manuscript for important intellectual content: Cornilis, El-Sohemy, Kabagambe, Campos. Statistical analysis: Cornilis, El-Sohemy, Kabagambe, Campos. Obtained funding: El-Sohemy, Campos. Administrative, technical, or material support: Campos. Study supervision: El-Sohemy, Campos. Financial Disclosures: None reported.

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REFERENCES


20. Butler MA, lwasaki M, Guengerich FP, Kadilbar F. Human cytochrome P450A (P-4501A2), the phen- d analysis. COFFEE, CYP1A2 GENOTYPE, AND RISK OF MYOCARDIAL INFARCTION

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20. Butler MA, lwasaki M, Guengerich FP, Kadilbar F. Human cytochrome P450A (P-4501A2), the phen...
Letter to the Editor

Endothelial function acutely worse after drinking energy beverage☆☆

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Energy products, especially energy drinks are being consumed more and more worldwide, and particularly concerning is that adolescents are often targeted in marking [1]. Energy drinks are flavored beverages that usually contain added amounts of sugar, artificial caffeine, as well as other additives such as taurine, B vitamins, herbal supplements, guarana (a natural source of caffeine), glucuronanone, and ginseng [1]. The consumption of energy drinks before or during exercise might be linked to an increased risk for myocardial ischemia [2,3]. In healthy individuals who consume caffeine and then exercise afterwards, significant reductions in myocardial blood flow have been noted by indirect laboratory measures [3,4]. Several mechanisms have been postulated to explain this effect, including the ability of caffeine to block adenosine receptors that modulate coronary vasomotor tone [5]. This vasoconstrictive effect might be more pronounced among caffeine-naïve individuals or those who acutely ingest higher doses of caffeine, such as are present in energy drinks [2].

Reduced coronary blood flow may be a symptom of endothelial dysfunction, which affects the ability of the endothelium to regulate vascular resistance [6]. Following exposure to stress, (including exposure to cold, mental arithmetic, anger, ingestion of a meal, or exercise, as well as cigarette smoking, cocaine, alcohol), the impaired ability to dilate the coronary arteries could result in supply–demand imbalance or coronary spasm, potentially leading to myocardial ischemia and/or cardiac arrhythmia [7]. In the worst case, this acute endothelial dysfunction could lead to ischemia resulting in serious arrhythmia, coronary vasospasm and myocardial infarction [8]. The current case describes acute endothelial dysfunction following consumption of an energy drink.

After fasting and not smoking for at least 8 h prior, an asymptomatic 47 year old Caucasian man received a baseline ECG and underwent testing of endothelial function using the technique of flow mediated dilatation (FMD) according to recommended guidelines by a single registered vascular ultrasonographer who was certified by the University of Wisconsin Atherosclerosis Imaging Research Program Core Laboratory [9]. After resting supine for 10-minutes in a temperature-controlled room, a blood pressure cuff was placed on the widest part of proximal right forearm approximately 1 cm distal to the antecubital fossa. Using a 10 MHz resolution linear array vascular ultrasound transducer with a Philips iE33 ultrasound machine, the brachial artery was located above the elbow and scanned in longitudinal sections. After recording baseline B-mode digital images of the brachial artery and spectral Doppler images of flow, the forearm cuff was inflated to 250 mm Hg for 5 min to induce reactive hyperemia. Immediately after deflation, spectral Doppler images are obtained to verify hyperemia. FMD of the brachial artery was measured 60 and 90 s after cuff deflation. The relative FMD (%) was calculated as the ratio between the largest post-cuff release and the baseline diameter. Each image was checked for quality control, and each artery diameter was measured from the media to media points by two experts, 3 measurements at the QRS complex, repeated on 3 separate beats, and then averaged.

The subject then drank a 24-oz Monster energy beverage in approximately 1 min. A 24 oz can of Monster Energy contents include 54 g Sucrose, glucose, sucralose, maltodextrin, Sodium 360 mg Sodium Citrate Sodium Chloride, Caffeine 240 mg, Taurine 2000 mg, Niacin 40 mg 200% RDA, Pyridoxine 4 mg 200% RDA, Cyanocobalamin (B12) 12 mcg 200% RDA, Riboflavin (B2) 3.4 mg 200% RDA, Ginseng Extract 400 mg, Glucuronolactone, L-Arginine, Guarana Extract, and L-Carnitine all listed as a part of a 5000 mg “Energy Blend,” & Sodium Benzoate.

The subject had FMD repeated at 50 min and 90 min after consumption. He reported no significant side effects following consumption of the beverage. The subject was in the supine position for all ECGs and FMD measurements.

The Baseline ECG showed sinus bradycardia 52 bpm, normal intervals, corrected QT interval (QTc; Bazett’s formula) 373 ms. Blood pressure was 103/68 mm Hg. Fifty (50) minutes following consumption of the energy beverage, the ECG revealed sinus bradycardia 48 bpm, normal intervals, QTc 378 ms; blood pressure 106/70 mm Hg. Ninety minutes...

☆☆ This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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post energy beverage, ECG showed sinus bradycardia 54 bpm, normal intervals and QTc 394 ms; blood pressure 112/71 mm Hg.

Reactive hyperemia (as measured by arterial FMD) became worse following consumption of an energy drink (Figs. 1 & 2 ). Of note, there was also an increase in the QTc interval by 5.6% (from 373 to 394 ms).

One group has reported that energy drinks have been shown to acutely reduce endothelial function [10]. In this study, subjects consumed either a 250-mL sugar-free energy drink (1 can) containing caffeine (80 mg), taurine (1000 mg), and glucuronolactone (600 mg), or 250 mL carbonated water (control). They found that energy drink consumption acutely increased platelet aggregation and decreased endothelial function in healthy young adults, measured 1 h after consumption of the energy drink [10].

Another case report of an otherwise healthy 28-year-old motorcross racing man had a cardiac arrest after a day of consuming excessive amounts of a caffeinated “energy drink” [11]. The authors noted severe coronary artery spasm, leading them to postulate that a combination of excessive ingestion of caffeine- and taurine-containing energy drinks and strenuous physical activity may produce myocardial ischemia by inducing coronary vasospasm [11].

Regarding the interesting finding of the QTc interval becoming longer, we noted several reports in the literature noting this also following energy beverages. The first was a case of a 22 year old female with out-of-hospital cardiac arrest (in a discotheque) due to initial torsades de pointes tachycardia secondary degenerating to ventricular fibrillation [12]. Prior to cardiac arrest the patient consumed six cans of a caffeinated energy drink within 4 h. Her QTc was noted to be 526 ms and 492 ms in the intensive care unit and eventually went back to 419 ms at baseline. Genetic testing revealed a long QT syndrome 1 (KCNQ1 mutation).

Another group presents the case of a 13-year-old referred to clinic with type 1 LQTS after noting a prolonged QTc on an outpatient ECG 2 months prior [13]. During the morning of her initial presentation, the patient consumed at least one 16-oz can of an energy drink (160 mg of caffeine) and reported palpitations, chest pain, shakiness, and dizziness. Chest pain and palpitations were persistent though not excessive, so she presented to the emergency department in the evening because of chest pain and palpitations, chest pain, shakiness, and dizziness.

Given the rise of emergency visits for complications of energy drinks as well as some deaths being associated with their consumption, it behooves us to do more research to identify the possible mechanisms by which they may act in certain healthy individuals to cause side effects. Given the possibility that endothelial dysfunction may play a role in morbidity with concomitant energy drink intake and exercise, more research is recommended to clarify the mechanisms of and significance of these effects.

Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor in-Chief of this journal.

“The author(s) of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology.”

References


Caffeine Reduces Myocardial Blood Flow During Exercise

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ABSTRACT

Caffeine consumption has been receiving increased interest from both the medical and lay press, especially given the increased amounts now available in energy products. Acute ingestion of caffeine usually increases cardiac work; however, caffeine impairs the expected proportional increase in myocardial blood flow to match this increased work of the heart, most notably during exercise. This appears to be mainly due to caffeine’s effect on blocking adenosine-induced vasodilatation in the coronary arteries in normal healthy subjects. This review summarizes the available medical literature specifically relating to pure caffeine tablet ingestion and reduced exercise coronary blood flow, and suggests possible mechanisms. Further studies are needed to evaluate this effect for other common caffeine-delivery systems, including coffee, energy beverages, and energy gels, which are often used for exercise performance enhancement, especially in teenagers and young athletes.

Caffeine (1,3,7-trimethylxanthine) is one of the most widely used pharmacologically active drugs in the world and is found in products such as colas, coffee, tea, energy beverages, dietary supplements, over-the-counter medications, and chocolate. It has been estimated that worldwide caffeine consumption is approximately 76 mg/d per person; in the United States and other developed countries, the average consumption rate exceeds 230 mg/d.1

As the popularity and consumption of energy beverages has increased, sudden cardiac death has been reported in teenagers and young athletes who have consumed these products.2 Most energy beverages also contain guarana, a rainforest vine with seeds that contain high levels of caffeine.3 High levels of caffeine, especially in individuals who do not consume caffeine on a regular basis, may play a role in caffeine toxicity and possible cardiac death.3 The use of energy beverages is often advertised in conjunction with amateur and professional sports, and cardiac arrest in the setting of caffeine use and exertion has been described.4

On October 17, 2012, a California Superior Court lawsuit was filed against Monster Energy (Monster Beverage Corporation, Corona, Calif) by the parents of Anais Fournier, a 14-year-old Maryland teenager who died after drinking two 24-ounce Monster Energy Drinks in 24 hours. After autopsy, the cause of death was noted as “cardiac arrhythmia due to caffeine toxicity.”5 Energy beverages have been shown to acutely reduce endothelial function; however, energy beverages also may contain a variety of constituents, and the contribution of caffeine alone is unclear.6

Given the increase in caffeine availability and reports of adverse events, an understanding of the cardiac effects of caffeine is urgently required. This review summarizes the available medical literature specifically relating to caffeine ingestion and reduced exercise coronary blood flow, suggesting possible mechanisms. This review specifically focuses on the effects of caffeine on the coronary arteries, especially the reduced coronary blood flow noted with exercise.7-9

MATERIALS AND METHODS

MEDLINE, Embase, and Google database searches were conducted in an iterative manner for the English-language scientific literature published from 1976 to December 2012.
Caffeine Pharmacology and Physiology

Caffeine is a methylxanthine whose use results in phosphodiesterase inhibition, adenosine receptor antagonism, and release of catecholamines. When taken orally, caffeine is absorbed rapidly, with time to maximum plasma concentration of 45 minutes (range, 15-120 minutes) and an average half-life of 5 hours (range, 2-12 hours). Caffeine is present in a number of commonly consumed beverages in varying amounts and concentrations (Table 1). Caffeine is mainly metabolized into paraxanthine (80%), theobromine, and theophylline. Higher caffeine doses, repeated dosing, and habitual caffeine intake generally result in prolongation of its half-life and reduced clearance of caffeine and its metabolites, creating the potential for caffeine toxicity.

Caffeine produces a number of cardiac effects that seem to occur in a more pronounced manner in caffeine-naïve subjects or those consuming higher doses of caffeine (Table 2). Excessive consumption of caffeine may acutely cause caffeine intoxication, resulting in tachycardia, elevated blood pressure, increased myocardial contractility, vomiting, hypokalemia (from beta-adrenergic stimulation), cardiac arrhythmias (atrial flutter, atrial fibrillation, atrioventricular-nodal reentry tachycardia, ventricular tachycardia, and ventricular fibrillation), seizures, and death.

Reduced coronary blood flow may be a symptom of endothelial dysfunction, which affects the ability of the endothelium to regulate vascular resistance (see Coronary Vasodilatation). During stress (including exposure to cold, mental arithmetic, anger, ingestion of a meal, or exercise) and with certain exposures (cigarette smoking, cocaine, alcohol), the impaired ability to dilate the coronary arteries could result in supply-demand imbalance or coronary spasm, potentially leading to myocardial ischemia or cardiac arrhythmia.

The effects of caffeine may be altered by genetic polymorphisms, metabolic induction/inhibition of cytochrome

<table>
<thead>
<tr>
<th>Table 1 Caffeine Content of Selected Beverages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beverage</td>
</tr>
<tr>
<td>Soft Drinks</td>
</tr>
<tr>
<td>Coca Cola, Coca Cola ZERO (The Coca-Cola Company, Atlanta, Ga)</td>
</tr>
<tr>
<td>Diet Coke (The Coca-Cola Company)</td>
</tr>
<tr>
<td>Dr Pepper (Dr Pepper Snapple Group, Plano, Tex)</td>
</tr>
<tr>
<td>Coffee and Tea</td>
</tr>
<tr>
<td>McDonald’s (Oak Brook, Ill) cup of brewed coffee</td>
</tr>
<tr>
<td>Starbucks (Seattle, Wash) Bold Pick of the Day Coffee</td>
</tr>
<tr>
<td>Starbucks Espresso Roast Coffee</td>
</tr>
<tr>
<td>Typical cup of tea</td>
</tr>
<tr>
<td>Starbucks Tazo Awake Brewed Tea</td>
</tr>
<tr>
<td>Energy beverages</td>
</tr>
<tr>
<td>AMP Energy (PepsiCo Inc, Purchase, NY)</td>
</tr>
<tr>
<td>Red Bull (Red Bull GmbH, Fuschl am See, Austria)</td>
</tr>
<tr>
<td>Monster Energy (Monster Beverage Corporation, Corona, Calif)</td>
</tr>
<tr>
<td>Rockstar (Rockstar Inc, Las Vegas, Nev)</td>
</tr>
<tr>
<td>NOS (Coca-Cola Company)</td>
</tr>
<tr>
<td>Energy shots</td>
</tr>
<tr>
<td>5-hour Energy (Living Essentials, Wabash, Ind)</td>
</tr>
</tbody>
</table>

Sources: Product labels, company reports, and independent post-marketing analyses.
system (medications, drugs), individual factors (age, weight, sex), the presence of hepatic diseases, environmental exposures, recent use of caffeine, chronic use of caffeine, and whether it is consumed at rest or with exercise.\textsuperscript{13} For example, caffeine-naïve subjects are more sensitive to the physiologic effects of caffeine and have a significantly increased resting heart rate compared with subjects habituated to caffeine.\textsuperscript{14} Further, subjects who have exercised in the past 5 hours or are consuming caffeine during exercise seem to be more sensitive to caffeine effects.\textsuperscript{8,13} Caffeine is mainly metabolized in the cytochrome system of the liver by the enzyme cytochrome 1A2.\textsuperscript{15} The activity of enzyme cytochrome 1A2 can be influenced by factors such as sex, age, and smoking.\textsuperscript{15} In general, women have lower enzyme cytochrome 1A2 activity than men.\textsuperscript{16} Further, women and nonsmokers with lower enzyme cytochrome 1A2 activity have been shown to experience more toxic effects after consuming caffeine when compared with individuals with higher enzyme cytochrome 1A2 activity.\textsuperscript{16} There is a significant interaction between caffeine and a selective serotonin reuptake inhibitor, fluvoxamine, a strong enzyme cytochrome 1A2 inhibitor, which may result in caffeine toxicity.\textsuperscript{17}

### Coronary Vasodilatation

Vasodilatation of the coronary artery is the net result of augmentation in relaxation of the smooth muscle surrounding the blood vessels, as well as removal of the stimuli for contraction.\textsuperscript{18} The endothelium (thin layer of cells that line the artery) is a key regulator of the vascular permeability, hemostasis, and underlying smooth muscle cell tone (resistance).\textsuperscript{19} Thus, the endothelium is critical to normal vessel tone and variation; therefore, normal endothelial function is important for coronary artery function and health in the short and long term, and endothelial dysfunction is detrimental to artery function.\textsuperscript{19} Of note, in cases where there is impairment in endothelium dependent vasodilatation, maximal coronary flow response to adenosine may be reduced even in the absence of stenosis.\textsuperscript{11}

The main intracellular stimuli that can result in the vasodilatation of blood vessels include the following:\textsuperscript{20,49,50}

- Hyperpolarization mediated: Changes in the resting membrane potential of the cell affect the level of intracellular calcium through modulation of voltage-sensitive calcium channels in the plasma membrane, mediated via adenosine.
- Cyclic adenosine monophosphate mediated: Adrenergic stimulation results in elevated levels of cyclic adenosine monophosphate and protein kinase A, which results in increasing calcium removal from the cytoplasm, mediated via prostacyclin.
- Cyclic guanosine monophosphate mediated: stimulation of protein kinase G, via nitric oxide.

To gauge normal coronary artery vasodilatation and contraction in vivo, we require surrogate measures of function. Myocardial blood flow can be measured indirectly using a number of methods, including the following:

- Positron emission tomography (PET) measuring myocardial perfusion reserve.\textsuperscript{21} The major limitations of PET include higher costs, availability of imaging agents, less apparent and more difficult to evaluate artifacts, and high radiation doses.\textsuperscript{21}
- Flow-mediated dilatation of the brachial artery induced by reactive hyperemia is an accurate method for measuring brachial artery endothelial function in humans and is an accepted noninvasive surrogate for coronary artery endothelial function.\textsuperscript{22} Limitations of flow-mediated dilatation include that it is not easily reproducible, it is not established to be directly related to cardiovascular events (ie, not established as a prognostic risk factor), and many extrinsic factors influence its results (recent exercise, food, drink, smoking, and medications).\textsuperscript{22}

### Table 2  Effects of Acute Caffeine Ingestion on the Heart

<table>
<thead>
<tr>
<th>Effect</th>
<th>Mechanism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>No change or increased heart rate</td>
<td>Increased sympathetic stimulation</td>
<td>39-41</td>
</tr>
<tr>
<td>Increased systolic and diastolic blood pressure by 5%-10%</td>
<td>Increased peripheral vascular resistance</td>
<td>39,40,42,43</td>
</tr>
<tr>
<td>Increased myocardial contractility</td>
<td>Increased sympathetic stimulation</td>
<td>39,40</td>
</tr>
<tr>
<td>Caffeine toxicity associated with cardiac arrhythmias, including atrial fibrillation, atrial flutter, atrioventricular-nodal reentry tachycardia, and ventricular tachycardia</td>
<td>Inhibits phosphodiesterase activity and decreased calcium uptake into the sarcoplasmic reticulum; direct effects on inhibiting sodium current in the sodium channel. Both lead to prolongation of signal-averaged QRS complexes, in addition to vagal stimulation.</td>
<td>44-48</td>
</tr>
<tr>
<td>Inhibition of intracellular enzyme phosphodiesterase</td>
<td>Reduced conversion of cyclic adenosine monophosphate to adenosine monophosphate causing prolonged and intensified beta-receptor activation with positive inotropic and chronotropic effects</td>
<td>49,50</td>
</tr>
<tr>
<td>Reduced myocardial blood flow with exercise</td>
<td>Reduced coronary vasodilatation secondary to blockage of adenosine receptors</td>
<td>7-9,36</td>
</tr>
</tbody>
</table>
Table 3  Caffeine Effects on Myocardial Blood Flow at Rest and Exercise.

<table>
<thead>
<tr>
<th>No. of Healthy Subjects Studied (Male)</th>
<th>Mean Age (y)</th>
<th>Caffeine Ingestion (Testing Performed)</th>
<th>Measuring Tool</th>
<th>Criteria</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 (5)</td>
<td>58 ± 13</td>
<td>200 mg (testing 50 min later)</td>
<td>Myocardial perfusion reserve using PET</td>
<td>Ratio of myocardial blood flow during bicycle stress divided by myocardial blood flow at rest.</td>
<td>Exercise-induced myocardial blood flow response decreased 14% after caffeine ingestion ($P &lt; .05$).</td>
<td>7</td>
</tr>
<tr>
<td>18 (11)</td>
<td>27 ± 6</td>
<td>200 mg (testing 50 min later)</td>
<td>Myocardial perfusion reserve using PET</td>
<td>Ratio of myocardial blood flow during bicycle stress divided by myocardial blood flow at rest.</td>
<td>Resting myocardial blood flow was not affected. Exercise-induced myocardial blood flow response decreased 22% after caffeine ingestion ($P &lt; .01$).</td>
<td>8</td>
</tr>
<tr>
<td>40 (33)</td>
<td>53 ± 6</td>
<td>200 mg (testing 60 min later)</td>
<td>Flow-mediated dilatation of the brachial artery</td>
<td>Percent flow-mediated dilation as (maximum diameter minus baseline diameter)/baseline diameter $\times$ 100.</td>
<td>Resting flow-mediated dilation increased 10% after caffeine ingestion ($P &lt; .001$).</td>
<td>23</td>
</tr>
<tr>
<td>10 (10)</td>
<td>26.8 ± 5.2</td>
<td>300 mg (testing 60 min later)</td>
<td>Forearm blood flow responses to acetylcholine, an endothelium-dependent vasodilator, and to sodium nitroprusside, an endothelium-independent vasodilator.</td>
<td>Forearm blood flow was measured by using a strain-gauge plethysmograph.</td>
<td>Resting forearm blood flow was not affected by oral caffeine ingestion. Resting forearm blood flow response to acetylcholine was increased 25% ($P &lt; .05$).</td>
<td>24</td>
</tr>
<tr>
<td>10 (3)</td>
<td>30 ± 3</td>
<td>360 mg (6 mg/kg)</td>
<td>Forearm blood flow was made at baseline and at 20-min intervals.</td>
<td>Forearm blood flow was measured by using the indirect plethysmographic venous occlusion technique.</td>
<td>Before exercise, caffeine increased both systolic blood pressure 17% and mean arterial pressure 11% but had no effect on forearm blood flow. During dynamic exercise, caffeine attenuated the increase in forearm blood flow by 53%, $P &lt; .05$.</td>
<td>9</td>
</tr>
</tbody>
</table>

$\text{PET} = \text{positron emission tomography.}$
Acute Caffeine Ingestion and Effects on Coronary Blood Flow During Exercise

The studies measuring the effects from acute caffeine ingestion on coronary blood flow with exercise are summarized (Table 3).

Myocardial Blood Flow Studies

After caffeine ingestion at rest, myocardial blood flow does not change significantly in PET studies.8 However, in healthy subjects who consume caffeine and then exercise afterward, there are significant reductions in myocardial blood flow.

The first study involved 10 women and 5 men, with a mean age of 58 years. The authors measured myocardial blood flow by PET after ingestion of 200 mg of caffeine, followed by 50 minutes of exercise (stationary bicycle).8 They did not show resting data. Exercise myocardial blood flow response was reduced 14% during exercise after caffeine ingestion.

The second study involved 18 healthy subjects, with a mean age of 27 years, who underwent both resting and exercise myocardial blood flow (stationary bicycle) with PET 50 minutes after taking 200 mg caffeine.7 Although resting myocardial blood flow was unaffected by caffeine, exercise myocardial blood flow was reduced by 22% after caffeine consumption. This study also looked at the effect during altitude simulation (hypobaric hypoxia) and noted that myocardial blood flow was reduced even more by 39%.

Taken together, these studies show that PET measurement of myocardial blood flow in individuals exercising approximately 1 hour after 200 mg of caffeine consumption is reduced in the range of 14% to 22%.7,8 This reduction is even greater if exercise is performed at hypoxia at altitude (39%).8 In patients with coronary artery disease who consume caffeine and then exercise, the reductions are even more significant (18%-25%).7 These studies suggest that the normal exercise-induced hyperemic flow response may at least be antagonized in part by caffeine.8

Flow-Mediated Vasodilatation Studies

The flow-mediated dilation studies noted no significant effect of caffeine or a minor improvement in flow-mediated dilation at rest.23,24 Unfortunately, several studies did not conduct evaluations of flow-mediated dilation during exercise. One study showed reduced forearm blood flow with exercise after caffeine consumption.9

The first study involved 40 subjects (33 men), with a mean age of 53 years, who received 200 mg caffeine. The flow-mediated dilation at rest was noted to be unchanged 1 hour after receiving 200 mg caffeine.23 These subjects had no significant change in their heart rate or systolic or diastolic blood pressure after this dose; this is interesting because caffeine ingestion usually results in an increase in heart rate and systolic and diastolic blood pressures in healthy, normotensive subjects.

Another study involved 10 young men (mean age, 27 years) and measured forearm blood flow by using a strain-gauge plethysmograph and averaging 3 measurements and expressed as milliliters per minute per 100 mL of forearm tissue volume.24 One hour after taking 300 mg of caffeine, there was no demonstrated change in resting forearm blood flow.24 Although heart rate did not change, the subjects’ systolic and diastolic blood pressures both increased after caffeine consumption. Also, there was an improved forearm blood flow response to acetylcholine, an endothelium-dependent vasodilator suggesting that endothelial function may have been enhanced.

Another study examined the acute effects of caffeine on forearm blood flow during dynamic leg exercise in 10 trained, caffeine-naïve cyclists (7 women and 3 men) who were studied at rest and during bicycle ergometry before and after the ingestion of 6 mg/kg caffeine (~300 mg caffeine) or 6 mg/kg fructose (placebo) with 250 mL of water.9 After consumption of caffeine or placebo, subjects rested for 100 minutes (rest protocol) or rested for 45 minutes followed by 55 minutes of cycle ergometry at 65% of maximal oxygen consumption (exercise protocol). Measurements of mean arterial pressure, forearm blood flow, heart rate, skin temperature, and rectal temperature and calculation of forearm vascular conductance were made at baseline and at 20-minute intervals. Plasma angiotensin II was measured at baseline and at 60 minutes post-ingestion in the exercise protocols. Before exercise, caffeine was noted to increase both systolic blood pressure (17%) and mean arterial pressure (11%) without affecting forearm blood flow or forearm vascular conductance. During dynamic exercise, caffeine attenuated the increase in forearm blood flow (53%) and forearm vascular conductance (50%) and accentuated exercise-induced increases in angiotensin II (44%).

Taken together, these studies suggest that caffeine in doses of 200 to 300 mg does not affect resting myocardial blood flow as measured by flow-mediated dilation or forearm blood flow. However, the one study including exercise suggested that caffeine can alter the cardiovascular response to dynamic exercise in a manner that may modify regional blood flow and conductance.9

Mechanisms for Caffeine Effects on Exercise Coronary Blood Flow

Possible mechanisms for caffeine’s effect to reduce myocardial blood flow are listed in Table 4 and discussed in more detail in this section. Caffeine ingestion has been shown to inhibit soluble guanylate cyclase with subsequent suppression of the conversion of guanosine triphosphate into cyclic guanosine monophosphate.25,26 Cyclic guanosine monophosphate serves as the second messenger of the l-arginine/nitric oxide system; consequently, a decrease in cyclic guanosine monophosphate levels could account for the impairment of nitric oxide–mediated effects.27 In blood vessels, cyclic guanosine monophosphate leads to relaxation of vascular smooth muscles, which would lead to vasodilatation
and increased blood flow. By blocking cyclic guanosine monophosphate formation, caffeine may thus prevent vasodilatation. Acute caffeine ingestion also may decrease insulin sensitivity in healthy adults, which could contribute to derangement of nitric oxide production and oxidative stress, possibly through uncoupling of the endothelial nitric oxide synthase.

Caffeine may augment vascular oxidant stress via increased production of angiotensin II, a powerful vasoconstrictor, by inhibiting the suppressing effect of adenosine A1 receptors on the production of renin. This inhibitory effect of caffeine is more potent at adenosine A2A receptors than A1 receptors. Levels of angiotensin II were measured in one study and shown to increase in subjects exposed to caffeine and subsequent exercise. Caffeine also can lead to an overall increase in coronary artery tone and constriction. Caffeine blocks both coronary adenosine A2 receptors and the downstream effects of endogenously produced adenosine on coronary artery vasodilatation in a dose-dependent fashion, leading to overall increased coronary artery tone and net vasoconstriction. Caffeine also has been shown to stimulate release of adrenal norepinephrine leading to stimulation of coronary alpha-2 adrenergic receptors, increasing coronary vasomotor tone with net vasoconstriction. In the sympathetic nerve endings in the heart, caffeine also facilitates norepinephrine release, leading again to increased coronary alpha-2 adrenergic receptor-mediated coronary vasomotor tone and net vasoconstriction.

### DISCUSSION

A dose of caffeine between 200 and 300 mg taken orally does not appear to have any acute detrimental effect on myocardial blood flow at rest. However, if the subject is exposed to exercise, it appears that the usual adenosine- and sympathetic-mediated increased coronary vasodilatation that should match the augmentation in cardiac work is significantly reduced. In light of the propensity of individuals to consume energy products that contain high levels of caffeine and then exercise, the resultant supply—demand mismatch in the heart could lead to possible cardiovascular complications, such as myocardial ischemia, spasm, and arrhythmia, even in healthy individuals. These deleterious effects may be more pronounced with exercise at altitude (e.g., climbing at high terrestrial altitude and skiing).

There was some variability noted in the effects of caffeine on myocardial blood flow as measured by surrogate tools. This is likely related to variations in patient population (age, sex), time of measurement after taking caffeine, and different measurement methodologies used to indirectly measure endothelial function. In addition, each measuring tool has its limitations, including reproducibility and being affected by intrinsic factors. However, in a well-run measuring center that follows strict dietary, exercise, and medication protocols for its test subjects and has well-trained technologists who use specific landmarks to perform ultrasound measurements, the reproducibility of the measures is good.

Caffeine also may have a dual role, so that it increases or decreases endogenous nitric oxide production, causing increased or decreased endothelial function under different circumstances. The balance of the vasodilator effect of caffeine as an endothelium-dependent vasodilator and the vasoconstrictor effect of caffeine as an adenosine-receptor antagonist may regulate vascular function. The latter effect appears to be important in those who have consumed caffeine and then exercise approximately 1 hour later, the point at which caffeine levels in the blood are at their peak.
Although there are several possible mechanisms by which acute caffeine ingestion may affect endothelial function and myocardial blood flow during exercise, it is likely that caffeine antagonizes the coronary artery vasodilator effects mediated by its inhibition of adenosine receptors.9,36-38

The significance of these changes is unknown at this time. However, one possibility is that if there is reduced myocardial blood flow; this may result in a supply—demand imbalance and ischemia that could lead to arrhythmia. In addition, during a period of endothelial dysfunction, coronary artery spasm could occur, leading again to ischemia and arrhythmia. One case report noted significant coronary artery spasm in a healthy adult after consuming high levels of caffeine in the form of an energy beverage.4 Given the possibility that endothelial dysfunction may play a role in morbidity with concomitant caffeine intake and exercise, more research is recommended to clarify the significance of these effects.

CONCLUSIONS
Acute caffeine ingestion in humans elicits various cardiovascular effects, some of which may be deleterious, especially in the setting of stress or exercise. These effects are frequently more pronounced in the caffeine-naive individual or in those acutely ingesting higher doses such as are present in energy beverages. In addition, there are important pharmacologic differences in individual caffeine levels affected by multiple variables, including age, sex, medications, and drugs. In healthy individuals who perform aerobic exercise 1 hour after consumption of 200 to 300 mg of caffeine, a reduction in myocardial blood flow has been noted by indirect tests. Additional research is needed to understand and characterize the underlying mechanisms of caffeine. Moreover, it is critical to assess the safety of high-dose caffeine ingestion in those who are younger, caffeine naïve, or planning to exercise in the next few hours. Specific research needs to be done on the impact of energy products on coronary blood flow, because many of the caffeine-containing sports liquids and gels contain other ingredients that may interact with caffeine and alter the net effect on the coronary artery function during exercise.

References


49. Movsesian MA. Beta-adrenergic receptor agonists and cyclic nucleotide phosphodiesterase inhibitors: shifting the focus from inotropy to cyclic adenosine monophosphate. *J Am Coll Cardiol*. 1999;34:318-324.

Coffee, Caffeine, and Risk of Hospitalization for Arrhythmias

Abstract

Context: Population study data about relations of coffee drinking to arrhythmia are sparse.

Objective: To study relations of coffee drinking to risk of cardiac arrhythmia in 130,054 persons with previous data about coffee habits.

Design and Outcome Measure: We used Cox proportional hazards models with 8 covariates to study coffee-related risk in 3137 persons hospitalized for cardiac arrhythmia. We conducted a similar analysis of total caffeine-related risk in a subgroup with data about other caffeine intake (11,679 study participants; 198 hospitalized).

Results: With non-coffee-drinkers as the referent, the adjusted hazard ratio (HR) for any arrhythmia at the level of <1 cup of coffee per day was 1.0 (95% confidence interval [CI] = 0.9–1.1; p = 0.7); for 1–3 cups/day, it was 0.9 (CI, 0.8–1.0; p = 0.2), and for ≥4 cups/day, it was 0.8 (CI, 0.7–0.9; p = 0.002). With coffee intake as a continuous variable, the HR per cup per day was 0.95 (CI, 0.95–0.99; p = 0.001). Results were similar for several strata, including persons with history or symptoms of possible cardiopulmonary disease and those without such history or symptoms. Coffee had similar relations to atrial fibrillation (48% of participants with arrhythmia) and most other specific arrhythmia diagnoses. Controlled for number of cups of coffee per day, total caffeine intake was inversely related to risk (HR highest quartile vs lowest = 0.6; p = 0.03).

Conclusion: The inverse relations of coffee and caffeine intake to hospitalization for arrhythmias make it unlikely that moderate caffeine intake increases arrhythmia risk.

Background

Cardiac rhythm disturbances are among the toxic effects from very large doses of caffeine administered in animal studies1–3 and taken in human suicide attempts.4 Patients frequently report palpitations after caffeine ingestion, and physicians often advise patients with arrhythmias to avoid caffeinated coffee. However, human experimental1–2 and prospective population study5–12 data about relations of commonly ingested amounts of coffee and caffeine to arrhythmia have yielded inconsistent results. Controlled experiments in humans have shown no relation to ventricular premature beats (VPBs), including data about caffeine restriction in persons with symptomatic VPBs,5 caffeine administration in patients with recent myocardial infarction,7 and inducibility of VPBs.8 A controlled experiment of ingestion of the caffeine equivalent of 4 to 5 cups of coffee showed no relationship to heart rate and number of atrial or VPBs on taped electrocardiograms.5 Two earlier population studies of coffee drinking and incident atrial fibrillation risk11,12 showed no association; another13 showed a weak positive association but with an inconsistent dose–response relationship.

In view of the need for more data about the association of arrhythmia risk with commonly ingested amounts of coffee, we examined and report here data about relations of reported coffee intake to subsequent risk of hospitalization for various arrhythmias in a large free-living population.

Methods

Study Participants and Data

The study protocols were approved by the institutional review board of the Kaiser Permanente Medical Care Program. Study participants were 130,054 members of a Northern California comprehensive health care plan who voluntarily underwent a health examination between 1978 and 1985. For many

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years, the examination was offered to adult members as a way to have a routine health checkup. Examination questionnaire items included ethnicity, other demographics, habits, and medical history. One query was “Do you drink coffee?” Options for answers to that question were “more than 6 cups/day, 4–6 cups/day, 1–3 cups/day, less than 1 cup/day, and never or seldom.” The questionnaire included a similar query about tea intake. Coffee and tea consumption were ascertained one time only. Measurements included body mass index (BMI), blood pressure, fasting blood glucose level, total blood cholesterol level, and leukocyte count. Total coffee and tea intake were ascertained for all 130,054 participants. Examinees in 1984–1985 (n = 11,679, or 9.3% of all participants) answered queries about type of coffee, with answer options being “none, caffeinated only, decaffeinated only, both caffeinated and decaffeinated.” These persons also supplied data about caffeine in tea, soft drinks, and medications, enabling estimation of total caffeine intake. Caffeine intake was studied among these 11,679 participants.

Analytic Methods
Participants were monitored until December 31, 2008, death or other cause for Health Plan termination, or first hospitalization in a program facility with a primary discharge diagnosis of cardiac dysrhythmia, code 427 in the International Classification of Diseases, 9th Revision. Incompleteness of comprehensive computerized data about outpatient arrhythmia diagnoses plus the impracticality of reviewing >130,000 paper records precluded study of arrhythmia events not resulting in hospitalization. The average duration of follow-up monitoring was 17.64 years, with a total of 2,224,214 person-years of observation. There were 3137 persons with an arrhythmia diagnosis. Table 1 shows selected unadjusted distributions.

We used Cox proportional hazards models determined by the PHREG procedure described in version 8 of the user’s guide for SAS Analytics (SAS Institute Inc, Cary, NC). Coffee was studied categorically, with never/seldom as referent and <1, 1–3, 4–6, ≥6 cups/day or <1, 1–3, ≥4 cups/day. With an assumption of linearity, coffee was also studied as a continuous per-cup-per-day variable with these assigned numbers: 0 for never/seldom, 0.5 for <1 cup/day, 2.0 for 1–3 cups, 5.0 for 4–6 cups, and 7 for ≥6 cups. Most multivariate models included age (continuous), sex, ethnicity (white as referent, with black, Asian, Hispanic, and other as additional groups), BMI (<25 kg/m² as referent, with 25–29 kg/m², ≥30 kg/m² as additional groups), education (no college as referent, with some college and college graduate as additional groups), cigarette smoking (never as referent, with ex-smokers, <1 pack/day, and ≥1 pack/day as additional groups), alcohol intake (never as referent, with ex-drinkers and 4 current drinking categories as additional groups), and a cardiorespiratory (CR) composite covariate.

The CR baseline risk covariate was considered positive if the participant answered yes to any of 27 queries involving current or past possible cardiovascular conditions (heart attack, angina, stroke, high blood pressure, diabetes, abnormal findings on electrocardiography, chest pain, palpitations, shortness of breath, blackouts, etc). The participants with a positive CR variable

<table>
<thead>
<tr>
<th>Trait</th>
<th>Percentage of study population (n = 130,054)</th>
<th>Percentage of participants with arrhythmia (n = 3137)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>44.1</td>
<td>47.2</td>
</tr>
<tr>
<td>Women</td>
<td>55.9</td>
<td>52.8</td>
</tr>
<tr>
<td>&lt;60 years old</td>
<td>83.2</td>
<td>56.6</td>
</tr>
<tr>
<td>≥60 years old</td>
<td>17.8</td>
<td>43.4</td>
</tr>
<tr>
<td>White</td>
<td>55.7</td>
<td>63.2</td>
</tr>
<tr>
<td>Black</td>
<td>26.8</td>
<td>21.0</td>
</tr>
<tr>
<td>Asian</td>
<td>10.6</td>
<td>6.5</td>
</tr>
<tr>
<td>Coffee</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>26.9</td>
<td>18.8</td>
</tr>
<tr>
<td>&lt;1 cup/day</td>
<td>14.0</td>
<td>11.9</td>
</tr>
<tr>
<td>1–3 cups/day</td>
<td>41.6</td>
<td>51.4</td>
</tr>
<tr>
<td>4–6 cups/day</td>
<td>12.0</td>
<td>14.4</td>
</tr>
<tr>
<td>≥6 cups/day</td>
<td>4.3</td>
<td>6.5</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>46.4</td>
<td>43.0</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>21.4</td>
<td>24.6</td>
</tr>
<tr>
<td>Current smoker</td>
<td>25.0</td>
<td>28.8</td>
</tr>
<tr>
<td>Cardiorespiratory composite (“yes” to any of 27 queries; see text for details)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiorespiratory “yes”</td>
<td>56.0</td>
<td>73.2</td>
</tr>
<tr>
<td>Cardiorespiratory “not yes”</td>
<td>44.0</td>
<td>26.8</td>
</tr>
</tbody>
</table>
comprised 56.0% of the total study population and 73.2% of participants with arrhythmia.

The subset of 11,679 participants that supplied information about coffee types was also studied by comparing persons taking specific coffee types to non-coffee-drinkers as referent. In the same subset, total caffeine intake was studied as a continuous (per decile) variable. These were studied both with and without control for total amount of coffee intake.

For coffee intake, we studied risk of any arrhythmia and of specific diagnoses. The latter included supraventricular tachycardia (code 427.0; n = 257), paroxysmal ventricular tachycardia (code 427.1; n = 232), atrial fibrillation (code 427.31; n = 1512), atrial flutter (code 427.32; n = 273), ventricular fibrillation/flutter/cardiac arrest (code 427.4–5; n = 91), premature beats (code 427.6; n = 91), and other arrhythmia (code 427.8; n = 755). Analyses yielded estimates of hazard ratios (HRs), 95% confidence intervals (CIs), and p values. We performed similar analyses of the relation of tea to arrhythmia risk.

Similar analyses of total coffee intake were performed, including—one at a time—systolic blood pressure, diastolic blood pressure, total blood cholesterol, glucose, and leukocyte count.

Finally, we used similar models to examine risk of hospitalization for the three most numerous cardiovascular diagnostic groups. These were coronary artery disease (ICD codes 410–414; n = 7658), cerebrovascular disease (codes 430–438; n = 5108), and heart failure (code 428; n = 3418).

### Results

In adjusted models (Table 2), we observed an inverse relation of coffee to risk of hospitalization for arrhythmia, especially supraventricular arrhythmias, that was statistically significant for heavier coffee drinkers (≥4 cups/day) and for coffee as a continuous variable. This inverse relation was progressive in the largest intake categories: for example, at 4–6 cups/day, the HR for all arrhythmias was 0.84 (p = 0.05), and at ≥6 cups/day, it was 0.75 (p = 0.02). The results were similar for most of the specific supraventricular arrhythmia diagnoses. The HR for heavy coffee drinkers was >1.0 for paroxysmal ventricular tachycardia, but it was 0.5 for the composite of “ventricular fibrillation/flutter/cardiac arrest.”

The inverse relation of coffee drinking to arrhythmia was consistent in men, women, whites, blacks, and persons younger than or older than 60 years of age at baseline (Table 3). The association was nearly identical in persons with a yes for CR composite and those without a yes for the composite (Table 3). The inverse coffee relation was slightly stronger for risk within 10 years of examination (vs ≥10 years) and for risk of never-smokers (vs ex-smokers or current smokers). Tea drinking was less prevalent in our study population and was unrelated to any endpoint. For the 24,945 persons reporting intake of ≥1 cup/day of tea (vs none), the HR was 1.01; the HR per cup per day was 1.00.

We performed most stratified analyses separately for atrial fibrillation, the diagnosis for half of all participants. The results for atrial fibrillation were consistently similar to those for all arrhythmias; for example, the HR of those drinking ≥4 cups/day were 0.83 for men, 0.78 for women, 0.78 for white persons, 0.65 for black persons, 0.79 if <60 years old at baseline, 0.83 if ≥60 years old at baseline, 0.64 if <10

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### Table 2. Adjusted hazard ratio (95% confidence interval) of arrhythmia diagnoses by coffee intake

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>N</th>
<th>&lt;1 cup/day</th>
<th>1–3 cups/day</th>
<th>≥4 cups/day</th>
<th>Per cup per day*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any arrhythmia</td>
<td>3137</td>
<td>0.97</td>
<td>0.93</td>
<td>0.82</td>
<td>0.97</td>
</tr>
<tr>
<td>Paroxysmal supraventricular</td>
<td>257</td>
<td>0.85</td>
<td>1.01</td>
<td>0.63</td>
<td>0.94</td>
</tr>
<tr>
<td>tachycardia</td>
<td></td>
<td>(0.88–1.11)</td>
<td>(1.02–1.10)</td>
<td>(0.73–0.93)</td>
<td>(0.88–1.02)</td>
</tr>
<tr>
<td>Paroxysmal ventricular tachycardia</td>
<td>232</td>
<td>0.99</td>
<td>1.04</td>
<td>1.22</td>
<td>1.05</td>
</tr>
<tr>
<td>(0.54–1.34)</td>
<td></td>
<td>(0.71–1.53)</td>
<td>(0.79–1.87)</td>
<td>(0.98–1.12)</td>
<td></td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>1512</td>
<td>0.82</td>
<td>0.88</td>
<td>0.81</td>
<td>0.97</td>
</tr>
<tr>
<td>(0.67–1.00)</td>
<td></td>
<td>(0.76–1.01)</td>
<td>(0.69–0.96)</td>
<td>(0.94–1.00)</td>
<td></td>
</tr>
<tr>
<td>Atrial flutter</td>
<td>273</td>
<td>0.99</td>
<td>0.86</td>
<td>0.80</td>
<td>0.97</td>
</tr>
<tr>
<td>(0.65–1.93)</td>
<td></td>
<td>(0.62–1.20)</td>
<td>(0.54–1.19)</td>
<td>(0.91–1.04)</td>
<td></td>
</tr>
<tr>
<td>Ventricular fibrillation/flutter</td>
<td>91</td>
<td>0.62</td>
<td>0.72</td>
<td>0.47</td>
<td>0.88</td>
</tr>
<tr>
<td>cardiac arrest</td>
<td></td>
<td>(0.28–1.34)</td>
<td>(0.42–1.22)</td>
<td>(0.23–0.96)</td>
<td>(0.78–1.00)</td>
</tr>
<tr>
<td>Premature beats</td>
<td>91</td>
<td>1.98</td>
<td>0.98</td>
<td>0.62</td>
<td>0.87</td>
</tr>
<tr>
<td>(1.02–3.84)</td>
<td></td>
<td>(0.54–1.79)</td>
<td>(0.28–1.35)</td>
<td>(0.77–0.99)</td>
<td></td>
</tr>
<tr>
<td>Other arrhythmia</td>
<td>755</td>
<td>1.02</td>
<td>0.93</td>
<td>0.72</td>
<td>0.94</td>
</tr>
<tr>
<td>(0.79–1.32)</td>
<td></td>
<td>(0.77–1.14)</td>
<td>(0.56–0.93)</td>
<td>(0.90–0.98)</td>
<td></td>
</tr>
</tbody>
</table>

* Versus nondrinkers as referent, with 8 covariates.

Hazard ratio for 4–6 cups/day = 0.82; hazard ratio for ≥6 cups/day = 0.81.

*p < 0.01.

"p < 0.05."
years to diagnosis, 0.84 if ≥10 years to diagnosis, 0.81 if CR “yes,” and 0.84 if CR “not yes.”

In the subset with caffeine data, total caffeine intake was related to lower arrhythmia risk, and the inverse relation to arrhythmia was stronger for persons reporting drinking only caffeinated coffee than for those reporting decaffeinated or both types (Table 4), associations little affected by controlling for total amount of coffee intake.

Among covariate relations, age, male sex, white ethnicity, adiposity (BMI ≥ 30 kg/m²), and blood pressure were all related to increased risk (data not shown). Cigarette smoking was weakly related to risk. For example, compared with never smokers, the HR for <1 pack/day smokers was 1.08 (CI, 0.96–1.21), and for ≥4 pack/day smokers, the HR was 1.13 (CI, 1.04–1.21; p = 0.002). The CR composite was related to risk with a HR of 1.64 (CI, 1.51–1.78; p < 0.001). Inclusion of total blood cholesterol level, systolic or diastolic blood pressure, blood glucose level, or leukocyte count in models had a negligible effect on the HRs for arrhythmia. Although both systolic and diastolic blood pressures were related to risk, the HR for ≥4 cups of coffee was 0.82 (CI, 0.73–0.93) with or without inclusion of either systolic or diastolic blood pressure.

As detailed in the “Study Participants and Methods” section, there were three broad causes of cardiovascular hospitalizations with greater numbers than arrhythmias. The HRs associated with ≥4 cups/day for these were as follows: CAD, 1.13 (CI, 1.04–1.21; p = 0.002); cerebrovascular disease, 0.95 (CI, 0.87–1.04; p = 0.3); and heart failure, 1.04 (CI, 0.93–1.17; p = 0.5).

**Discussion**

It is unclear what, if any, rhythm disturbances are represented by anecdotal reports of “palpitations” after caffeine ingestion. Yet we are unaware of previous population studies that examine all arrhythmia in relation to coffee drinking. The validity of the inverse relation of coffee drinking to risk of hospitalization for arrhythmias in

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**Table 3. Adjusted hazard ratio (95% confidence interval) of arrhythmia in selected groups by coffee consumption**

<table>
<thead>
<tr>
<th>Group (no. hospitalized)</th>
<th>N</th>
<th>≥4 cups/day vs no coffee</th>
<th>Per cup per day (continuous)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>1565</td>
<td>0.84 (0.71–0.99)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.97 (0.95–1.00)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Women</td>
<td>1572</td>
<td>0.80 (0.67–0.95)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.96 (0.93–0.99)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>White</td>
<td>2098</td>
<td>0.84 (0.73–0.97)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.98 (0.95–1.00)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Black</td>
<td>697</td>
<td>0.71 (0.59–0.95)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.93 (0.89–0.98)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Asian</td>
<td>217</td>
<td>0.92 (0.51–1.65)</td>
<td>0.97 (0.89–1.07)</td>
</tr>
<tr>
<td>Never smoked</td>
<td>1426</td>
<td>0.76 (0.64–0.92)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.96 (0.93–0.99)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ex-smokers</td>
<td>959</td>
<td>0.95 (0.76–1.18)</td>
<td>0.98 (0.99–1.01)</td>
</tr>
<tr>
<td>Current smokers</td>
<td>621</td>
<td>0.86 (0.64–1.14)</td>
<td>0.97 (0.94–1.01)</td>
</tr>
<tr>
<td>&lt;10 years to hospitalization</td>
<td>425</td>
<td>0.72 (0.52–0.99)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.95 (0.90–1.00)</td>
</tr>
<tr>
<td>≥10 years to hospitalization</td>
<td>2752</td>
<td>0.84 (0.74–0.95)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.97 (0.89–1.07)</td>
</tr>
<tr>
<td>&lt;60 years old at baseline</td>
<td>1879</td>
<td>0.84 (0.74–0.99)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.97 (0.95–0.99)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>≥60 years old at baseline</td>
<td>1258</td>
<td>0.82 (0.67–0.99)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.96 (0.93–0.99)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cardiorespiratory composite “yes”</td>
<td>2296</td>
<td>0.83 (0.72–0.95)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.97 (0.94–0.99)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cardiorespiratory composite “not yes”</td>
<td>841</td>
<td>0.83 (0.65–1.05)</td>
<td>0.96 (0.92–0.99)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Versus nondrinkers in separate models, with 8 covariates.

<sup>b</sup>p < 0.05.

<sup>c</sup>p < 0.01.

---

**Table 4. Adjusted hazard ratio (confidence interval) of arrhythmia by coffee type and total caffeine**

<table>
<thead>
<tr>
<th>Model</th>
<th>No coffee (n = 2837)</th>
<th>Caffeinated only (n = 4409)</th>
<th>Decaffeinated only (n = 1545)</th>
<th>Caffeinated and decaffeinated (n = 3307)</th>
<th>Caffeine continuous&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total coffee uncontrolled</td>
<td>1.0 (referent)</td>
<td>0.70 (0.51–0.98); p = 0.04</td>
<td>0.90 (0.62–1.30); p = 0.6</td>
<td>0.83 (0.59–1.16); p = 0.3</td>
<td>0.96 (0.92–0.99); p = 0.02</td>
</tr>
<tr>
<td>Total coffee controlled</td>
<td>1.0 (referent)</td>
<td>0.68 (0.42–1.10); p = 0.1</td>
<td>0.87 (0.54–1.39); p = 0.6</td>
<td>0.80 (0.49–1.29); p = 0.4</td>
<td>0.95 (0.90–1.00); p = 0.05</td>
</tr>
</tbody>
</table>

<sup>a</sup>Models with 8 covariates, either without or with control for total coffee, among 11,679 study participants who supplied coffee type and caffeine data; 198 of these were hospitalized for arrhythmia.

<sup>b</sup>Per decile of caffeine score.
these data is reinforced by consistency in most strata. Although the pathophysiologic mechanisms of various rhythm disturbances are not identical, it seems noteworthy that we found similar coffee associations with various supraventricular rhythm diagnoses. It is also important that other common cardiovascular causes of hospitalization, especially CAD and heart failure, did not show similar inverse associations with coffee intake. The relative specificity of the inverse coffee–arrhythmia association may provide further support for the results.

Although of borderline statistical significance, the coffee type and caffeine score data suggest that caffeine is involved. In this population, heavier coffee drinkers drink mostly the caffeinated type. Caffeine from coffee comprised 80% of all caffeine in the subset with caffeine data. Thus, caffeine intake may be a marker for total coffee consumption. Considerable detail about distributions and traits of persons in this population reporting caffeinated and decaffeinated coffee has been published. Tea drinking was unrelated to arrhythmia risk. On average, tea contains less caffeine than coffee, and tea drinkers in this population consume fewer cups per day than coffee drinkers do. Thus, the absence of a relation to tea does not rule out a role for caffeine.

A study of risk of hospitalization for atrial fibrillation in men reported by Wilhelmsen et al showed, in 754 cases, a borderline increase in risk at 1–4 cups/day (odds ratio = 1.24 [CI, 1.00–1.54]) but no significantly increased risk at ≥5 cups/day (odds ratio = 1.09 [CI, 0.87–1.38]). Frost and Vestergaard presented data from 555 cases of atrial fibrillation/flutter in 160,000 men and women, studying caffeine intake, mostly from coffee, by quintile. The HR of quintile 5 versus quintile 1 was 0.91 (CI, 0.70–1.19). A report by Conen et al of 945 incident atrial fibrillation events in relation to caffeine intake in 33,638 women showed an HR of quintile 5 versus quintile 1 of 0.89 (CI, 0.72–1.09). In view of overlapping CIs and differences in methodology, our results for atrial fibrillation are not incompatible with these reports.

**Potential Confounders**

Persons at increased risk of rhythm disturbances because of symptoms or a diagnosis of heart disease might have quit drinking coffee before giving baseline data. Such a “sick quitter” phenomenon could raise the risk of the non-coffee-drinking referent group and cause a spurious inverse coffee association. The similar inverse relation in CR “yes” and “not yes” strata substantially refutes this explanation. We made the CR composite a broadly inclusive one, with the result that it included a majority of all participants. Some of the CR “yes” participants probably had no CR illness, but the “not yes” stratum is likely to be composed of healthy participants with few sick quitters. The specificity of the relation for arrhythmia also reduces the likelihood of this explanation. The weakening of the inverse relation after ten years could be interpreted as support for the sick-quitter phenomenon. However, it might also be because of reduction of coffee intake by some participants as they aged, thus supporting a true inverse coffee association.

Cigarette smoking is correlated with coffee drinking and is an important potential confounder of studies of coffee and health endpoints. Smoking was not strongly related to arrhythmias in our data, and the inverse coffee–arrhythmia relation was strongest in never-smokers, points against the likelihood of confounding by smoking. Both nicotine and caffeine are metabolized by the hepatic cytochrome P4501A1 enzyme. Benowitz et al called acceleration of hepatic metabolism of caffeine by cigarette smoking “a well-established observation.” We speculate that more prolonged presence of caffeine in the blood of never-smokers might be a factor in their stronger inverse coffee–arrhythmia relation.

Persons with rhythm problems are often not hospitalized. For the years of follow-up monitoring, we had no data about rhythm disturbances not resulting in hospitalization. Thus, it is likely that our participants disproportionately include persons with arrhythmias causing hemodynamic compromise or other reasons for concern about the rhythm disturbance. It is possible that the relation of coffee drinking to more benign rhythm problems might be different from the results reported here. We have no data that cast light on this possibility.

We have no data about follow-up coffee use, but loss to follow-up monitoring is not systematically related to coffee habits in this population. Other limitations of this analysis include incomplete caffeine data; lack of validation of diagnoses by actual paper-chart review; and absence of data about circumstances leading to hospitalization, method of coffee preparation, size of cups, time of day coffee was imbibed, and dietary habits. Strengths include the large multiethnic study cohort, diversity of coffee habits, and presence of data about demographics and smoking.
Possible Mechanisms
Biologic mechanisms for a protective effect of coffee are speculative. Caffeine, the most prominent pharmacologically active ingredient in coffee, induces catechol release, but primarily in caffeine-naïve persons and not in regular ingesters.9 Probably the caffeine action of most hypothetical relevance is antagonism of adenosine by competitive binding to receptors. Pharmacologic doses of adenosine decrease atrioventricular nodal conduction, reduce atrial muscle contractility, decrease sinoatrial node activity, and cause coronary artery dilation.21
It is unclear how antagonism of these adenosine actions by caffeine might reduce risk of arrhythmias. Adenosine also shortens the refractory period of atrial tissue and can trigger atrial fibrillation and other supraventricular arrhythmias during pharmacologic coronary disease testing20–25; these aspects may more plausibly support a protective effect of adenosine antagonism by caffeine.
Noncaffeine ingredients abound in coffee. Relevant to cardiovascular disease is a diterpene compound, cafestol, in coffee prepared without additives. Cafestol, in coffee, induces catechol release, and caffeine is a rich source of antioxidants,6–7 a possible source of health benefit, but not specifically for arrhythmias. We will not speculate further here.

Conclusion
In a large cohort, coffee drinking and caffeine intake are inversely related to risk of hospitalization for arrhythmias, especially atrial fibrillation and other supraventricular arrhythmias. These observational data do not establish causality, and thus a protective effect is not proven. It is highly unlikely that moderate caffeine intake increases arrhythmia risk. 

Disclosure Statement
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Acknowledgment
Katharine O’Moore-Klopf, ELS, of KOK Edit provided editorial assistance.

References

A Useful Medicine

Coffee, though a useful medicine, if drank constantly, will at length induce a decay of health, and hectic fever.
— The Moral Instructor, Pt IV, Sect II, Ch 10; Jesse Torrey, 1787-1834
CYP1A2 genotype modifies the association between coffee intake and the risk of hypertension

Paolo Palatini, Giulio Ceolotto, Fabio Ragazzo, Francesca Dorigatti, Francesca Saladini, Italia Papparella, Lucio Mos, Giuseppe Zanata and Massimo Santonastaso

Objectives The longitudinal relationship between coffee use and hypertension is still controversial. Cytochrome P450 1A2 (CYP1A2) is the main responsible enzyme for the metabolism of caffeine. The aim of the present study was to investigate the effect of coffee intake on the risk of developing hypertension needing antihypertensive treatment in individuals stratified by CYP1A2 genotype.

Design We assessed prospectively 553 young White individuals screened for stage 1 hypertension. Coffee intake was ascertained from regularly administered questionnaires. Incident physician-diagnosed hypertension was the outcome measure. Genotyping of CYP1A2 SNP was performed by real time PCR.

Results During a median follow-up of 8.2 years, 323 individuals developed hypertension. For carriers of the slow *1F allele (59%), hazard ratios of hypertension from multivariable Cox analysis were 1.00 in abstainers (reference), 1.72 (95%CI, 1.21–2.44) in moderate coffee drinkers (P = 0.003), and 3.00 (1.53–5.90) in heavy drinkers (P = 0.001). In contrast, hazard ratios for coffee drinkers with the rapid *1A/*1A genotype were 0.80 (0.52–1.23, P = 0.29) for moderate drinkers and 0.36 (0.14–0.89, P = 0.026) for heavy drinkers. In a two-way ANCOVA, a gene × coffee interactive effect was found on follow-up changes in systolic (P = 0.000) and diastolic (P = 0.007) blood pressure. Urinary epinephrine was higher in coffee drinkers than abstainers but only among individuals with slow *1F allele (P = 0.001).

Conclusion These data show that the risk of hypertension associated with coffee intake varies according to CYP1A2 genotype. Carriers of slow *1F allele are at increased risk and should thus abstain from coffee, whereas individuals with *1A/*1A genotype can safely drink coffee. J Hypertens 27:1594–1601 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Keywords: caffeine, coffee, CYP1A2, hypertension, polymorphism

Introduction As caffèinated coffee has been reported to increase blood pressure (BP) in cross-sectional studies [1–4], prospective studies examining the association between coffee consumption and risk of hypertension have been inconclusive. Two meta-analyses of randomized controlled trials whose duration ranged from one to 12 weeks, demonstrated a small BP increase for coffee intake [5,6]. The effect of coffee consumption on the risk of developing hypertension in the long term has been explored in a few prospective studies, which provided conflicting results [7–9]. In a cohort of young White men from the United States, coffee use was associated with risk of hypertension, but the association was no longer significant after adjustment for confounders [7]. In a more recent analysis of the Nurses’ Health Studies I and II of 155,594 the United States normotensive women, regular coffee intake was not associated with increased risk of hypertension [8]. At variance with those results, coffee abstinence was found to be associated with lower risk of hypertension in the Doetinchem Cohort Study [9].

Cytochrome P450 1A2 (CYP1A2) is the main responsible enzyme for the metabolism of caffeine [10]. An A → C substitution at position 734 (CYP1A2*1F) in the CYP1A2 gene decreases enzyme activity and inducibility [11,12]. Carriers of the variant *1F allele are ‘slow’ caffeine metabolizers, whereas individuals homozygous for the CYP1A2*1A allele are ‘rapid’ caffeine metabolizers [11,12]. Recently, it has been demonstrated that coffee consumption increases the risk of myocardial infarction only among individuals with slow metabolizer genotype [13], whereas among those with the *1A/*1A genotype coffee even decreases the risk. Whether CYP1A2 genotype modifies the association between coffee intake and risk of hypertension is not known. Thus, the aim of the present study was to examine whether CYP1A2 genotype modifies the long-term association between...
intake of caffeinated coffee and risk of hypertension needing antihypertensive treatment in the participants of the HARVEST (Hypertension and Ambulatory Recording VEnetia Study), a prospective longitudinal study of young individuals screened for stage 1 hypertension [14].

Methods
Study population
The HARVEST is a prospective study of 18–45 year old individuals, initiated in 1990, investigating the origin of hypertension with regard to physiological [14], genetic [15], and clinical [16] characteristics. Never treated individuals screened for stage 1 hypertension (systolic BP between 140 and 159 mmHg and/or diastolic BP between 90 and 99 mmHg) were enrolled. Patients with diabetes mellitus, nephropathy, and cardiovascular disease were excluded [14–16]. The 553 individuals taking part in this subproject were all those recruited and followed up in the four HARVEST centers, which agreed to participate in the genetic study [15]. Their characteristics are shown in Table 1. The higher prevalence of men among our study participants (72.5%) confirms previous observations of a much higher prevalence of men in the young segment of the hypertensive population [17]. Patients’ recruitment was obtained with the collaboration of the local general practitioners who were instructed during local meetings. Consecutive patients with the earlier-mentioned clinical characteristics seen in the offices of the general practitioners and willing to participate in the study were eligible for recruitment and were sent to the referral centers. Blood and urine samples are periodically collected and taken to the coordinating office in Padova, where they are processed. The study was approved by the HARVEST Ethics Committee and the Ethics Committee of the University of Padova, and written informed consent was given by the participants.

Procedures
At baseline, all individuals underwent physical examination, anthropometry, blood chemistry, and urine analysis. Participants completed questionnaires about their lifestyle, including coffee consumption, physical activity, alcohol use and cigarette smoking. Coffee intake was defined according to the number of caffeine-containing coffees drunk per day. The caffeine content per cup of ‘expresso’ Italian coffee, which was the most abundantly consumed type of coffee by the HARVEST participants, averages 100 mg [18]. Decaffeinated coffee was not taken into account. Tea and other caffeinated drinkings were not taken into account in the present study being unusual and irregular in this area of Italy (Venetia). A positive family history of hypertension was defined as one or two parents having hypertension and/or taking antihypertensive treatment [15]. Details about the interview, lifestyle assessment and criteria used for subjects’ classification according to lifestyle were reported elsewhere [14–16]. Baseline BP was the mean of six readings obtained during two visits performed 2 weeks apart [14–16]. BMI was considered as an index of adiposity (weight divided by height squared). Individuals with the metabolic syndrome were identified by applying AACE criteria [19]. In 298 participants, urine was collected over 24 h for epinephrine and norepinephrine assessment by a HPLC method [14]. Urine specimens were frozen (–20°C) and sent to the Coordinating Office at the University of Padova, where they were processed [14].

Genotyping
Genomic DNA was extracted from whole blood through the NucleoSpin Blood kit (Macherey-Nagel, Düren, Germany). Primers and probes for allelic discrimination analysis of CYP1A2 polymorphism, designed from sequences derived from the GenBank database using Primer 3 (provided by the Whitehead Institute Cambridge, Massachusetts, USA) and Operon’s Oligo software (Operon Technologies Inc., Alameda, California, USA), were as follows: forward primer AGAGAGCCA GCGTTCATGTT, reverse primer CTGATGCGTGT TCTGTGCTTT, CYP1A2*1F probe (FAM-labelled)-5’-TCTGTGCGCCACAGGTTG-3’ (BLACK HOLE1), CYP1A2*1A (TEXAS RED labelled)-5’-TCTGCGCCACAGGTTG-3’.

Table 1 Clinical characteristics of the study individuals by CYP1A2 genotype

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total (N = 553)</th>
<th>*1A/*1A (N = 226)</th>
<th>*1A/*1F-1/*1F-1/*1F (N = 327)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33.2 (8.6)</td>
<td>33.2 (8.6)</td>
<td>33.5 (8.6)</td>
</tr>
<tr>
<td>Sex (m/f, %)</td>
<td>72.5/27.5</td>
<td>72.1/27.9</td>
<td>72.6/27.2</td>
</tr>
<tr>
<td>Parental hypertension (no/yes, %)</td>
<td>37.8/62.2</td>
<td>41.7/58.3</td>
<td>35.2/64.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.2 (3.5)</td>
<td>25.2 (3.5)</td>
<td>25.3 (3.6)</td>
</tr>
<tr>
<td>Baseline systolic blood pressure (mmHg)</td>
<td>145.9 (10.5)</td>
<td>144.9 (10.3)</td>
<td>146.6 (10.6)</td>
</tr>
<tr>
<td>Baseline diastolic blood pressure (mmHg)</td>
<td>93.4 (5.6)</td>
<td>93.2 (5.6)</td>
<td>93.6 (5.3)</td>
</tr>
<tr>
<td>Coffee intake (no intake/moderate/ heavy, %)</td>
<td>26.9/62.9/10.2</td>
<td>26.1/64.6/9.3</td>
<td>27.5/68.1/8.0</td>
</tr>
<tr>
<td>Physical activity (no/yes, %)</td>
<td>59.9/40.1</td>
<td>58.4/41.6</td>
<td>60.8/39.1</td>
</tr>
<tr>
<td>Cigarette smoking (no/yes, %)</td>
<td>80.5/19.5</td>
<td>78.8/21.2</td>
<td>81.7/18.3</td>
</tr>
<tr>
<td>Oral contraceptive use in women (no/yes, %)</td>
<td>85.4/14.6</td>
<td>87.3/12.7</td>
<td>84.1/15.9</td>
</tr>
<tr>
<td>Alcohol drinking (no/0–50 g/day&gt; 50 g/day, %)</td>
<td>53.2/41.6/5.2</td>
<td>52.7/42.0/5.3</td>
<td>53.5/41.3/5.2</td>
</tr>
<tr>
<td>Metabolic syndrome (%)</td>
<td>23.9</td>
<td>21.7</td>
<td>25.4</td>
</tr>
</tbody>
</table>

Values are given as mean (SD) or percentage. All differences between genotypes are nonsignificant.
Follow-up and study end-point
Office BP and lifestyle habits were assessed monthly during the first 3 months of follow-up, then after 6 months, and every 6 months thereafter. In the HARVEST study, the endpoint was development of hypertension eligible for antihypertensive medication after a period of lifestyle modification. After baseline examination, individuals were given general information about nonpharmacological measures by the HARVEST investigators, following the suggestions of current guidelines on the management of hypertensive patients [21–25]. To ensure homogeneous counselling by doctors participating in the study, training in current international guidelines was provided to them throughout the study duration. As the criteria for treatment were revised several times by the Scientific Committees from 1990 to 2006 [21–24], the definition of the end point changed accordingly. Before 1999, the criteria for treating low-risk individuals with stage 1 hypertension, such as those enrolled in the HARVEST study, were primarily based on the BP level [21,22]. In 1999, stratification of patients according to global cardiovascular risk became crucial for deciding whether antihypertensive drug treatment was needed [23]. However, being the participants in this study young to middle-aged individuals mostly at low-cardiovascular risk, also after 1999 most end points were based on the BP level.

Individuals who do not meet the criteria for treatment are checked at 6-month intervals unless they drop out. Enrollment of individuals with *1A/*1A genotype versus those with *1A/*1F + *1F/*1F genotype and of coffee drinkers versus abstainers was equally distributed throughout the years. The definition of sustained hypertension is based on at least six clinic BP readings taken on two subsequent visits within 1 month [14–16]. The second end-point visit was performed immediately before starting antihypertensive treatment. Thus, final BP was the average of the last six clinic readings before starting treatment in the individuals who reached the endpoint and was the average of the three readings obtained at the last available visit in the individuals who remained untreated. Other details on follow-up procedures were reported elsewhere [14–16].

Data analysis
The present study was performed in the 589 individuals for whom BP and information on lifestyle habits were available both at baseline and final assessments and who had at least 6 months of follow-up. Thirty-six individuals who were past coffee drinkers or drank less than 1 cup/day were excluded leaving a total of 553 individuals for analysis. For individuals lost to follow-up, the last available BP values were taken into account. Results are presented using a dominant *1F allele model with *1A/*1F and *1F/*1F genotypes combined, because both groups have a similar rate of caffeine metabolism [11,12]. Participants were grouped into three categories of coffee drinking, nondrinkers (none), moderate drinkers (one to three cups daily) and heavy drinkers (four or more cups daily), a classification used in previous analyses [4,24]. Current smokers were those who reported smoking one or more cigarettes per day. Four categories of alcohol drinking were considered (0 g, <50 g, 50–100 g, >100 g of alcohol/day) [14]. As there were only two individuals in the more than 100 g/day alcohol class, the two upper classes of alcohol were subsequently combined. The significance of differences in categorical variables was assessed with the \( \chi^2 \) test. Differences between individuals with CYP1A2 *1A/*1A genotype and carriers of *1F allele were assessed with unpaired \( t \) test adjusting for age and sex. The distribution of clinical variables were compared across classes of coffee consumption by ANCOVA adjusting for age and sex. We evaluated potential gene x coffee interactions on baseline catecholamines and follow-up changes in systolic and diastolic BPs in a two-way ANCOVA after adjustment for age and sex. Changes in BPs were adjusted also for follow-up time and baseline BPs. For catecholamines, significance was given for log-transformed data. Within each CYP1A2 group, the risk of hypertension related to coffee intake was assessed in multivariable Cox proportional hazards models adjusting for sex, age, BMI, family history for hypertension, duration of hypertension, physical activity, smoking status, alcohol consumption, and baseline BP. Analyses were also performed within strata of alcohol use and smoking status. Coffee intake was also modelled as a time-dependent categorical variable in Cox proportional hazards analysis. No violations to the proportional hazards assumption were detected by inspection of survival curves. Estimates of hazard ratio and corresponding two-sided 95% confidence intervals (CIs) relating coffee consumption to risk of hypertension were computed from the Cox models. A two-tailed probability value less than 0.05 was considered significant. Data are presented as mean ± SD unless specified. Analyses were performed using Statistica version 6 (Stat Soft, Inc, Tulsa, Oklahoma, USA) and Systat version 10 (SPSS Inc., Evanston, Illinois, USA).
Results

Twenty-seven percent of participants did not drink coffee, 62.9% were moderate coffee drinkers (1–3 cups/day), and 10.1% were heavy coffee drinkers (≥4 cups/day). Genotypes frequencies (*1A/*1A = 41%, *1A/*1F = 43%, *1F/*1F = 16%) were in agreement with the Hardy-Weinberg equilibrium ($\chi^2 = 0.81, P = 0.36$). The proportion of *1F carriers did not differ between the categories of coffee intake ($P = 0.7$, Table 1). Coffee drinkers were older ($P = 0.000$) and slightly heavier ($P = 0.072$) than abstainers, and were more likely to smoke cigarettes ($P = 0.005$) and drink alcohol ($P = 0.000$). Coffee intake was not related to gender and baseline BP. Demographic and risk factor characteristics of participants divided by genotypes are presented in Table 1. Baseline systolic BP was slightly higher in carriers of *1F allele than in homozygous for the *1A allele but the difference was of borderline significance ($P = 0.063$). The other clinical variables did not differ between the groups. Plasma glucose, total cholesterol, high-density lipoprotein cholesterol, and triglycerides did not differ across categories of coffee consumption or CYP1A2 genotypes (data not shown).

Follow-up

During a median follow-up of 8.2 years only 24 individuals (4.3%) changed their habits. Of these, 11 individuals (2%) started to drink coffee or increased coffee consumption, whereas 13 individuals (2.5%) quit or reduced coffee use. During the follow-up, 323 individuals (58.4%) developed sustained hypertension needing antihypertensive treatment whereas 230 individuals did not meet the criteria for treatment. In the whole cohort, only small changes in clinic BP were observed during the follow-up end declined to $8.1/85.3$ mmHg. In the individuals who did not reach the endpoint BP at follow-up end declined to $133.6 \pm 81.8/85.3 \pm 6.1$ mmHg. In the individuals stratified by CYP1A2 genotype, an increased incidence of hypertension was found in coffee drinkers compared with abstainers among the carriers of the slow *1F allele (64.6 versus 47.8%, respectively, $P = 0.006$) but not among the individuals homozygous for the *1A allele (55.1 versus 59.3%, respectively, $P = 0.29$). In a two-way ANCOVA, both CYP1A2 genotype and coffee were independent predictors of the follow-up increase in systolic BP with a highly significant interactive effect of the two variables on the BP change (Table 2). An interactive effect of CYP1A2 genotype and coffee was found also for diastolic BP. No effect of coffee or CYP1A2 genotype was found on adjusted follow-up changes in body weight, plasma glucose, total cholesterol, high-density lipoprotein cholesterol, or triglycerides. In a Cox proportional hazards analysis assessing the risk of hypertension needing antihypertensive treatment associated with coffee drinking in the whole group, coffee was a weak predictor of outcome ($P = 0.04$). However, when participants were stratified by CYP1A2 genotype, after taking into account age, sex, parental hypertension, hypertension duration, physical activity, smoking status, alcohol intake, BMI, and BP at baseline, the increased risk of hypertension associated with coffee intake was observed only among carriers of the slow *1F allele (Fig. 1). In this group, the hazard ratio (95% CI) of hypertension was 1.72 (95% CI 1.21–2.44, $P = 0.028$) for 1 to 3 cups/day and was 3.00 (95% CI 1.53–5.90, $P = 0.001$) for 4 cups/day or more, as compared with abstainers. Among the participants who were homozygous for the rapid *1A allele there was even an inverse association between coffee intake and hypertension. The hazard ratios were 0.80 (95% CI 0.52–1.23, $P = 0.29$) for 1–3 cups/day and 0.36 (95% CI 0.14–0.89, $P = 0.026$) for 4 cups/day or more. Inclusion of oral contraceptive use in women into the regression model did not materially alter the results. When coffee intake during follow-up was included in the model as a time-dependent covariate, or when more recent coffee intake was taken into account, results were similar to those seen for baseline coffee drinking. The association between coffee intake and risk of hypertension was not influenced by gender, smoking status at baseline, or alcohol use in either CYP1A2 group.

Urinary catecholamines according to coffee use and CYP1A2 genotype

Urinary epinephrine was higher among coffee drinkers than nondrinkers ($P = 0.012$). However, the between-category difference in urinary epinephrine was significant among carriers of the slow *1F allele and not among individuals with the *1A/*1A genotype (Fig. 2). In a two-way ANCOVA, an interactive effect of coffee and CYP1A2 genotype was observed on urinary epinephrine ($P = 0.050$). Similar results were found for urinary norepinephrine (Fig. 2), but the between-category differences did not attain the level of statistical significance for either CYP1A2 group.

Table 2 Follow-up changes in blood pressure in the participants subdivided according to CYP1A2 genotype and coffee consumption. Results of two-way ANCOVA

<table>
<thead>
<tr>
<th></th>
<th>Cups/day coffee (*1A/*1A genotype)</th>
<th>Cups/day coffee (*1A/*1F = 1F/*1F genotypes)</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0  1–3  ≥4</td>
<td>0  1–3  ≥4</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>59 146 21</td>
<td>90 202 35</td>
<td></td>
</tr>
<tr>
<td>Systolic BP</td>
<td>0.0±2.2  −0.4±1.3  −5.9±2.9</td>
<td>−2.8±1.7  1.0±1.1  2.8±2.3</td>
<td>0.050</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>1.3±1.4  0.4±0.8  −5.6±1.9</td>
<td>0.2±1.1  2.6±0.7  3.0±1.5</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Data are means ± SEM, and are adjusted for age, sex, baseline blood pressure, and follow-up length. BP denotes blood pressure in mmHg.
study in a subset of those individuals to investigate whether the CYP1A2 genotype modifies the relationship between coffee intake and risk of developing hypertension needing antihypertensive treatment. We found a linear relationship between coffee use and risk of hypertension among carriers of the *1F variant of the CYP1A2 gene and an inverse association among individuals homozygous for the *1A allele.

All guidelines recommend several dietary changes for preventing development of hypertension [21–24]. Coffee consumption is not included among the nonpharmacological measures, which should be instituted because the adverse effects of coffee on hypertension have constituted a controversial subject for several decades. Meta-analyses examining the relationship between coffee intake and risk of hypertension have observed a positive though weak association among short-term clinical trials [5,6] but not among prospective cohort studies. Two large prospective studies failed to demonstrate an independent association between coffee intake and incidence of hypertension [7,8]. In the study by Klag et al. [7] the association of coffee drinking with hypertension incidence ceased to be statistically significant after taking into account parental hypertension and lifestyle factors. In the study conducted in the Nurses’ Health Studies I and II, a positive association was found between risk of hypertension and frequency of caffeinated soft drinks [8]. However, coffee was not associated with incident hypertension in either cohort. In a recent report by Funatsu et al. [26], coffee intake of more than three cups per day for 4 weeks even lowered BP in a small

Discussion
In a previous analysis of the whole cohort of the HARVEST participants, we found a nonlinear association between coffee consumption and development of sustained hypertension [25]. We undertook the present
group of prehypertensive men. At variance with the above data, in a recent study by Uiterwaal et al. [9] abstinence from coffee was found to be associated with lower risk of hypertension compared with moderate coffee drinking. In women, a lower risk of hypertension was found also for heavy consumers [9].

Ninety-five percent of caffeine is detoxified through an initial N\textsuperscript{3}-demethylation catalyzed by CYP1A2, and caffeine is an inducer of the enzyme [10–12]. However, this enzyme has a wide interindividual variability in activity, which is regulated by a genetic polymorphism [27]. Thus, CYP1A2 genotype may have important influences on the pressor effect of coffee. Indeed, our results demonstrated that carriers of the *1F allele, who are slow caffeine metabolizers [11,12], had an increased risk of developing hypertension needing antihypertensive treatment which was proportional to coffee intake. Within the individuals with the fast *1A/*1A genotype an opposite trend was found. The CYP1A2 genotype-related difference was particularly striking among the individuals who drank at least 4 cups/day. If coffee exerts opposite effects on BP according to CYP1A2 genotype, it is not surprising that most studies, including our previous analysis [25], found a nonlinear association between coffee intake and risk of hypertension. In the Klag et al. study [7], the relative risk estimates increased only slightly with successive levels of coffee drinking, and decreased somewhat in the heaviest drinkers. In the women of both Nurses' Health Studies cohorts [8], and those of the Uiterwaal et al. study [9], even a modest inverse U-shaped relation was found between coffee intake and the incidence of hypertension.

Coffee is a complex ‘blend’ of a vast number of different bioactive chemicals, any of which may be responsible for its effect on BP [28]. Among these, polyphenols seem to act as protective antioxidants and have other beneficial actions on the cardiovascular system [28]. The polyphenols chlorogenic acid and dihydrocaffeic acid (both found in coffee) have been shown to increase nitric oxide synthase activity in a dose-dependent manner in cultured cells, which is associated with a comparable increase in endothelial nitric oxide synthase protein [29]. In spontaneously hypertensive rats, the development of hypertension was inhibited when rats were fed diets containing 0.5% chlorogenic acid for 8 weeks [30]. A BP-lowering effect of chlorogenic acid has been observed also in human studies. In 28 mild hypertensive individuals who were randomized in a double-blind placebo-controlled study to receive either 140 mg of chlorogenic acid or placebo for 12 weeks, the chlorogenic acid regimen caused a significant 10 mmHg lowering of systolic BP and 7 mmHg lowering of diastolic BP [31]. Thus, there seems to be a Jekyll and Hyde aspect to coffee whose overall action on the cardiovascular system appears to be regulated by the CYP1A2 gene. In individuals with the fast *1A/*1A genotype, the effect of caffeine on BP seems to be negligible and outweighed by the hypotensive action of polyphenols or other bioactives. In carriers of CYP1A2*1F allele, who are slow caffeine metabolizers, the pressor effect of caffeine seems to prevail. Indeed, in the present study, after 8.2 years of follow-up our heavy coffee drinkers homozygous for the fast *1A/*1A genotype had a 9 mmHg lower BP than their counterparts with the slow *1F allele. This interpretation explains why in a meta-analysis of randomized controlled trials on the effect of intake of coffee and caffeine, BP elevations were reported to be much larger for caffeine than coffee [6]. In the Nurses Health Study I and II [8], caffeinated colas but not coffee were associated with hypertension, which may be due to the lack of polyphenols in the colas and the presence of them in the coffee. This mechanism may explain the differential effect of coffee found also in patients with myocardial infarction according to CYP1A2 genotype. Also in this clinical setting carriers of *1F allele had an increased risk of myocardial infarction with increasing coffee consumption and homozygous for *1A allele had a decrease in risk [13].

Urinary epinephrine and norepinephrine and vanillylmandelic acid have been shown to increase after caffeine administration in humans [32,33], and increased sympathetic activity is considered one main mechanism through which caffeine raises BP. In agreement with our previous results [24], in the present study there was a relationship between coffee intake and urinary epinephrine. However, the increase in epinephrine related to coffee consumption was found only among individuals with the CYP1A2*1F allele, whereas no effect of coffee on catecholamines was found among the individuals with the fast *1A/*1A genotype.

The absence of an association between coffee and risk of hypertension in some studies may have been due to a relatively low frequency of *1F carriers in the populations that were examined. In the HARVEST study, the frequency of carriers of the *1F allele was 59%, but lower frequencies have been reported in other populations [34,35].

Our results were obtained in a cohort of individuals screened for stage 1 hypertension with a low cardiovascular risk profile. According to international guidelines, these individuals should be followed for extended periods with nonpharmacological treatment [21–24]. Indeed, in many of the HARVEST participants, clinic BP decreased to below 140/90 mmHg during the first few months of observation, and in the group that did not meet the criteria for treatment mean final clinic BP was 133.6/85.3 mmHg. In the HARVEST study, the diagnosis of sustained hypertension was made on the basis of several visits encompassing an average of over 50 BP measurements per person using uniform criteria. However, a limitation of our study is that the definition of the
endpoint changed during the follow-up because treatment criteria for this category of individuals changed from 1990 to 2006. However, individual’s recruitment was well balanced throughout the years across categories of either coffee intake or CYP1A2 genotype. In addition, besides categorical endpoint we also considered absolute changes in BP during the follow-up as outcome measure obtaining consistent results.

Limitations
Several limitations of this study should be noted. In the present study we used a short food frequency questionnaire and the results can only be interpreted as indicators of the dietary habits in general. Therefore, misclassification must be considered. Other weaknesses of our study include the incomplete nature of our questionnaire without information on dietary factors associated with hypertension incidence such as dietary intake of sodium (or measurement of sodium urinary output), potassium, and fiber. However, previous studies have shown that coffee consumption was associated to alcohol use, smoking and physical activity, which were available in our study, and not to other dietary habits [36]. In addition, we did not measure caffeine, polyphenols, or direct markers of CYP1A2 catalytic activity. However, urinary epinephrine concentration, which is related to caffeine intake [25,32,33], was proportional to coffee consumption and was more elevated in IF allele carriers. These limitations notwithstanding, the major strength of our analyses is that they offer data-linking coffee intake and change in BP over time from a homogeneous cohort of young-to-middle-age individuals included within a narrow BP range. However, because we do not know of any other study on the effects of the CYP1A2 polymorphisms on the BP effects of chronic coffee use, the present findings should be confirmed in other studies.

In conclusion, the present data show that coffee has differential effects on BP among individuals screened for stage 1 hypertension according to CYP1A2 genotype. Abstinence from caffeinated coffee should be included among the dietary changes that should be instituted in prehypertensive or hypertensive patients who are carriers of the 1F allele. In individuals homozygous for the 1A allele coffee consumption can be allowed.

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List of the centers participating in the HARVEST study:

Trial Coordinator: P Palatini.

References
Caffeine and Cardiac Arrhythmias: A Review of the Evidence

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ABSTRACT

Limited data exist on the safety and physiologic effects of caffeine in patients with known arrhythmias. The studies presented suggest that in most patients with known or suspected arrhythmia, caffeine in moderate doses is well tolerated and there is therefore no reason to restrict ingestion of caffeine. A review of the literature is presented.

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KEYWORDS: Arrhythmia; Caffeine

Over half of Americans drink coffee daily, and more ingest caffeine in either coffee or another form such as tea, soda, and chocolate. The topic of the effects of caffeine on the cardiovascular system has been a source of much debate. Case reports have linked caffeine overdose to coronary vasospasm and a variety of supraventricular and ventricular arrhythmias. A survey of 697 providers revealed that 80%-90% recommended moderating caffeine intake in patients with known tachycardia, palpitations, and arrhythmia, yet it is unclear from where this impressive consensus arises. A common question in the care of patients with known arrhythmia is the safety of caffeine and whether there is a need to curtail caffeine intake.

Currently, our limited understanding is based on case reports, cohort studies, physiologic studies, and large epidemiologic studies. Case reports must be cautiously considered due to the inherent bias of reporting only cases in which caffeine ingestion was coincident with an arrhythmia; these do not demonstrate causality. Cohort studies that evaluate this topic examine at-risk populations such as those with known ventricular arrhythmias, but the number of subjects is small, making it difficult to draw conclusions. Physiologic studies provide insights into the effects of caffeine on the cardiac conduction system, but study designs do not necessarily translate into true conditions. Epidemiologic studies evaluate healthy but not at-risk populations. Unfortunately, there are no large-scale randomized controlled trials on the effect of caffeine in populations of patients with arrhythmias.

The purpose of this article is to review the evidence relating caffeine and arrhythmia in order to provide an understanding of our knowledge and the limitations of our knowledge, as well as to provide recommendations for patients with known or suspected arrhythmia with regard to caffeine intake.

CAFFEINE AND CARDIOVASCULAR DISEASE

Caffeine is a nonselective competitive antagonist of adenosine receptor subtypes A1 and A2A in concentrations typically consumed by humans. At higher concentrations, caffeine can induce intracellular calcium release and phosphodiesterase inhibition, and at higher doses not typically consumed, can cause gamma-aminobutyric acid inhibition. It is suggested that 100 mg can increase alertness in humans. Increases in blood pressure are noted at 250 mg, and the lethal dose has been estimated to be 10 g. A cup of drip coffee typically provides 115-175 mg of caffeine, while instant coffee contains 65-100 mg. The average consumption ranges from 200-300 mg/day in the United States.

Several human studies have examined the effect of caffeine use on cardiovascular disease. A 1965 cohort study of...
5858 Japanese men found a relationship between caffeine use and serum cholesterol, but the difference between those consuming no coffee and those consuming 7 or more cups per day was only 13 mg/dL. A recent study revealed no association between caffeine and coronary artery calcium score, carotid intima-media thickness, or progression of atherosclerotic lesions in a population of 5115 adults aged 18-30 years. Additionally, in a study in which exercise stress tests were performed on patients with coronary artery disease, caffeine (250 mg) was shown to have no effect on exercise duration, time to onset of angina, and time to onset of ST-segment depression, although peak blood pressure increased by 7 mmHg. Further echocardiographic evaluation in this study showed no effect on left ventricular function.

Studies have shown that caffeine increases blood pressure and catecholamine levels, and lowers heart rate after acute ingestion although energy drinks can have differential effects on the heart rate, possibly due to other substances. However, the blood pressure effect resolves within 4 hours in most patients. Furthermore, these effects are attenuated in habitual coffee drinkers, suggesting that there is tolerance to the adrenergic effects. In fact, habitual caffeine use has been shown to have minimal effect on long-term blood pressure. Interestingly, the physiologic effects of caffeine differ in men and women.

Large outcome-based studies of caffeine have been performed in humans. A 1990 2-year cohort study of 45,589 healthy men found no increased risk of myocardial infarction, stroke, or need for coronary revascularization in patients who drank coffee, even 4 or more cups per day. Another group has performed multiple large epidemiologic studies finding no increased risk of coronary artery disease, stroke, or mortality with coffee consumption in cohorts of healthy and diabetic subjects. A 2007 study even found a dose-dependent beneficial effect of caffeine on cardiovascular mortality in the elderly, with a 52% reduction in risk in those consuming 4 or more servings per day.

Overall, these studies are consistent, with minimal to no effect of caffeine on coronary artery disease or stroke. To examine the potential role of caffeine on arrhythmias, the following sections will explore its effects on the conduction system and arrhythmias in animals and humans.

**EFFECTS OF CAFFEINE ON THE HUMAN ELECTROCARDIOGRAM**

Donnerstein et al performed signal-averaged electrocardiograms (ECGs) in 12 subjects given a 5 mg/kg dose of caffeine. Compared with placebo, there was an increase in QRS duration with caffeine ingestion; the small magnitude of this change, approximately 1 ms, is likely clinically insignificant, although it reached statistical significance. P-wave duration and heart rate remained unchanged. Caron et al performed a study in which 10 healthy volunteers were given caffeine (400 mg) and underwent ECG. Compared with the baseline ECG, there was no change in P-wave indices. The same group performed a similar study in which they showed that caffeine (400 mg) did not change electrocardiographic parameters, including PR interval, QRS duration, corrected QT interval, RR interval, or corrected QT interval dispersion. Furthermore, when healthy volunteers drank a high-caffeine “energy drink,” electrocardiographic parameters (except for heart rate) were unchanged.

**ANIMAL STUDIES OF CAFFEINE AND ARRHYTHMIA**

Animal studies allow investigators to probe the link between caffeine and arrhythmia in ways that are impossible using human subjects. Higher-dose caffeine protocols are typically used. Additionally, invasive studies and evaluations of the concomitant effects of ischemia that would be impossible to perform on human subjects are frequently employed. Bellet et al performed a pivotal study in 1972 examining the effect of caffeine on the ventricular fibrillation threshold. In this experiment, the supraphysiologic caffeine dose of 12.5 mg/kg was injected into dogs, and the amount of energy required to induce ventricular fibrillation by delivering sequential impulses during the QT segment was determined. Caffeine decreased the ventricular fibrillation threshold in nonischemic and ischemic models in this study. This was seen as evidence that caffeine could induce or lower the threshold for arrhythmia in susceptible patients. Interestingly, the change in ventricular fibrillation threshold was prevented by beta blockade. In a rabbit study, high (1 mg/kg/min) but not moderate (0.3 mg/kg/min) doses of caffeine facilitated induction of ventricular tachycardia when animals were subjected to a ventricular pacing protocol, demonstrating a dose-dependent effect of caffeine on initiation of ventricular tachycardia. Again, caffeine administration was accompanied by a large increase in plasma norepinephrine, and propranolol (as well as verapamil and adenosine) suppressed induction of ventricular tachycardia. In another study, increasing doses of caffeine (1-5 mg/kg) were given to dogs, resulting in increased incidence of both supraventricular and ventricular arrhythmias. Ventricular tachycardia, atrial flutter, and atrial fibrillation were present only with high doses. Another study used programmed electrical stimulation in mice, finding that 1 mM of caffeine...
introduced into a buffer perfusing the heart increased the incidence of ventricular tachycardia from 0% to 100%. The role of triggered activity was suggested based on the response to diltiazem. Further evaluating the mechanism of the link between caffeine and arrhythmia, Paspa and Vassalle performed a canine study in 1984 that recognized that caffeine induces an oscillatory potential in the Purkinje fibers that increases with concentration of caffeine and time of exposure. In this animal model, caffeine also increased the rate of spontaneous discharge in active fibers, and was seen to initiate spontaneous repetitive activity. Other mechanistic insights were provided by a study in which rats were injected with toxic doses of caffeine-sodium salicylate (15 mg/kg/min) and died with progressive ventricular ectopy starting at 22.8 minutes, eventually leading to ventricular fibrillation, an effect that was stalled but not ameliorated by beta-adrenergic or calcium-channel blockade. All these data support the notion that at high doses, caffeine may produce catecholamine-induced triggered activity.

HUMAN STUDIES OF CAFFEINE AND ARRHYTHMIA

Several human studies have been performed to investigate the effect of caffeine on atrial and ventricular arrhythmias (Table). Both physiologic and epidemiologic studies have been performed.

Early human studies were performed using invasive electrophysiology studies. Gould et al obtained His bundle electrograms in 12 patients with known cardiovascular disease before and after ingestion of a 150-mg dose of caffeine. There were no changes in intra-atrial, atrioventricular (AV) nodal, and His-Purkinje conduction. However, the effective and functional refractory periods of the AV node decreased after ingestion of coffee, an effect that was attributed to release of catecholamines. Dobmeyer et al

<table>
<thead>
<tr>
<th>Table</th>
<th>Human Studies Examining the Effect of Caffeine on Arrhythmia</th>
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<tbody>
<tr>
<td>Reference Year n Description</td>
<td></td>
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<tr>
<td>Frost and Vestergaard 2005 47,949</td>
<td>No increased risk of atrial fibrillation or flutter in healthy patients followed for an average of 5.7 years</td>
</tr>
<tr>
<td>Conen et al 2010 33,638</td>
<td>No increased risk of atrial fibrillation in healthy women followed for an average of 14.4 years</td>
</tr>
<tr>
<td>Prineas et al 1980 7311</td>
<td>Ingestion of &gt;9 cups of coffee per day associated with twice the risk of PVC in healthy patients</td>
</tr>
<tr>
<td>de Vreede-Swagemakers et al 1999 117</td>
<td>Ingestion of &gt;10 cups of coffee per day associated with OR of 55.7 for sudden cardiac death in population with coronary artery disease</td>
</tr>
<tr>
<td>DeBacker et al 1979 81</td>
<td>No decrease in PVC frequency with avoidance of caffeine in healthy men with symptomatic PVC</td>
</tr>
<tr>
<td>Myers et al 1987 70</td>
<td>No increase in ventricular arrhythmias in patients given 300 mg caffeine with recent MI as compared with placebo</td>
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<tr>
<td>Clee et al 1979 50</td>
<td>No increase in ectopy with caffeine ingestion in elderly patients with high prevalence of baseline ectopy</td>
</tr>
<tr>
<td>Graboyes et al 1989 50</td>
<td>No increase in arrhythmias with caffeine during stress test in patients with known ventricular arrhythmias</td>
</tr>
<tr>
<td>Myers and Harris 1990 35</td>
<td>No increase in ventricular arrhythmias in patients given 450 mg caffeine with recent MI as compared with placebo</td>
</tr>
<tr>
<td>Sutherland et al 1985 18</td>
<td>Increased frequency of PVC in patients with frequent PVC at baseline</td>
</tr>
<tr>
<td>Newby et al 1996 13</td>
<td>No decrease in symptoms or frequency of PVC with avoidance of caffeine in patients with symptomatic PVC</td>
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</table>

PVC = premature ventricular complex; OR = odds ratio; MI = myocardial infarction.
found that 200 mg of caffeine shortened the refractory periods of the right atrium, AV node, and right ventricle. Paradoxically, the effective refractory period of the left atrium increased with caffeine. Of note, there was an increase in sustained atrial arrhythmias in response to programmed atrial extrastimuli after ingestion of caffeine, an effect that was noted in patients who reported caffeine sensitivity. However, the stage of the stimulation protocol at which the arrhythmias were induced was not indicated, and therefore the absolute sensitivity of these patients to caffeine cannot be clearly defined.

Further studies have administered caffeine to populations of patients followed with continuous electrocardiography. A 1985 study in 18 patients with frequent premature ventricular complexes (PVC) and 18 controls found that when patients were given caffeine at a dose of 1 mg/kg each half-life for a 24-hour period, those with frequent PVCs at baseline had an increase in the number of PVC/hour from 207 ± 350 PVC/hour to 307 ± 414 PVC/hour, while controls did not have an increase in ectopy. A randomized, double-blinded physiologic study was performed in which 70 patients were given a 300-mg dose of caffeine within 7 days of myocardial infarction. Although epinephrine levels increased and the systolic blood pressure increased, there was no increase in frequency or severity of ventricular arrhythmias in these high-risk patients. A later study administered either higher-dose (450 mg) caffeine or placebo to 35 patients with recent myocardial infarction and observed with 24-hour electrocardiography. No increase in ventricular ectopy or arrhythmia was noted. Another study of 50 patients with known malignant ventricular arrhythmias who underwent bicycle stress testing with ingestion of caffeine (200 mg) showed no increase in ventricular arrhythmias.

Chelsky et al. performed invasive electrophysiologic studies in 22 patients with a history of symptomatic nonsustained ventricular tachycardia. These patients underwent electrophysiology study 1 hour before and after caffeine ingestion. This study showed that caffeine did not significantly alter inducibility or severity of arrhythmias, suggesting that caffeine has little effect on the substrate supporting ventricular arrhythmias. Thus, despite increases in epinephrine levels, these studies demonstrate no proarrhythmic effect of caffeine. It is interesting to note that although epinephrine levels increase with caffeine ingestion, the magnitude of the release is 6-fold less elevated than the increase noted during exercise.

Several human epidemiologic studies have been performed to probe the link between caffeine and arrhythmia, with mixed results. An epidemiologic survey of 7311 healthy men aged 37-57 years who underwent 2-minute electrocardiographic recording revealed that drinking >9 cups of coffee per day was associated with twice the risk of PVCs after adjusting for other risk factors. A self-reported questionnaire associated caffeine with triggering of right ventricular outflow tract tachycardia in men, but not in women. A retrospective case-control study of 117 patients with a history of coronary artery disease who suffered sudden cardiac arrest revealed an impressive association of heavy coffee consumption (>10 cups per day) with increased risk of sudden cardiac death, with an odds ratio of 55.7 (95% confidence interval, 6.4-483) compared with matched controls with coronary artery disease. However, only 2 of the control subjects drank more than 10 cups of coffee per day, and therefore the size of the control group may have been inadequate. Larger-scale epidemiologic studies have been performed showing no association of caffeine intake with increased arrhythmias. In the Danish Diet, Cancer, and Health study, 47,949 subjects with a mean age of 56 years were followed on average for 5.7 years. This study found no association between caffeine use and development of atrial arrhythmias. In the Women’s Health Study, in which 33,638 women were followed for an average of 14.4 years, caffeine consumption also was not associated with an increased incidence of atrial fibrillation. A trial of 81 healthy men with frequent PVCs who abstained from caffeine found no change in frequency of PVC as measured by 24-hour electrocardiography. Additionally, in a study of 50 healthy elderly subjects followed by a continuous ECG, caffeine was not linked to an increase in atrial or ventricular ectopy despite a high prevalence of baseline ectopy in the study population.

Several human epidemiologic studies have been reviewed suggest that this advice is unnecessary. Although animal studies have shown a proarrhythmic effect of caffeine, this effect typically appears at much higher doses than consumed by human subjects, and has only been documented in abnormal clinical situations such as programmed stimulation and massive ischemia. Although these studies provide insights into the physiologic effects of caffeine on the cardiac conduction system, they do not provide real-world experience in patients who consume typical doses of caffeine.

Human studies have shown that caffeine has minimal effect on the ECG. Invasive electrophysiology studies have shown that there is an effect of caffeine on the refractory periods of atrial, ventricular, and nodal tissue, however it is uncertain if these findings translate into a susceptibility for arrhythmia. Given the results of large human epidemi-
ologic studies, it is unlikely that the majority of patients are sensitive to the effects of caffeine. Nevertheless, there may be individuals who are susceptible to the small electrophysiologic changes induced by caffeine and therefore may experience arrhythmias with caffeine.

Although it has been shown that patients with frequent ventricular ectopy can have an increase in frequency of their arrhythmia with caffeine ingestion,46 high-risk patients such as those with recent myocardial infarction,42,45 nonsustained ventricular tachycardia,43 and malignant ventricular arrhythmia44 have been studied with no increase in frequency or severity of arrhythmia. These studies provide evidence that caffeine may not be harmful even in patients who are at risk of malignant arrhythmia.

There are significant limitations in our knowledge about the relationship between caffeine and arrhythmia. Although large-scale epidemiologic human studies do exist, they did not study patients with malignant arrhythmias. Therefore, we rely on smaller studies to make inferences about outcomes in these patients. Several studies did evaluate the arrhythmias of interest; they were limited by a short time period of results and therefore cannot capture infrequent outcome events. In addition, specific arrhythmias such as catecholaminergic polymorphic ventricular tachycardia have not been studied, although survey evidence exists to suggest that caffeine increases the frequency of right ventricular outflow tract tachycardia.52 Patients who state that their arrhythmias are triggered by caffeine intake have not been studied in adequate depth to draw conclusions about the safety of caffeine ingestion. In these cases, it is prudent to abstain from caffeine until further information is available.

Overall, the data suggest that in most patients, even those with known or suspected arrhythmia, caffeine in moderate doses is well tolerated and there is therefore no reason to restrict ingestion of caffeine. Care should be taken to avoid caffeine in situations in which catecholamines are thought to drive the arrhythmia, as well as in patients who note sensitivity to caffeine.

References

47. Newby DE, Neilson JM, Jarvie DR, Boon NA. Caffeine restriction has no role in the management of patients with symptomatic idiopathic ventricular premature beats. *Heart*. 1996;76(4):355-357.
Dietary factors and incident atrial fibrillation: the Framingham Heart Study


ABSTRACT

Background: There have been conflicting reported associations between dietary factors and incident atrial fibrillation (AF).

Objective: We evaluated associations between consumption of alcohol, caffeine, fiber, and polyunsaturated fatty acids (PUFAs) and incident AF in the Framingham Heart Study.

Design: Participants without AF (n = 4526; 9640 examinations; mean age: 62 y; 56% women) from the original and offspring cohorts completed food-frequency questionnaires and were followed prospectively for 4 y. We examined the associations between dietary exposures and AF with Cox proportional hazards regression.

Results: A total of 296 individuals developed AF (177 men, 119 women). In multivariable analyses, there were no significant associations between examined dietary exposures and AF risk. Hazard ratios (HRs) for increasing quartiles of dietary factors were as follows: for alcohol, 0.73 (95% CI: 0.5, 1.05), 0.85 (95% CI: 0.61, 1.18), and 1.12 (95% CI: 0.83, 1.51) (P for trend = 0.48); for caffeine, 0.84 (95% CI: 0.62, 1.15), 0.87 (95% CI: 0.64, 1.2), and 0.98 (95% CI: 0.7, 1.39) (P for trend = 0.84); for total fiber, 0.86 (95% CI: 0.61, 1.2), 0.64 (95% CI: 0.44, 0.92), and 0.81 (95% CI: 0.54, 1.2) (P for trend = 0.16); and for n−3 (omega-3) PUFAs, 1.11 (95% CI: 0.81, 1.54), 0.92 (95% CI: 0.65, 1.29), and 1.18 (95% CI: 0.85, 1.64) (P for trend = 0.57; quartile 1 was the reference group).

Conclusions: Consumption of alcohol, caffeine, fiber, and fish-derived PUFA was not significantly associated with AF risk. The observed adverse association between the consumption of dark fish and AF merits further investigation. Our findings suggest that the dietary exposures examined convey limited attributable risk of AF in the general population.

INTRODUCTION

Atrial fibrillation (AF) is the most commonly encountered arrhythmia in clinical practice (1) and conveys increased risks of stroke, dementia, heart failure, and overall mortality (2). Its increasing prevalence poses great challenges to public health and the health care system (3). Therefore, identifying novel, modifiable risk factors for AF is a priority for preventive efforts (4). Lifestyle factors, in particular dietary intake, have been recognized as important, modifiable risk factors for cardiovascular disease (CVD). Studies have shown that dietary components, such as alcohol, caffeine, fiber, and fish-derived long-chain polyunsaturated fatty acids (PUFAs), influence CVD morbidity and mortality (5–8).

However, the effect of dietary factors on risk of AF is not well established. The associations between AF risk and alcohol and caffeine intakes have had conflicting results (9–13). Reports on the relation of fish and fish-derived fatty acid intakes on AF risk have also been inconsistent: studies have shown an alternately protective effect (14) or null to unfavorable relation (15–17). The association between dietary fiber intake and incident AF is largely unexplored. Thus, whether dietary factors predispose individuals to AF risk remains uncertain. In the setting of a community-based cohort study, we conducted longitudinal analyses of the associations of alcohol, caffeine, fiber, and fish-derived n−3 PUFAs with incident AF.

SUBJECTS AND METHODS

Study design

The Framingham Heart Study (FHS) is a longitudinal study that was initiated in 1948 to identify CVD risk factors. Offspring of Original cohort participants and their spouses were recruited to participate in the Framingham Offspring Study in 1971. The detailed design and methodology of the study have been pre-

1 From the Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging, Tufts University, Boston, MA (JS, PFJ, MJ, and JMO); the Departments of Biostatistics (VMJ and LMS) and Epidemiology (PAQ and EJB), School of Public Health, the Sections of Cardiology (JWM, SP, DL, RSV, and EJB) and Preventive Medicine (RSV and EJB), Evans Memorial Department of Medicine, and the Department of Health Sciences, Sargent College of Health and Rehabilitation Sciences (PAQ), Boston University, Boston, MA; the National Heart, Lung, and Blood Institute’s Framingham Heart Study, Framingham, MA (DL, JVM, and EJB); and the Cardiovascular Research Center, Massachusetts General Hospital, Boston, MA (SAL).

2 Supported by the National Heart, Lung, and Blood Institute (grants HL-54776; to JS and JMO), the National Institute of Diabetes and Digestive and Kidney Diseases (grant DK075030; to JMO), the US Department of Agriculture Research (contracts 53-K06-5-10 and 58-1950-9-001; to JS and JMO), the American Heart Association (award 09FFT2190028; to JVM), and the National Institutes of Health (grants N01-HC 25195, 6R01-NS 17950, R01HL101056, HL092577, and 1R01HL102214; to EJB).

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Various described (18, 19). We selected participants who attended the Original Cohort 20th (n = 1401; 1986–1989) or 22nd (n = 1166; 1991–1993) examinations and the Offspring Cohort fifth (n = 3799, 1991–1995), sixth (n = 3532; 1996–1997), or seventh (n = 3267; 1998–2001) examinations. FHS participants who resided in institutional facilities (n = 328), were <45 y of age (n = 809), lacked clinical covariates (n = 372), had an invalid (n = 599) or incomplete food-frequency questionnaire (FFQ) (n = 708), or had prevalent AF at the baseline (n = 378) were excluded. A total of 4526 participants (n = 9640 examinations) were included in the final analyses. The study protocol was approved by the Boston University Medical Center Institutional Review Board. Written informed consent was obtained from participants.

Case and clinical ascertainment
AF was ascertained from interim medical evaluations at hospitals and external clinicians. The first AF event was recorded during up to two 4-y follow-up intervals. AF was validated by FHS cardiologists who reviewed and classified all available electrocardiograms from FHS clinic and outside records.

Clinical covariates
The FHS clinical examination includes an assessment for AF cardiovascular risk factors and is well described elsewhere (20). Body mass index was determined by weight (in kg) divided by height squared (in m). Seated systolic blood pressure was measured twice by the FHS physician and averaged. Hypertension treatment was determined by self-report. A heart murmur was considered significant if any diastolic murmur or ≥3 of 6 intensity systolic murmurs were auscultated by a Framingham clinic physician. Cardiovascular disease and heart failure were adjudicated by a panel of 3 investigators by using previously published criteria (20).

Dietary assessment
Dietary intakes were assessed by a validated 126-item semi-quantitative FFQ (21). Participants were asked to report the frequency of consumption of listed foods during the previous year. Nutrient intakes were determined by multiplying the frequency of consumption for each food item by the nutrient content of the portion. Data from the FFQ were considered valid if <13 food items were missing and the total energy intakes reported by men and women were ≥600 and <4200 kcal/d for men or <4000 kcal/d for women. Alcohol intakes (g/d) were determined from beer, wine, and liquor consumption. Caffeine ingestion was determined from coffee, tea, and caffeinated beverage intake. Total fiber ingestion was calculated from cereals, fruit, vegetables, and legumes. Salmon, swordfish, bluefish, mackerel, and sardines were classified as dark fish. Canned tuna consumption was reported separately. Eicosapentaenoic acid and docosahexaenoic acid were grouped as n−3 PUFAs. Linoleic acid and arachidonic acid were grouped as n−6 PUFAs.

Statistical analyses
Descriptive statistics were calculated for demographic characteristics, clinical risk factors, and each dietary factor for men and women. Dietary factors were organized into quartiles on the basis of the total sample. Tests for trend across categories of dietary factors were based on Cox proportional hazards regression by assigning the median intake for each quartile category to each individual in that category and then treating it as a continuous variable. The numbers of AF cases and the total number of person-years of follow-up were computed for each quartile. Cox proportional hazards regression analysis, which was shown to be robust applied to pooled data (22), was then used to generate age-, sex- and energy-adjusted and multivariable-adjusted hazards ratios of AF for each quartile of the dietary risk factors by using the lowest quartile as the reference group. The multivariable models included age, sex, energy intake, body mass index, systolic blood pressure, hypertension treatment, electrocardiographic PR interval, significant heart murmur, and history of heart failure on the basis of a previously published Framingham AF risk prediction model (20). We conducted a series of prespecified secondary analyses. We checked for interactions between sex and each dietary risk factor and showed no substantive evidence of effect modification. Considering the potential threshold effects for alcohol exposure (10, 11), we classified individuals into moderate-to-heavy alcohol drinker and nondrinker groups by using 35 g alcohol/d (equivalent to 3 drinks/d) as the cutoff for men and 25 g alcohol/d (equivalent to 2 drinks/d) as the cutoff for women. Then, we compared AF risk between the 2 groups. The proportionality of hazards assumption was not violated. All analyses were conducted with SAS for Windows, version 9 (SAS Institute, Cary, NC).

RESULTS
Of the 9640 person examinations representing 4526 individuals included in the analysis, the mean age of participants was 62 ± 10 y, and 56% of participants were women. Baseline characteristics and dietary intakes are presented in Table 1 (see supplemental Table 1 under “Supplemental data” in the online issue for intakes of specific sources of fiber, alcohol, and caffeine). During the 4-y follow up, 296 cases of incident AF were identified (177 men and 119 women). In AF cases, there were potentially 28 individuals with lone AF aged <66 y and without a history of prevalent myocardial infarction, heart failure, significant heart murmur, hypertension, and electrocardiographic left ventricular hypertrophy.

Age-, sex-, and energy-adjusted and multivariable-adjusted associations between dietary factors and AF are described in Table 2. We did not observe significant associations between the selected dietary risk factors and incident AF (P for trend = 0.48 for alcohol, 0.84 for caffeine, 0.16 for fiber, and 0.57 for n−3 PUFAs, respectively). We further showed that moderate-to-heavy alcohol consumption was associated with a 1.35-fold risk or AF than nondrinkers, albeit without significance (95% CI: 0.97, 1.86; P = 0.07). In further analysis, the exclusion of participants who took fish-oil supplements (3.75% of participants) did not substantially alter the association between n−3 PUFAs and AF risk. There were no significant associations of other types of dietary fat including saturated fatty acids, monounsaturated fatty acids, total PUFAs, and n−6 PUFAs with AF risk (see supplemental Table 2 under “Supplemental data” in the online issue). The results of our primary analyses were not significantly altered after the exclusion of participants with prevalent or interim CVD (n = 965) (data not shown).
We conducted secondary analyses to test the associations between total fish and types of fish intakes and AF risk. We did not observe a significant association between total fish intake and AF risk. However, participants who consumed >4 servings of dark fish/wk were at increased risk of developing AF (hazard ratio: 1.65 when the risk of AF in the first quartile of a given nutrient was compared to risk of AF in the fourth quartile of that nutrient).

In a longitudinal analysis conducted in the FHS, we observed little evidence of an association between dietary exposures from alcohol, caffeine, fiber, and fatty acids and incident AF during 4-7 years of follow up. In exploratory analyses, we observed that the high weekly consumption of dark fish was associated with a significantly increased risk of AF.

Acute alcohol intake and binge drinking have been related to AF (23, 24). Epidemiologic data of the long-term relation of alcohol intake have been inconsistent. Data from the early analysis of the FHS and the Cardiovascular Health Study showed no effect of alcohol intake on AF (9, 25). In recent analyses from the FHS, moderate-to-heavy alcohol consumption (≥3 drinks/d; =36 g alcohol) was significantly associated with increased AF risk in men (11). The association with the consumption of ≥2 drinks/d (equivalent to 25 g alcohol/d) and AF risk also has been identified in women (10). In our study, we observed only a marginal association between moderate-to-heavy drinking and increased risk of AF; possibly because of a small group of moderate-to-heavy drinkers in this population (only 9–15% of women and men in our cohort). Whereas prior evidence suggested that moderate-to-heavy drinking contributes to AF risk, the evidence from our study could neither confirm nor rule out that hypothesis. The arrhythmogenicity of chronic alcohol exposure may stem from alcoholic cardiomyopathy (26), increased oxidative stress, neurohormonal activation, and altered calcium homeostasis (27).

Caffeine is widely present in many dietary sources and may mediate AF by resulting in neurohormonal stimulation and sympathetic activation (28, 29). The clinical effect of intakes of caffeine from coffee, tea, and caffeinated soda on AF remains inconclusive. In young healthy participants in a clinical trial, acute ingestion of caffeinated instant coffee did not result in supraventricular arrhythmias (30). In contrast, an acute increase in coffee intake was associated with the recurrence or persistence of AF in patients with a first episode of AF (31). As for the habitual intake, our study and a Danish cohort showed no association of daily caffeine intake with incident AF (12). However, one study identified moderate coffee consumption (1–4 cups coffee/d) as a contributor to AF risk (32), whereas another study showed that caffeine was associated with less successful cardioversion in participants with hypertension (13). Further studies are necessary to clarify the relation of caffeine exposure to risk of incident and recurrent AF in healthy individuals and patients with a predisposition for AF.

The consumption of whole grains and dietary fiber, especially cereal fiber, reduces CVD morbidity and mortality (33, 34). The rich contents of fiber, vitamins, minerals and various phytochemicals in whole grains (35) decrease inflammation and oxidative stress (36). High intakes of whole grains also are associated with lower risk of death attributed to inflammatory diseases (37). Because emerging evidence indicates that inflammation and oxidative stress may play a role in the pathophysiology of AF (38), the high intake of whole grains and fiber may have an antiarrhythmic potential. As an important source of dietary magnesium, whole grains may provide additional protection because magnesium has been suggested to be effective in the acute management of rapid AF in clinics (39). Our data did not support the hypothesis of an antiarrhythmic relation between

### Table 1

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Men (n = 4231 examinations)</th>
<th>Women (n = 5409 examinations)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>62.0 ± 9.82</td>
<td>62.8 ± 10.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.3 ± 4.2</td>
<td>27.1 ± 5.5</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>131 ± 18</td>
<td>130 ± 21</td>
</tr>
<tr>
<td>Current smoker (%)</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Moderate-to-heavy alcohol drinker (%)</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>Heart failure (%)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Significant heart murmur (%)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>PR interval (ms)</td>
<td>170 ± 3</td>
<td>161 ± 2</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Hypertension treatment (%)</td>
<td>31</td>
<td>30</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dietary factors</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total energy (kcal/d)</td>
<td>1951 ± 643</td>
<td>1724 ± 559</td>
</tr>
<tr>
<td>Total fiber (g/d)</td>
<td>18.5 ± 7.9</td>
<td>18.6 ± 8.0</td>
</tr>
<tr>
<td>Alcohol (g/d)</td>
<td>14.7 ± 19.3</td>
<td>6.6 ± 11.3</td>
</tr>
<tr>
<td>Caffeine (mg/d)</td>
<td>274 ± 214</td>
<td>232 ± 194</td>
</tr>
<tr>
<td>Saturated fatty acid (g/d)</td>
<td>23.0 ± 10.8</td>
<td>19.8 ± 9.1</td>
</tr>
<tr>
<td>Monounsaturated fatty acid (g/d)</td>
<td>24.9 ± 11.1</td>
<td>20.9 ± 9.1</td>
</tr>
<tr>
<td>PUFA (g/d)</td>
<td>12.4 ± 5.6</td>
<td>11.3 ± 5.1</td>
</tr>
<tr>
<td>n-3 PUFA, 20:5 and 22:6 (mg/d)</td>
<td>1.2 ± 0.5</td>
<td>1.3 ± 0.5</td>
</tr>
<tr>
<td>Fish intake (servings/wk)</td>
<td>1.9 ± 1.7</td>
<td>1.9 ± 1.7</td>
</tr>
<tr>
<td>Dark fish (servings/wk)</td>
<td>0.4 ± 0.6</td>
<td>0.3 ± 0.6</td>
</tr>
<tr>
<td>Canned tuna fish (servings/wk)</td>
<td>0.9 ± 1.1</td>
<td>1.0 ± 1.1</td>
</tr>
<tr>
<td>Shrimp and shellfish (servings/wk)</td>
<td>0.3 ± 0.4</td>
<td>0.3 ± 0.4</td>
</tr>
</tbody>
</table>

1 PUFA, polyunsaturated fatty acid.
2 Mean ± SD (all such values).
3 Defined as >35 g alcohol/d for men and >25 g alcohol/d for women.
whole grains and dietary fiber at the relatively modest amounts consumed in the FHS cohort.

Consumption of fish and fish-derived n–3 PUFAs decreases the risk of sudden cardiac death (7) mainly through preventing cardiac arrhythmia, particularly ventricular fibrillation (40). The anti-arrhythmic effects of n–3 PUFAs could be mediated through their regulation of the calcium channel, eicosanoid metabolism, inflammation, and cardiac muscle metabolism (41). In our study, dark-fish intake and n–3 PUFA intake from fish or fish-oil supplements were not associated with a lower incidence of AF. Our findings are consistent with many earlier cohort studies (15–17) but contradict a previous longitudinal cohort that reported a beneficial association between AF and n–3 PUFA from fish or fish supplements in elderly participants of the Cardiovascular Health Study (14). The inconsistent results across observational studies may reflect differences in study populations and dietary assessment methodologies. Also, the heterogeneous nature of AF could potentially differentiate individual responses to fish-derived n–3 PUFAs (42, 43). This notion has been supported by observations that showed the consumption of n–3 PUFAs increases parasympathetic tone (44) and may mediate lone AF risk in susceptible individuals, whereas n–3 PUFAs may have a protective role in older individuals at risk of AF secondary to structural heart disease (45). Divergent associations of fish oil with AF have also been shown in experimental studies that demonstrated that n–3 PUFAs suppress the type of AF

TABLE 3
Risk of atrial fibrillation according to total fish and dark-fish intake

<table>
<thead>
<tr>
<th>Frequency of fish intake</th>
<th>Never or &lt;1 serving/wk</th>
<th>1–4 servings/wk</th>
<th>&gt;4 servings/wk</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total fish</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of cases/participants</td>
<td>107/3365</td>
<td>156/5460</td>
<td>33/815</td>
<td>—</td>
</tr>
<tr>
<td>Person-years of follow-up</td>
<td>12,872</td>
<td>20,919</td>
<td>3093</td>
<td>—</td>
</tr>
<tr>
<td>Age, sex, and energy adjusted</td>
<td>1 (reference)</td>
<td>0.91 (0.71, 1.17)</td>
<td>1.33 (0.89, 1.98)</td>
<td>0.57</td>
</tr>
<tr>
<td>Multivariable adjusted 4</td>
<td>1 (reference)</td>
<td>0.88 (0.69, 1.13)</td>
<td>1.25 (0.84, 1.86)</td>
<td>0.81</td>
</tr>
<tr>
<td>Dark fish</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of cases/participants</td>
<td>248/8321</td>
<td>43/1298</td>
<td>5/21</td>
<td>—</td>
</tr>
<tr>
<td>Person-years of follow-up</td>
<td>31,886</td>
<td>4932</td>
<td>67</td>
<td>—</td>
</tr>
<tr>
<td>Age, sex, and energy adjusted</td>
<td>1 (reference)</td>
<td>1.02 (0.74, 1.41)</td>
<td>8.77 (3.61, 21.27)</td>
<td>0.15</td>
</tr>
<tr>
<td>Multivariable adjusted 4</td>
<td>1 (reference)</td>
<td>1.01 (0.72, 1.39)</td>
<td>6.53 (2.65, 16.06)</td>
<td>0.21</td>
</tr>
</tbody>
</table>

1 Hazard ratio; 95% CI in parentheses (all such values).
2 Cox proportional hazards regression model adjusted for age, sex, BMI, systolic blood pressure, hypertension treatment, electrocardiogram, PR interval, significant heart murmur, and heart failure.
associated with heart failure–induced atrial structural remodeling but not the type related to atrial tachycardia–induced electrical remodeling in dogs (46).

Interestingly, our data suggest that high consumption of dark fish (>4 servings/wk) paradoxically may be associated with an increased AF risk. Because of the few cohort members in this high-consumption category (21 individuals and only 5 incident cases of AF), our finding may merely represent a chance association. Because high fish intake as part of a healthier diet is often associated with other healthier behaviors, we cannot rule out the possibility of a higher rate of screening and detection of AF in participants with high fish intake, which could have introduced a bias. However, the unfavorable effect on AF risk has also been observed in a Danish population in which the mean intake of fish-derived n-3 PUFAs was higher than that in our population (17). These observations may suggest a true adverse effect of dark fish and fish oil on certain subtypes of AF. In addition, potential toxins such as dioxins and methyl mercury accumulated in certain fish may have a negative effect on cardiac arrhythmia. Nonetheless, these hypotheses require further investigation.

The current study had several limitations. First, dietary information was collected through self-reports by questionnaires. For some dietary factors, such as alcohol intake, potential underreporting may lead to underestimation of the effect of heavy drinking on risk of AF. Likewise, the associations between dietary factors and AF may be small and require larger samples with more events or longer follow-up to detect the relatively narrow range of intake in the FHS cohort further limited our ability to detect associations between certain dietary exposures, such as fiber and fiber sources, and AF risk. In addition, there may have been threshold effects whereby higher dietary exposure amounts than were observed in our sample may be necessary to affect AF risk. Another consideration is that AF that was asymptomatic, intermittent, or did not lead to a clinical encounter may not have been captured by the FHS investigators. Our participants were middle-aged to elderly and virtually all of European ancestry. The generalizability of our data to other races/ethnicities or younger individuals is unknown. Similarly, in our study, we were not able to definitively separate lone AF from AF associated with CVD, which likely involves different disease mechanisms. As a result, the heterogeneous AF in this study may have complicated our ability to detect a potential association between dietary factors and AF in specific subgroups. Finally, the dark-fish finding should be viewed as a hypothesis-generating finding because it may represent a false-positive discovery in the context of an exploratory subgroup analysis.

In conclusion, we did not observe major associations between alcohol, caffeine, fiber, fatty acids, or total fish consumption with AF risk but rather may suggest an adverse effect of a high weekly consumption of dark fish. Our findings, together with previous inconsistent reports, require further investigation of dietary factors in relation to various subtypes of AF in other cohorts.

The authors’ responsibilities were as follows—LMS, PFJ, DL, RSV, MJ, JMO, and EJB: designed the study; JS, LMS, PFJ, PAQ, JMO, and EJB: conducted research; VMI and LMS: analyzed data; JS: wrote the manuscript; JS, LMS, PFJ, JWM, SAL, SP, PAQ, JMO, and EJB: revised the manuscript; and all authors: read and approved the final manuscript. None of the authors had a conflict of interest.

REFERENCES

Speaker Recommended References

DAY 1, SESSION 4: CAFFEINE EFFECTS ON THE CENTRAL NERVOUS SYSTEM

An update on the mechanisms of the psychostimulant effects of caffeine

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Abstract
There has been a long debate about the predominant involvement of the different adenosine receptor subtypes and the preferential role of pre- versus post-synaptic mechanisms in the psychostimulant effects of the adenosine receptor antagonist caffeine. Both striatal A1 and A2A receptors are involved in the motor-activating and probably reinforcing effects of caffeine, although they play a different role under conditions of acute or chronic caffeine administration. The present review emphasizes the key integrative role of adenosine and adenosine receptor heteromers in the computation of information at the level of the striatal spine module (SSM). This local module is mostly represented by the dendritic spine of the medium spiny neuron with its glutamatergic and dopaminergic synapses and astroglial processes that wrap the glutamatergic synapse. In the SSM, adenosine acts both pre- and post-synaptically through multiple mechanisms, which depend on heteromerization of A1 and A2A receptors among themselves and with D1 and D2 receptors, respectively. A critical aspect of the mechanisms of the psychostimulant effects of caffeine is its ability to release the pre- and post-synaptic brakes that adenosine imposes on dopaminergic neurotransmission by acting on different adenosine receptor heteromers localized in different elements of the SSM.

Keywords: adenosine receptors, caffeine, dopamine receptors, local modules, psychostimulants, receptor heteromers.


Psychostimulant effects of caffeine
Caffeine is the most consumed psychoactive drug in the world, with about 90% of the population (including children) in the United States regularly consuming caffeine-containing beverages or foods (Frary et al. 2005). Caffeine produces the same behavioral effects as classical psychostimulants, such as cocaine and amphetamine, mainly motor activation, arousal, and reinforcing effects (Fredholm et al. 1999; Griffiths et al. 2003). Furthermore, in humans, caffeine produces subjective effects that are qualitatively similar to classical psychostimulants (Griffiths et al. 2003). In fact, in drug discrimination experiments in animals (which provide reliable laboratory models for studying the interoceptive effects of central acting drugs; see Solinas et al. 2006), classical psychostimulants can substitute for behaviorally relevant doses of caffeine and the other way around (Mumford and Holtzman 1991; Munzar et al. 2002; Solinas et al. 2005).

Although the motor activating and arousal effects of caffeine are very well established (Fredholm et al. 1999), there has been some resistance in the literature to accept the reinforcing effects of caffeine, in spite of the fact that its considerable worldwide consumption provides a compelling circumstantial evidence. Nevertheless, there is already enough unambiguous experimental evidence supporting that caffeine can function as a reinforcer under certain conditions both in laboratory animals and humans (Griffiths and Woodson 1988; Griffiths et al. 2003).

Drug reinforcement can be defined as the ability of a drug to maintain self-administration or choice behavior. The reinforcing efficacy of a drug refers to the relative effective-
ness in maintaining behavior on which the delivery of the drug is dependent. The experiments with self-administration (oral or intravenous) of caffeine or with choice behavior of caffeinated versus non-caffeinated foods in animals and humans have given very similar results. Those results indicate that caffeine has a weaker reinforcing efficacy than classical psychostimulants (Griffiths and Woodson 1988; Griffiths et al. 2003). In fact, when considering the criteria for drug dependence and abuse established by the Diagnostic and Statistical Manual of Mental Disorders, 4th edition, caffeine users often fulfill the criteria for drug dependence, but not for drug abuse (Nehlig 1999; Griffiths et al. 2003).

Drugs with reinforcing properties can also produce secondary reinforcing effects and, for instance, flavors that have been paired with the drug also become reinforcing. Thus, the subject develops a preference for drug-paired flavors. Caffeine has been shown to induce flavor preference both in humans and in the experimental animal (Griffiths et al. 2003; Fedorchak et al. 2002; Myers and Izbicki 2006). In naïve rats, low doses of caffeine induce conditioned flavor preference, while high doses produce taste aversion (Brockwell and Beninger 1996; Myers and Izbicki 2006). Importantly, acclimatization to daily caffeine consumption altered the preference-aversion function, apparently by reducing the aversiveness of high doses (Myers and Izbicki 2006). The same dose-dependent preference-aversion function shown in conditioned flavor preference experiments has been observed in conditioned place preference experiments (Brockwell and Beninger 1996; Bedingfield et al. 1998; Patkina and Zvartau 1998).

Physical dependence through suppression of the aversive effects of withdrawal symptoms has also been demonstrated to play an important role in human habitual caffeine consumption (Griffiths et al. 2003; Juliano and Griffiths 2004; Tinley et al. 2004; James and Rogers 2005). Caffeine withdrawal syndrome includes headache, fatigue, decreased energy and alertness, increased mood, difficulty concentrating, irritability, and not cleared away (Juliano and Griffiths 2004). Onset of symptoms already occurs 12–24 h after abstinence and, although the incidence or severity of the symptoms increases with increases in daily dose, symptoms can appear with doses as low as 100 mg/day (equivalent to the content of caffeine in a regular cup of drip coffee) (Juliano and Griffiths 2004). Experiments in humans even suggest that the performance and mood enhancing effects of caffeine can only be appreciated in caffeine-dependent individuals and, therefore, just represent withdrawal reversal (James and Rogers 2005). Some studies suggest that caffeine can only produce conditioned flavor preference in caffeine-dependent individuals (Tinley et al. 2004), supporting a key role of negative reinforcing in caffeine consumption. In summary, both positive reinforcing effects along with suppression of withdrawal symptoms after short periods of abstinence can explain caffeine dependence by humans.

**Caffeine and the central dopaminergic system**

According to their main mechanism of action, psychostimulants are often classified as ‘dopamine uptake blockers’ and ‘dopamine releasers.’ Dopamine uptake blockers, such as cocaine, bind to and inhibit dopamine transporter function (Torres et al. 2003; Elliott and Beveridge 2005). Dopamine releasers, such as amphetamine, also bind to dopamine transporter and they enhance neurotransmitter release by reversing dopamine transport (Elliott and Beveridge 2005; Sulzer et al. 2005). Furthermore, dopamine uptake blockers and dopamine releasers exert differential effects on vesicular monoamine transporter-2 function (Hanson et al. 2004). The common final effect of both major types of psychostimulants is a significant increase of dopamine in the extracellular space of dopamine innervated brain areas. Thus, classically, psychostimulants have been considered as indirect dopamine receptor agonists, to differentiate them from direct dopamine receptor agonists, which directly activate dopamine receptors.

After a unilateral lesion of the ascending dopaminergic pathways in rodents, the systemic administration of indirect dopamine agonists induces an asymmetric motor activation, a turning behavior ipsilateral to the lesioned side, because of the stronger stimulation of dopamine receptors in the non-denervated compared with the dopamine-denervated striatum. On the other hand, the administration of direct dopaminergic agonists induces contralateral turning, because of the development of adaptive changes that increase the sensitivity of dopamine receptors to agonists in the denervated striatum (Ungerstedt 1971; Pycock 1980). The effects of caffeine in this so-called Ungerstedt’s model have been very difficult to interpret. Thus, caffeine (and other methylxanthines) was initially found to induce contralateral turning behavior (Fuxe and Ungerstedt 1974). However, it was also found that caffeine potentiates both the ipsilateral and contralateral turning induced by indirect and direct dopamine receptor agonists (Fuxe and Ungerstedt 1974). More recently, Morelli and coworkers have demonstrated that the ability of caffeine to induce contralateral turning (when systemically administered) depends on the previous repeated administration, ‘priming,’ of direct dopamine receptor agonists (Cauli and Morelli 2005). In fact, when administered to non-primed animals, caffeine induces a weak although significant ipsilateral turning behavior (Cauli et al. 2003; Cauli and Morelli 2005).

The results from turning behavior experiments illustrate what it has been confirmed with several other animal models, that caffeine strongly interacts with the central dopaminergic systems and that it mimics and potentiates the behavioral effects of direct or indirect dopamine receptor agonists (for
reviews, see Ferre et al. 1992; Garrett and Griffiths 1997; Fisone et al. 2004; Cauì and Morelli 2005). Furthermore, an important amount of experimental evidence supports a key role of dopamine in the behavioral effects of caffeine in animals and humans. Thus, dopamine depletion or blockade of dopamine receptors significantly impairs the motor and discriminative stimulus effects of caffeine (Ferre et al. 1992; Garrett and Griffiths 1997). The results obtained with Ungerstedt’s model, with the ability of eliciting either ipsilateral or contralateral turning depending on the experimental paradigm, suggest that both pre- and post-synaptic mechanisms are involved in the ability of caffeine to influence dopaminergic neurotransmission. But these mechanisms should definitively involve the blockade of endogenous adenosine. Thus, it is now well established that the multiple central and non-central effects of methylxanthines are mostly because of antagonism of endogenous adenosine (Fredholm 1980; Rall 1982; Snyder 1985). Therefore, the key to elucidate the mechanisms of action responsible for the psychostimulant effects of caffeine is to understand how adenosine modulates dopaminergic neurotransmission in the brain.

Adenosine A<sub>1</sub> or A<sub>2A</sub> receptor antagonism?

Among the four cloned adenosine receptors (A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub>, and A<sub>3</sub> receptors), A<sub>1</sub> and A<sub>2A</sub> receptors are the ones predominantly expressed in the brain. Caffeine is a non-selective adenosine receptor antagonist, with reported similar in vitro affinities for A<sub>1</sub>, A<sub>2A</sub>, and A<sub>2B</sub> receptors and with lower affinity for A<sub>3</sub> receptors (Fredholm et al. 2001a; Solinas et al. 2005). Physiological extracellular levels of adenosine can be sufficient to occupy and, therefore, stimulate A<sub>1</sub> and A<sub>2A</sub> receptors. On the other hand, A<sub>2B</sub> receptors have a lower affinity for adenosine and they are only activated by high pathological extracellular levels of adenosine (Fredholm et al. 2001a). Thus, A<sub>1</sub> and A<sub>2A</sub> receptors seem to be the preferential targets for caffeine in the brain, although their involvement in the psychostimulant effects of caffeine still remains controversial. A<sub>1</sub> receptors are widely expressed in the brain, while A<sub>2A</sub> receptors are highly concentrated in the striatum (Fredholm et al. 2001a; Schiffmann et al. 2007). The striatal localization of both receptors can underlie the motor-activating and reinforcing effects of caffeine (see below). On the other hand, A<sub>1</sub> receptors localized in the brainstem and basal forebrain and A<sub>2A</sub> receptors localized in the hypothalamus have been suggested to be involved in caffeine-induced arousal (see below).

It was initially suggested by Snyder et al. (1981) that A<sub>1</sub> receptors are preferentially involved in the motor stimulant effects of caffeine, based upon a correlation between potencies of a series of methylxanthines in stimulating locomotor activity of mice and in competing at adenosine receptors labeled with a tritiated A<sub>1</sub> receptor agonist. Later studies in mice and monkeys suggested that there was a better correlation between the locomotor depressant effects of several adenosine receptor agonists and their ability to bind to A<sub>2A</sub> receptors (Durcan and Morgan 1989; Spealman and Coffin 1986). Also, there seemed to be a better correlation between the ability of several methylxanthines to produce motor stimulation in monkeys (increase in response rate in schedule-controlled behavior) and their ability to bind A<sub>2A</sub> receptors (Spealman 1988). It was then suggested that blockade of A<sub>2A</sub> receptors was mainly responsible for the motor stimulating effects of caffeine (Spealman 1988). This was supported by more recent findings demonstrating the ability of A<sub>2A</sub> receptor antagonists and the inability of some A<sub>1</sub> receptor antagonists to reproduce some biochemical and behavioral effects of caffeine (Svenningsson et al. 1977; El Yakoubi et al. 2000) and the inability of caffeine to induce motor activation in A<sub>2A</sub> receptor knockout mice (Ledent et al. 1997). Furthermore, some results even suggested that A<sub>1</sub> receptor antagonism induces motor depression, which could be responsible for the motor depressant effect observed with high doses of caffeine (El Yakoubi et al. 2000). However, other studies showed strong evidence that A<sub>1</sub> receptor antagonists can induce motor activation and potentiate the motor activating effects of A<sub>2A</sub> receptor antagonists (Jacobson et al. 1993; Popoli et al. 1998).

The involvement of A<sub>1</sub> and A<sub>2A</sub> receptors in the motor-activating properties of caffeine has recently been revisited with more comprehensive quantitative and qualitative analysis. In a systematic study that compared the counteraction of the motor depressant effects of selective A<sub>1</sub> and A<sub>2A</sub> receptor agonists by caffeine and selective A<sub>1</sub> and A<sub>2A</sub> receptor antagonists in rats, caffeine showed a profile of a non-selective adenosine receptor antagonist with preferential A<sub>1</sub> receptor antagonism (Karcz-Kubicha et al. 2003). Factor analysis of a detailed qualitative study of motor activity also depicted a very similar profile for caffeine and an A<sub>1</sub> receptor antagonist, which was significantly different from the profile of an A<sub>2A</sub> receptor antagonist (Antoniou et al. 2005). Recent experiments in wild-type mice or in mice with genetic inactivation of A<sub>1</sub>, A<sub>2A</sub> or both receptors also indicate that A<sub>1</sub> receptor contributes to the stimulatory but not the inhibitory motor-activating effects of caffeine (Haldenr et al. 2004; Kuzmin et al. 2006). Also, recent drug discrimination experiments support a key role of A<sub>1</sub> receptors in the psychostimulant effects of caffeine (Solinas et al. 2005). A selective A<sub>1</sub>, but not A<sub>2A</sub>, receptor antagonist produced significant caffeine-like discriminative effects in rats trained to discriminate an injection of a motor-activating dose of caffeine from saline. Furthermore, a selective A<sub>1</sub>, but not A<sub>2A</sub>, receptor agonist dose-dependently reduced caffeine’s discriminative effects (Solinas et al. 2005). In conclusion, both A<sub>1</sub> and A<sub>2A</sub> receptors are involved in the motor-
activating effects of caffeine, while A1 receptor is the adenosine receptor subtype mostly involved in its discriminative-stimulus effects.

Importantly, chronic exposure to caffeine differentially modifies its motor effects dependent on A1 and A2A receptor blockade. Thus, chronic exposure to caffeine in the drinking water results in partial tolerance to the motor effects of an additional acute administration of caffeine and total cross-tolerance to the motor effects of an A1 but not an A2A receptor antagonist (Karcz-Kubicha et al. 2003). This suggests that tolerance to the effects of A1 receptor blockade is mostly responsible for the tolerance to the motor-activating effects of caffeine and that the residual motor-activating effects of caffeine in tolerant individuals might be mostly because of A2A receptor blockade. These results agree with other experimental findings showing a lack of tolerance to the motor activating effects of an A2A receptor antagonist after its repeated administration (Hallånder et al. 2000). Similarly, in rats with a unilateral lesion of the ascending dopaminergic pathways, chronic treatment with caffeine or repeated administration of an A2A receptor antagonist was not associated with tolerance to the A2A receptor antagonist-induced potentiation of turning behavior produced by dopamine receptor agonists (Popoli et al. 2000; Pinna et al. 2001).

The well-known arousal-enhancing properties of caffeine depend on its ability to antagonize the sleep-promoting effects of adenosine (Porkka-Heiskanen et al. 2000; Basheer et al. 2004; Huang et al. 2007; Ferré et al. 2007a). Although previous results suggested that A1 receptors are involved in sleep regulation by inhibiting ascending cholinergic neurons of the basal forebrain (Porkka-Heiskanen et al. 2000; Basheer et al. 2004), more recent studies, which include experiments with A2A and A1 receptor knockout mice, indicate that A2A receptors play a crucial role in the sleep-promoting effects of adenosine and the arousal-enhancing effects of caffeine (Huang et al. 2007; Ferré et al. 2007a). Those A2A receptors are localized in the ventrolateral preoptic area of the hypothalamus and their stimulation promotes sleep by inducing GABA release in the histaminergic tuberomammillary nucleus, thereby inhibiting the histaminergic arousal system (Huang et al. 2007; Ferré et al. 2007a).

Finally, the relative involvement of A1 and A2A receptors in the reinforcing effects of caffeine still needs to be determined. But, most probably, the same basic dopamine-mediated mechanisms (see below) are involved in the motor-activating and reinforcing effects of caffeine, as it happens with classical psychostimulants (Wise 2004; Ikemoto 2007).

Adenosine in the striatal spine module

The striatal efferent neuron, also called the medium spiny neuron, constitutes more than 95% of the striatal neuronal population (Gerfen 2004). It is an inhibitory neuron that uses GABA as its main neurotransmitter. There are two subtypes of medium spiny neurons, which selectively express one of two peptides, enkephalin or dynorphin. Enkephalineric medium spiny neurons predominantly express dopamine D2 (D2L isoform) and A2A receptors, while dynorphineric medium spiny neurons, which also express the peptide substance P, predominantly express dopamine D1 receptors and adenosine receptors of the A1 subtype (Ferré et al. 1997; Agnati et al. 2003; Gerfen 2004) (Fig. 1). It must be pointed out that a detailed analysis of the mRNA expression of the different receptor subtypes indicated that there is a limited subset of striatal neurons (about 15% of all GABAergic efferent neurons) with a mixed phenotype of GABAergic enkephalineric and GABAergic substance P-dynorphineric neurons, with D1 and D2 receptors (Surmeier et al. 1996). The same degree of D1–D2 receptor co-localization has been suggested from recent experiments using bacterial artificial chromosome transgenic mice that express an enhanced green fluorescence protein under the control of the D1 or D2 receptor promoter (Lee et al. 2006).

![Fig. 1 Scheme of striatal spine modules represented by the dendritic spines of the enkephalineric (ENK) or dynorphineric (DYN) medium spiny neurons with glutamate (Glu) and dopamine (DA) nerve terminals that make synaptic contact with the head and neck of the dendritic spines, respectively, and astroglial processes that wrap the glutamatergic synapse. In the striatal spine module adenosine originates mostly from ATP co-released with glutamate from glutamate nerve terminals and astroglia. A1–A2A receptor heteromers are localized in glutamate terminals, A2A–D2 receptor heteromers are localized in the ENK dendritic spine, and A1–D1 receptor heteromers are localized in the DYN dendritic spine. A1 receptors that modulate dopamine release are also localized in a fraction of striatal dopamine terminals.](image-url)
The medium spiny neuron receives two main afferents: glutamatergic afferents from cortical, thalamic and limbic areas and dopaminergic afferents from the mesencephalon (substantia nigra and ventral tegmental area). Both sets of afferents converge on the dendritic spine of the medium spiny neuron. Glutamatergic and dopaminergic terminals make preferential synaptic contacts with the head and the neck of the dendritic spine, respectively (Gerfen 2004) (Fig. 1). ‘Local module’ has been recently defined as the minimal portion of one or more neurons and/or one or more glial cells that operates as an independent integrative unit (Ferré et al. 2007b). The dendritic spine, glutamatergic terminal, dopaminergic terminal, and astroglial processes that wrap the glutamatergic synapse constitute the most common striatal local module, which can be called the striatal spine module (SSM) (Ferré et al. 2007b) (Fig. 1). It must, however, be pointed out that the segregation of dopamine and adenosine receptors in the two subtypes of medium spiny neurons implies the existence of at least two subtypes of SSMs, which could be called enkephalinergic SSM and dynorphinergic SSM (without forgetting the small percentage of mixed enkephalinergic–dynorphinergic SSM; see above).

In the SSM, adenosine is a key modulator of dopaminergic and glutamatergic neurotransmission. Until recently it was believed that the main source of extracellular adenosine was a paracrine-like formation. Extracellular adenosine would come mostly from intracellular adenosine, the concentration of which depends upon the breakdown and synthesis of ATP, which is metabolized to AMP and, then, by means of 5’ nucleotidases it is converted to adenosine, which can be transported to the extracellular space by means of equilibrative transporters (Ferré et al. 2005). However, recent studies suggest that astroglia play a fundamental role in the formation of extracellular adenosine which affects synaptic transmission. Astrocytes express glutamate receptors (mostly metabotropic) and ATP receptors which, when activated, induce astrocytes to release glutamate and ATP (Newman 2003; Hertz and Zielke 2004) (Fig. 1). Astroglial-released ATP can be converted to adenosine in the extracellular space by means of ectonucleotidases (Pascual et al. 2005). Finally, there is an increasing amount of data suggesting the existence of a neurotransmitter-like formation of adenosine, a synaptic pool of adenosine. In this case, adenosine would come from ATP co-released with glutamate, which is metabolized to adenosine by means of ectonucleotidases (Ferré et al. 2005) (Fig. 1).

**Post-synaptic mechanisms: adenosine–dopamine receptor heteromers**

In the SSM, A_{2A} receptors are localized post-synaptically in the dendritic spine of enkephalinergic medium spiny neurons, co-localized with D_{2} receptors and pre-synaptically in glutamatergic terminals, co-localized with A_{1} receptors (Ferré et al. 1997, 2005, 2007b; Rosin et al. 2003; Schiffmann et al. 2007) (Fig. 1). In addition to the glutamatergic terminals, A_{1} receptors are localized in a fraction of dopaminergic nerve terminals (Borycz et al. 2007) and, post-synaptically, in the dendritic spine of dynorphinergic medium spiny neurons, co-localized with D_{1} receptors (Ferré et al. 1997, 2005) (Fig. 1). Although most of these findings have been demonstrated in the dorsal striatum, functional studies indicate that the same distribution of adenosine receptors can be found in the ventral striatum (Ferré et al. 2007a). Antagonistic interactions between A_{2A} and D_{2} receptors modulate the function of the enkephalinergic medium spiny neuron and antagonistic interactions between A_{1} and D_{1} receptors modulate the function of the dynorphinergic medium spiny neuron (Ferré et al. 1993, 1996). This gives the explanation at the neuronal level of an important number of pharmacological findings indicating a selective modulation of A_{1} and A_{2A} receptor ligands on D_{1} and D_{2} receptor-mediated behavioral effects, respectively (reviewed in Ferré et al. 1992, 1997, 2001). For instance, in relation to A_{1} and A_{2A} receptor blockade, selective A_{1} or A_{2A} receptor antagonists are not able to induce turning behavior in rats with a unilateral lesion of the ascending dopaminergic pathways, but they selectively potentiate the contralateral turning induced by low equipotent doses of D_{1} and D_{2} receptor agonists, respectively (Popoli et al. 2000; Ferré et al. 2001). Predictably, non-selective adenosine receptor antagonists, such as caffeine and theophylline potentiate both D_{1} and D_{2} receptor-mediated behavioral responses (Ferré et al. 1991a; Jiang et al. 1993).

The molecular mechanisms responsible for the selective antagonistic A_{1}–D_{1} and A_{2A}–D_{2} receptor interactions have been found to involve ‘intramembrane receptor–receptor interactions,’ a common property of neurotransmitter receptor heteromers (Agnati et al. 2003; Ferré et al. 2007c). In fact, there is compelling evidence for the existence of A_{1}–D_{1} and A_{2A}–D_{2} receptor heteromers in artificial cell systems (Gimés et al. 2000; Hillion et al. 2002; Canals et al. 2003) and in the striatum (Ferré et al. 2007c). In the present review, I adopt the broad definition of ‘neurotransmitter’ by Snyder and Ferris (2000), i.e. a molecule, released by neurons or glia, which physiologically influences the electrochemical state of adjacent cells. Neurotransmitter receptor heteromers are functional entities with distinctive biochemical properties different from those of the individual components of the heteromer. These biochemical characteristics include changes in ligand binding characteristics and signaling (Marshall 2001; George et al. 2002; Prinster et al. 2005; Ferré et al. 2007c). A receptor unit in the heteromer can display several biochemical properties, which can be simply dependent on the presence of the other unit, i.e. just as a consequence of the heteromerization, or on co-stimulation of the two (or more) receptor units in the heteromer. In case of dependence on co-stimulation, the neurotransmitter receptor heteromer acts as a
‘processor’ of computations that modulate cell signaling (Ferre´ et al. 2007c).

Intramembrane receptor–receptor interactions are changes in binding characteristics that are dependent on co-activation of the receptor units of the receptor heteromer (Ferre´ et al. 2007c). They imply an intermolecular crosstalk between both receptor units in the heteromer at the membrane level, without intervention of signaling pathways (Agnati et al. 2003; Ferre´ et al. 2007c). In the A2A–D2 receptor heteromer, the stimulation of the A2A receptor decreases the binding of dopamine to the D2 receptor (Ferre´ et al. 1991b). This intramembrane interaction seems to control neuronal excitability and, consequently, neuronal firing and neurotransmitter (GABA) release by the enkephalinergic medium spiny neurons (Ferre´ et al. 1993; Stromberg et al. 2000). This is related to the ability of D2 receptors to suppress Ca2+ currents through L-type voltage-dependent calcium channels by a cAMP–protein kinase A (PKA)-independent and Gq/11–phospholipase C-dependent signaling pathway (Hernandez-Lopez et al. 2000; Schiffmann et al. 2007) (Fig. 2a). Thus, stimulation of striatal A2A receptor does not produce a significant effect on its own, but it strongly counteracts the depressant effects of D2 receptor stimulation on neuronal firing and neurotransmitter release (Ferre´ et al. 1993; Stromberg et al. 2000; Schiffmann et al. 2007).

In addition to the intramembrane interaction, a strong antagonistic interaction between A2A and D2 receptors has been found at the second messenger level, by which stimulation of D2 receptors counteracts the activation of adenylyl-cyclase induced by stimulation of A2A receptors (Kull et al. 1999; Hillion et al. 2002). Stimulation of A2A receptor can potentially stimulate adenylyl-cyclase, with consequent activation of cAMP–PKA signaling pathway and induction of the expression of different genes, such as c-fos and preproenkephalin by activating the constitutive transcription factor cAMP-response element binding protein and the mitogen-activated protein kinase pathway (Kull et al. 1999; Ferre´ et al. 2005, 2007b; Schiffmann et al. 2007) (Fig. 2a). A2A receptor-mediated activation of PKA can also induce phosphorylation of dopamine and cAMP-regulated second messenger levels allow stimulation of A1 receptor to counteract the effects dopamine D1 receptor stimulation, which also signals through cAMP–PKA and promotes the expression of genes, such as c-fos and preprodynorphin. (c) In the adenosine A2A–A1 receptor heteromer, adenosine modulates glutamate release by acting on the presynaptic protein machinery involved in vesicle exocytosis. Low concentrations of adenosine predominantly stimulate A1 receptors, which decreases the probability of glutamate release by inhibiting the entrance of calcium through N- and P/Q-type VDCCs. High concentrations of adenosine also bind to A2A receptors, which dampens A1 receptor signalling through an intramembrane interaction and stimulates glutamate release by a cAMP–PKA-mediated mechanism, by phosphorylation of synaptic vesicle proteins.

Fig. 2 Scheme of the processing of information in the A2A–D2, A1–D1, and A1–A2A receptor heteromers. (a) In the A2A–D2 receptor heteromer, stimulation of the A2A receptor decreases the binding of dopamine to the D2 receptor; this intramembrane A2A–D2 interaction seems to involve a D2 receptor–Gq11–phospholipase C (PLC) signaling pathway, which modulates L-type voltage-dependent calcium channels (L-type VDCC); in addition to the intramembrane interaction, A2A and D2 receptors interact at the second messenger level, with stimulation of Gq-coupled D2 receptors counteracting the activation of adenylyl-cyclase (ACyclase) induced by stimulation of A2A receptors and, therefore, the consequent activation of the cAMP–PKA pathway, with phosphorylation of DARPP-32, AMPA receptors and promotion of the expression of genes, such as c-fos and preproenkephalin. (b) In the A1–D1 receptor heteromer, interactions at the intramembrane and second messenger levels allow stimulation of A1 receptor to counteract the effects dopamine D1 receptor stimulation, which also signals through cAMP–PKA and promotes the expression of genes, such as c-fos and preprodynorphin. (c) In the adenosine A2A–A1 receptor heteromer, adenosine modulates glutamate release by acting on the presynaptic protein machinery involved in vesicle exocytosis. Low concentrations of adenosine predominantly stimulate A1 receptors, which decreases the probability of glutamate release by inhibiting the entrance of calcium through N- and P/Q-type VDCCs. High concentrations of adenosine also bind to A2A receptors, which dampens A1 receptor signalling through an intramembrane interaction and stimulates glutamate release by a cAMP–PKA-mediated mechanism, by phosphorylation of synaptic vesicle proteins.
phosphoprotein 32 kDa (DARPP-32) at threonine 34 (Thr34) and a phosphatase 2-mediated dephosphorylation of DARPP-32 at Thr75 (Fisone et al. 2004; Schiﬀmann et al. 2007). Furthermore, A2A receptor-mediated activation of PKA can phosphorylate α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) glutamate receptors (Schiﬀmann et al. 2007), which plays a crucial role in the initial plastic changes of glutamatergic synapses, which includes synaptic recruitment of AMPA receptors (Song and Huganir 2002) (Fig. 2a).

From experiments with gene inactivation of Gαolf or DARPP-32, which show a signiﬁcant decrease in caffeine-induced motor activation, it has been suggested that the psychostimulant effects of caffeine depend mostly on the blockade of a basal adenosine tone able to activate A2A receptor–Gαolf–cAMP–PKA signaling (Hervé et al. 2001; Lindskog et al. 2002). However, under basal conditions, stimulation of A2A receptors can poorly activate cAMP–PKA signaling and gene expression, because of a strong tonic inhibitory effect of endogenous dopamine and D2 receptor stimulation on adenylyl-cyclase (reviewed in Ferre´ et al. 2005, 2007b; Schiﬀmann et al. 2007). For instance, the systemic administration of A2A receptor antagonists do not signiﬁcantly modify the basal PKA-dependent phosphorylation of DARPP-32 or AMPA receptors, but it signiﬁcantly counteracts DARPP-32 and AMPA receptor phosphorylation induced by D2 receptor antagonists (Svenningsson et al. 2000; Hakansson et al. 2006). Furthermore, genetic inactivation of AC5, the main adenylyl-cyclase isoform coupled to adenosine and dopamine receptors in the striatum, practically eliminates the inhibitory effects of D2 and stimulatory effects of A2A receptor stimulation on cAMP accumulation, but does not modify the motor depressant effects of A2A agonists and motor activating effects of caffeine (Lee et al. 2002).

We have therefore evidence for the coexistence of two reciprocal and apparently incompatible interactions between A2A and D2 receptors in the GABAergic enkephalinergic neurons, which could be involved in the psychostimulant effects of caffeine. Thus, either co-stimulation of A2A and D2 receptors results in blockade of the D2 receptor–Gαolf11–phospholipase C signaling pathway, by means of the intramembrane A2A–D2 interaction, or it results in a blockade of the A2A receptor–Gαolf–cAMP–PKA signaling, by means of the A2A–D2 interaction at the adenylyl-cyclase level. It is possible that when the D2 receptor is not forming heteromers, but homomers, it couples preferentially to Gβ, while when forming heteromers with the A2A receptor the D2 receptor couples preferentially to Gβ11. This would be a similar situation to that of the recently described D1–D2 receptor heteromer (Rashid et al. 2007). However, in the D1–D2 receptor heteromer, both receptors couple and signal through Gα11 (Rashid et al. 2007), while in the A2A–D2 receptor heteromer the main function of the A2A receptor seems to be the control of D2 receptor signaling through Gα11. In this case, A2A receptor does not couple to Gαolf, or else there should be a clear activation of the cAMP–PKA signaling under basal conditions. Another possibility for A2A receptors to activate Gαolf–cAMP–PKA signaling is to couple to another receptor, such as the metabotropic mGlu5 glutamate receptor. There is evidence indicating that A2A receptors heteromerize with mGlu5 receptors and that their co-activation synergistically potentiate A2A receptor function (Ferre et al. 2002), including A2A receptor-mediated PKA activation with phosphorylation of DARPP-32 (Nishi et al. 2003). Thus, in vivo experiments have demonstrated that co-activation of A2A and mGlu5 receptor agonists induce an increase in the striatal expression of c-fos. It is therefore possible that in the mGlu5–A2A–D2 receptor heteromer, D2 receptor predominantly uses Gβ–cAMP–PKA signaling.

In summary, A2A–D2 receptor interactions play a key role in the motor-activating effects of caffeine. In fact, these interactions have been given a lot of attention in the literature, more recently with the application of A2A receptor antagonists as an adjuvant therapy for L-DOPA in Parkinson’s disease (for recent review, see Muller and Ferre´ 2007). But, as mentioned before, A1–D1 receptor interactions are also of significant functional and pharmacological importance. Thus, A1 receptor antagonists selectively potentiate the motor activating effects of D1 receptor-mediated motor activation in different animal models (Popoli et al. 1996, 2000). Similarly to what happens with the A2A–D2 receptor heteromers, A1 and D1 receptors antagonistically interact both at intramembrane level and at the adenylyl-cyclase level (Fig. 2b). However, in this case, the interactions are not reciprocal, and stimulation of A1 receptors inhibits both the binding of dopamine to the D1 receptor (Ferre’ et al. 1994, 1998) and the D1 receptor-mediated activation of cAMP–PKA signaling pathway and the expression of genes, such as c-fos and preprodynorphin (Ferre´ et al. 1998, 1999) (Fig. 2b).

Pre-synaptic mechanisms: glutamate and dopamine release

It was first postulated that the post-synaptic A1 and A2A receptors, which form heteromers with D1 and D2 receptors, respectively, were mostly responsible for the interactions between adenosine and dopamine involved in the motor and reinforcing effects of caffeine (Ferre’ et al. 1992, 1997). However, previous studies had repeatedly shown that A1 and A2A receptors exert opposite modulatory roles on extracellular levels of glutamate and dopamine in the striatum, with activation of A1 receptors inhibiting and activation of A2A receptors stimulating glutamate and dopamine release (Wood et al. 1989; Lupica et al. 1990; Zetterstrom and Fillenz 1990; Ballarin et al. 1995; Popoli et al. 1995; Okada et al. 1996, 1997; Golembiowska and Zylewska 1997; Corsi et al. 2000). More recently, systemic administration of caffeine...
was found to produce a significant increase in the extracellular concentrations of dopamine and glutamate in the ventral striatum, particularly in the most medial part, the medial shell of the nucleus accumbens (Solinas et al. 2002; Quarta et al. 2004a; Borycz et al. 2007). The same effect was observed with the systemic administration of an A1 receptor antagonist, while an A2A receptor antagonist produced the opposite effect, a small but significant decrease in the extracellular concentration of dopamine and glutamate in the nucleus accumbens (Quarta et al. 2004a). Furthermore, chronic administration of caffeine in the drinking water completely counteracted the effects of caffeine or the A1 receptor antagonist on dopamine and glutamate, while the effect of A2A receptor antagonist was not modified (Quarta et al. 2004a). Thus, these biochemical changes correlate with previous studies on motor activity (Karcz-Kubicha et al. 2003), suggesting an involvement of pre-synaptic mechanisms in the psychostimulant effects of caffeine. This interpretation is strongly supported by results of in vivo microdialysis experiments where caffeine is administered through the dialysis probe (reverse dialysis). Local perfusion of caffeine or an A1 receptor antagonist, but not an A2A receptor antagonist, significantly increased the extracellular concentration of dopamine in the dorsal striatum (Okada et al. 1996, 1997). The same increasing effects on dopamine and also glutamate were obtained when caffeine or an A1 receptor antagonist, but not an A2A receptor antagonist, were perfused in the shell of the nucleus accumbens (Quarta et al. 2004b). Furthermore, caffeine-induced dopamine release in the shell of the nucleus accumbens was found to be dependent on glutamate release and stimulation of NMDA receptors most probably localized in dopaminergic terminals (Quarta et al. 2004b).

There has been some debate about the ability of caffeine to modulate dopamine release in the nucleus accumbens. Another experimental group claimed that the increase in dialysate dopamine by caffeine we observed in the shell of the nucleus accumbens is due to contamination by dopamine arising from the adjacent medial prefrontal cortex (De Luca et al. 2007). However, this is not a valid interpretation if we consider the difference in basal levels of extracellular dopamine between striatal compartments, which, in agreement with both research groups, is four to five times higher in the shell of the nucleus accumbens compared with the adjacent cortex (Borycz et al. 2007; De Luca et al. 2007). If anything, increases in the extracellular dopamine level in the shell of the nucleus accumbens could cause dopamine diffusion with contamination of samples in the adjacent cortex. A more detailed mapping analysis showed that systemic administration of caffeine or local perfusion of an A1 receptor antagonist could only produce a significant elevation of extracellular dopamine in the dorsal but not in the ventral part of the most medial aspect of the shell of the nucleus accumbens (Borycz et al. 2007). In fact, our ‘dorsal shell of the nucleus accumbens,’ which contains much higher basal levels of dopamine than the adjacent cortex, very much overlaps with the striatal compartment described by De Luca et al. (2007) as the ‘anterior and medial border of the shell of the nucleus accumbens,’ where they also found a significant caffeine-induced increase in extracellular dopamine. On the other hand, our ‘ventral shell of the nucleus accumbens’ very much overlaps with the striatal compartment simply described by De Luca et al. (2007) as ‘nucleus accumbens shell,’ where both studies show that caffeine does not modify the extracellular levels of dopamine. Therefore, the results of both experimental groups are basically the same and point to differential effects of caffeine in different striatal subcompartments. In fact, analyzing the effects of the intrastriatal perfusion of an A1 receptor antagonist in several other striatal compartments showed striking differences compared with the shell of the nucleus accumbens. Thus, A1 receptor blockade significantly increased the extracellular concentration of dopamine, but not glutamate, in the core of the nucleus accumbens and in the caudate–putamen and the effect was more pronounced in the most medial compartments (Borycz et al. 2007). In summary, a subregional difference in the A1 receptor-mediated control of glutamate and dopamine release exists in the striatum, most probably related to subregional differences in the level of tonic activation by endogenous adenosine (Borycz et al. 2007).

Many experimental findings indicate that dopamine release in the medial striatal compartments is involved in invigoration of approach and in some aspects of incentive learning (for recent review, see Ikemoto 2007). In relation to psychostimulants, dopamine release in the very medial striatal compartments seems to be involved in both their motor-activating and reinforcing effects (Ikemoto 2007). Therefore, the pre- and post-synaptic dopaminergic mechanisms mentioned above (striatal dopamine release and adenosine–dopamine receptor–receptor interactions) taking place in the medial striatal compartments are most probably involved in the motor and reinforcing effects of caffeine. In relation to its dopamine-releasing effects, at least two factors could explain the weaker reinforcing properties of caffeine when compared with other psychostimulants: its specific subregional effects (Borycz et al. 2007) and the development of tolerance (Quarta et al. 2004a). Also, even when considering both pre- and post-synaptic mechanisms, at behaviorally significant doses, caffeine does not reproduce the same pattern of striatal biochemical changes induced by classical psychostimulants and other drugs of abuse (Bennett and Semba 1998; Nehlig and Boyet 2000; Valjent et al. 2004). It is also important to mention that non-dopaminergic mechanisms, related to the ability of adenosine to directly or indirectly modulate the release of different neurotransmitters, such as acetylcholine, serotonin, and histamine (see below), can also be involved in the motor and rewarding effects of caffeine.
The existence of an A1 receptor-mediated glutamate-independent modulation of dopamine release suggested the presence of functional A1 receptors in striatal dopaminergic terminals. Recent studies, using a combination of immunological and pharmacological techniques in striatal nerve terminal preparations have demonstrated that A1 receptors are present in a significant fraction of dopaminergic terminals in the rat striatum, and that activation of these receptors directly inhibits dopamine release (Borycz et al. 2007). On the other hand the existence of an A1 and A2A receptor-mediated regulation of glutamate release suggested the existence of both receptors in striatal glutamatergic terminals. This was recently demonstrated with electron-microscopy and immunocytochemical experiments, which showed that the majority of glutamatergic nerve terminals contain both A1 and A2A receptors (Ciruela et al. 2006). In functional studies with striatal nerve terminal preparations, stimulation of the A1 receptor was found to decrease while stimulation of A2A receptors potentiated potassium-induced glutamate release (Ciruela et al. 2006). Importantly, when both A1 and A2A receptors were stimulated, the response was not a counteractive effect, but the same than results from A2A receptor stimulation, i.e. a potentiation of glutamate release (Ciruela et al. 2006). Furthermore, in the same kind of preparations, low concentrations of adenosine inhibited while high concentrations stimulated glutamate release (Ciruela et al. 2006). This would agree with a higher affinity for adenosine of the A1 compared with the A2A receptor (Fredholm et al. 2001b) and would provide a mechanism for a fine-tuned modulation of glutamate release by adenosine, with low concentrations inhibiting and high concentrations stimulating glutamate release.

The ability of A1 receptors to inhibit glutamate or dopamine release most probably involves a βγ-dependent inhibition of N- and P/Q-type voltage-dependent calcium channels, which is the most commonly reported mechanism for inhibition of neurotransmitter release by G1-coupled receptors (Jarvis and Zamponi 2001) (Fig. 2c). On the other hand, the stimulatory effect of A2A receptor on striatal glutamate release is probably related to their ability to activate cAMP–PKA signaling, as this mechanism has been shown for A2A receptor-induced acetylcholine in the striatum, GABA release in the globus pallidus and serotonin release in the hippocampus (Gubitz et al. 1996; Shindou et al. 2002; Okada et al. 2001) (Fig. 2c). This effect is related to the ability of PKA to phosphorylate different elements of the machinery that is involved in vesicular fusion (Leenders and Sheng 2005). The mechanism by which A2A receptor stimulation shuts down the effects of A1 receptor stimulation is related to their ability to heteromerize and to exert intermolecular interactions of the same kind as that previously described for the A2A–D2 receptor heteromer (see above). Thus, A1–A2A receptor heteromers have been identified in transfected cells an in rat striatum (Ciruela et al. 2006). A biochemical fingerprint of the A1–A2A receptor heteromer is an intramembrane receptor–receptor interaction, by which stimulation of A2A receptors decreases the affinity of A1 receptor for agonist binding (Ciruela et al. 2006). Furthermore, the affinity of the A2A receptor for caffeine was found to be significantly lower in the A2A–A1 receptor heteromer compared with the A2A–D2 receptor heteromer or the non-heteromerized A2A receptor (Ciruela et al. 2006). The A1–A2A receptor heteromer provides a ‘concentration-dependent switch’ mechanism by which low and high concentrations of synaptic adenosine produce the opposite effects on glutamate release. The A1–A2A receptor heteromer also provides a target for caffeine that explains at least the A1 receptor-mediated glutamate-dependent modulation of dopamine release.

Under chronic caffeine exposure, different factors might contribute to the predominant tolerance of the effects of A1 receptor blockade. Up-regulation of A1 receptors has been repeatedly demonstrated (Jacobson et al. 1996; Karcz-Kubichka et al. 2003), but its significance as a mechanism involved in caffeine tolerance has been seriously questioned (Holtzman et al. 1991). Modifications in the function of A1–A2A receptor heteromers might play a key role in the development of caffeine tolerance. In fact, as mentioned before, chronic caffeine exposure counteracts both motor activation and dopamine release in the nucleus accumbens induced by caffeine or an A1 receptor antagonist (but not and A2A receptor antagonist) (Karcz-Kubichka et al. 2003; Quarta et al. 2004b). Thus, radioligand binding experiments have shown that chronic treatment with caffeine causes an increase in the potency of A2A receptor agonist-mediated inhibition of A1 receptor agonist binding and a significant reduction in the affinity of the striatal A2A receptor for caffeine (Ciruela et al. 2006). An additional factor that might play a significant role in caffeine tolerance is the significant increase in plasma and extracellular concentrations of adenosine with chronic caffeine exposure (Conlay et al. 1997). The higher adenosine levels and low affinity of the A2A receptor for caffeine could allow endogenous adenosine to stimulate A2A receptors even in the presence of caffeine, which would not reach enough concentration to compete with adenosine for binding A2A receptors. Under these conditions, A1 receptor signaling in the A1–A2A receptor heteromer would be expected to be chronically turned down, because of its continuous blockade by caffeine and because of the strong A2A receptor-mediated inhibition of A1 receptor agonist binding. On the other hand, an additional administration of caffeine would be expected to produce a blockade of the residual A2A receptor signaling.

Conclusions and future perspectives

There has been a long debate about the predominant involvement of the different adenosine receptor subtypes
and the preferential role of pre- versus post-synaptic mechanisms in the psychostimulant effects of the adenosine receptor antagonist caffeine. The present review emphasizes the key integrative role of adenosine and adenosine receptor heteromers in the computation of information at the level of the SSM. In the SSM, adenosine acts pre- and post-synaptically through multiple mechanisms, which depend on heteromerization of A1 and A2A receptors among themselves and with D1 and D2 receptors, respectively. Caffeine produces its motor and reinforcing effects by releasing the pre- and post-synaptic brakes that adenosine imposes on dopaminergic neurotransmission in the SSM. By releasing the pre-synaptic brake, caffeine induces glutamate-dependent and glutamate-independent release of dopamine. These pre-synaptic effects of caffeine are potentiated by the release of the post-synaptic brake imposed by antagonistic adenosine–dopamine receptor interactions.

This review has focused on the role of adenosine in the SSM, which is a main target for the motor and, most probably, reinforcing effects of caffeine. We still need to study in detail the local modules localized in the basal forebrain and hypothalamus that are targets for the arousal effects of caffeine (Porkka-Heiskanen et al. 2000; Basheer et al. 2004; Huang et al. 2007; Ferré et al. 2007a). Furthermore, more research still needs to be performed about the role of adenosine in the SSM. We have not taken into account other neurotransmitters, such as acetylcholine, serotonin, histamine, and endocannabinoids. Several studies suggest that A1 and A2A receptors localized in cholinergic nerve terminals, which form very few synaptic specializations and release acetylcholine in a volume transmission mode (reviewed in Ferré et al. 1997), modulate striatal acetylcholine release (Gubitz et al. 1996; Preston et al. 2000). Serotonin also plays an important functional role in the striatum and, in the hippocampus, A1 and A2A receptors have Serotonin also plays an important functional role in the hippocampus, A1 and A2A receptors have

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## References


No claim to original US government works


Mechanisms of action of caffeine in the brain


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No claim to original US government works
Role of the Central Ascending Neurotransmitter Systems in the Psychostimulant Effects of Caffeine

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Abstract. Caffeine is the most consumed psychoactive drug in the world. It is a non-selective adenosine receptor antagonist that in the brain targets mainly adenosine A$_1$ and A$_{2A}$ receptors. The same as classical psychostimulants, caffeine produces motor-activating, reinforcing and arousing effects. This depends on the ability of caffeine to counteract multiple effects of adenosine in the central ascending neurotransmitter systems. Motor and reinforcing effects depend on the ability of caffeine to release pre- and postsynaptic brakes that adenosine imposes on the ascending dopaminergic system. By targeting A$_1$–A$_{2A}$ receptor heteromers in striatal glutamatergic terminals and A$_1$ receptors in striatal dopaminergic terminals (presynaptic brake), caffeine induces glutamate-dependent and glutamate-independent release of dopamine. These presynaptic effects of caffeine are potentiated by the release of the postsynaptic brake imposed by antagonistic interactions in the striatal A$_{2A}$–D$_2$ and A$_1$–D$_1$ receptor heteromers. Arousing effects of caffeine depend on the blockade of multiple inhibitory mechanisms that adenosine, as an endogenous sleep-promoting substance, exerts on the multiply interconnected ascending arousal systems. Those mechanisms include a direct A$_1$-receptor mediated modulation of the corticopetal basal forebrain system and an indirect A$_{2A}$-receptor mediated modulation of the hypothalamic histaminergic and orexinergic systems.

Keywords: Adenosine A$_1$ receptor, adenosine A$_{2A}$ receptor, ascending arousal systems, caffeine, dopaminergic system, local modules, receptor heteromers

INTRODUCTION

In the experimental animal as well as in humans, caffeine produces the same qualitative pharmacological effects as classical psychostimulants, such as cocaine and amphetamine: an increase in motor activity, arousal and reinforcing effects [1–3]. There has been some resistance to the claim that caffeine exerts reinforcing effects, in spite of the fact that its considerable worldwide consumption provides compelling circumstantial evidence. Nevertheless, there is enough unambiguous experimental evidence indicating that caffeine functions as a reinforcer under certain conditions both in laboratory animals and humans [2,4]. However, it is important to point out that caffeine has a weaker reinforcing efficacy than classical psychostimulants [2,4]. In fact, caffeine users often fulfill the criteria for drug dependence, but not for drug abuse, established by the Diagnostic and Statistical Manual of Mental Disorders (DSM IV; 4th edition) [2]. Furthermore, in humans, caffeine produces similar subjective stimuli to classical psychostimulants [2] and, in the experimental animal, caffeine and classical psychostimulants produce similar discriminative-stimulus effects [5–7].

Caffeine is a non-selective competitive adenosine receptor antagonist and produces its psychostimulant effects by counteracting the tonic effects of endogenous...
Adenosine on central adenosine receptors. This depends largely on the ability of adenosine to modulate the function of multiple central ascending neurotransmitter systems, which are involved in motor activation and reward (dopaminergic systems) and arousal (cholinergic, noradrenergic, histaminergic, orexinergic systems). Among the four cloned adenosine receptors (A_1, A_2A, A_2B, and A_3 receptors), A_1 and A_2A receptors are the ones predominantly expressed in the brain. Caffeine has similar in vitro affinities for A_1, A_2A, and A_2B receptors and much lower affinity for A_3 receptors [8]. A_1 and A_2A receptors are the preferential targets for caffeine in the brain, since physiological extracellular levels of adenosine are sufficient to occupy and, therefore, stimulate A_1 receptors localized in the basal forebrain and A_2A receptors localized in the hypothalamus are believed to be mostly responsible for the arousing properties of caffeine, which depend on the adenosine-mediated modulation of the ascending dopaminergic system. On the other hand, A_2B receptors have a lower affinity for adenosine and are only activated by high pathological extracellular levels of adenosine [7,8]. A_1 receptors are widely expressed in the brain, including the striatum, while A_2A receptors are highly concentrated in the striatum [8,9]. The striatal localization of both receptors seems to underlie the motor-activating and reinforcing effects of caffeine, which depend on the adenosine-mediated modulation of the ascending dopaminergic system. On the other hand, A_1 receptors localized in the basal forebrain and A_2A receptors localized in the hypothalamus are believed to be mostly responsible for the arousing properties of caffeine, which depend on the adenosine-mediated modulation of the ascending dopaminergic system.

**CAFFEINE AND THE ASCENDING DOPAMINERGIC SYSTEM**

An important amount of experimental evidence supports a key role of dopamine in the psychostimulant effects of caffeine in animals and humans. For instance, dopamine depletion or blockade of dopamine receptors significantly impairs the motor and discriminative stimulus effects of caffeine [10,11]. Most probably the same basic dopamine-mediated mechanisms are involved in the motor-activating and reinforcing effects of caffeine, as it happens with classical psychostimulants [12,13]. The key to understand these mechanisms of action is understanding how adenosine modulates dopaminergic neurotransmission in the brain.

*The striatum and the striatal spine module*

The ascending dopaminergic systems originate in the mesencephalon (substantia nigra and ventral tegmental area) and innervate the striatum (caudate-putamen, nucleus accumbens and olfactory tuberculum), cortex (mostly prefrontal), amygdala, and hippocampus [14]. These systems are involved in motor activation and learning and expression of goal-directed behaviors. The striatum is the main input structure of basal ganglia and contains the highest innervation of dopamine and the highest density of dopamine receptors in the brain [14]. In the striatum, the GABAergic striatal efferent neuron, also called the medium spiny neuron (MSN), constitutes more than 95% of the striatal neuronal population [14]. There are two subtypes of MSNs, which selectively express one of two peptides, enkephalin or dynorphin. Enkephalinergic MSNs predominantly express dopamine D_2 and A_2A receptors, while dynorphinergic MSNs predominantly express dopamine D_1 receptors and adenosine receptors of the A_1 subtype [15,16] (Fig. 1). The MSN receives two main afferents: glutamatergic afferents from cortical, thalamic, and limbic areas and the dopaminergic afferents from the mesencephalon. Both sets of afferents converge on the dendritic spine of the GABAergic efferent neuron. Glutamatergic and dopaminergic terminals make preferential synaptic contacts with the head and the neck of the dendritic spine, respectively [14,16] (Fig. 1). The dendritic spine, the glutamatergic terminal, the dopaminergic terminal and astroglial processes that wrap the glutamatergic synapse constitute the most common local module in the striatum, which we recently named striatal spine module (Fig. 1). We define “local module” as the minimal portion of one or more neurons and-or one or more glial cells that operates as an independent integrative unit [16]. The segregation of dopamine and adenosine receptors in the two subtypes of medium spiny neurons implies the existence of at least two subtypes of striatal spine module (Fig. 1).

Adenosine is a key modulator of dopaminergic and glutamatergic neurotransmission in the striatal local module. Recent studies indicate that astroglia plays a fundamental role in the formation of extracellular adenosine which can influence synaptic transmission. Astrocytes express glutamate receptors (mostly metabotropic) and ATP receptors which, when activated, induce astrocytes to release glutamate and ATP [17,18]. Astroglial-released ATP is then converted to adenosine in the extracellular space by means of ectonucleotidases [19]. Furthermore, there also seems to be a neurotransmitter-like formation of adenosine, a synaptic pool of adenosine. Adenosine can be produced from ATP co-released with glutamate, which
Fig. 1. Caffeine targets in the striatal spine modules of the GABAergic enkephalinergic neuron (a) and GABAergic dynorphinergic neuron (b). Adenosine A$_{2A}$-dopamine D$_2$ receptor heteromers are localized in the dendritic spines of the GABAergic enkephalinergic neuron and adenosine A$_1$-dopamine D$_1$ receptor heteromers are localized in the dendritic spines of the GABAergic dynorphinergic neurons. Presynaptic A$_{2A}$ receptors form heteromers with A$_1$ receptors and are localized in the striatal glutamatergic terminals that make contact with the dendritic spines of the GABAergic dynorphinergic neuron. A$_1$ receptors are also localized in a fraction of dopaminergic terminals (modified from ref. [16]).
is also metabolized to adenosine by means of ectonucleotidases [20]. In the striatal spine module, A2A receptors are localized postsynaptically in the dendritic spine of enkephalinergic MSNs, colocalized with D2 receptors and presynaptically in glutamatergic terminals to the dynorphinergic MSNs [3,9,15–17,20–23] (Fig. 1). Dopamine D2 receptors are also localized in dopaminergic and glutamatergic terminals, where they do not seem to form heteromers with A2A receptors [24]. A2A receptors are also expressed in dopaminergic and GABAergic terminals, but with lower density than postsynaptic A2A receptors in enkephalinergic MSN and presynaptic A2A receptors in glutamatergic terminals, [25]. Thus, some studies suggest the existence of functional striatal A2A receptors in the dopaminergic terminals, which do not directly mediate dopamine release, but that control dopamine release induced by glial cell line-derived neurotrophic factor (GDNF) [26,27]. Recent studies support the existence of A2A receptors in the striatal collaterals of the enkephalinergic MSNs, activation of which facilitates GABA release [28]. Less clear is the significance of some studies suggesting that activation of striatal A2A receptors in striatal GABA terminals inhibits GABA release [29]. Finally, functional A2A receptors have also been suggested to be localized in nerve terminals from cholinergic interneurons (which should also be included in the striatal spine modules, mostly as synaptic varicosities that release acetylcholine by volume transmission [16]) where they are co-localized with A1 receptors [30]. Presynaptically, A1 receptors are localized in glutamatergic terminals, colocalized with A2A receptors in those terminals which contact dynorphinergic MSNs, and in a fraction of dopaminergic nerve terminals [21,22,31]. Postsynaptically, A1 receptors are localized in the dendritic spine of dynorphinergic MSNs, colocalized with D1 receptors [15,20] (Fig. 1). In order to understand the role of adenosine in the striatum, we have to understand the role that the different adenosine receptor subtypes play in the different elements of the striatal spine module.

**Postsynaptic mechanisms: Adenosine-dopamine receptor heteromers**

In the striatal spine, antagonistic interactions between A2A and D2 receptors modulate the function of the enkephalinergic MSN, and antagonistic interactions between A1 and D1 receptors modulate the function of the dynorphinergic MSN [15,16,20]. This gives the explanation at the neuronal level of an important number of pharmacological findings indicating a selective modulation of A1 and A2A receptor ligands on D1 and D2 receptor agonists-induced behavioral effects, respectively. Thus, A1 and A2A receptor agonists counteract, respectively, the motor activating effects induced by dopamine D1 and D2 receptor agonists. Similarly, A1 and A2A receptor antagonists potentiate, respectively, the motor activating effects induced by dopamine D1 and D2 receptor agonists [15,16,20]. The molecular mechanisms responsible for the selective antagonistic A1-D1 and A2A-D2 receptor interactions seem to depend on allosteric interactions between adenosine and dopamine receptors forming receptor heteromers. In fact, there is compelling evidence for the existence of A1-D1 and A2A-D2 receptor heteromers in artificial cell systems and in the striatum [16,20,24,32]. In the A2A-D2 receptor heteromer, the stimulation of the A2A receptor decreases the binding of dopamine to the D2 receptor [33]. This allosteric interaction in the A2A-D2 receptor heteromer controls neuronal excitability and, consequently, neuronal firing and neurotransmitter release (GABA) release by the enkephalinergic MSN [34,35]. This is related to the ability of D2 receptors to suppress Ca2+ currents through L-type voltage-dependent calcium channels by a cAMP-protein kinase A (PKA)-independent calcium and phospholipase C (PLC)-dependent signaling pathway [24,32]. Thus, stimulation of striatal A2A receptor does not produce a significant effect on its own, but it strongly counteracts the depressant effects of D2 receptor stimulation on neuronal firing and neurotransmitter release in the enkephalinergic MSN [34,35].

In addition to the allosteric interaction in the A2A-D2 receptor heteromer, a reciprocal antagonistic interaction between A2A and D2 receptors has been found at the second messenger level. In this case stimulation of D2 receptors counteracts the activation of adenylcyclase induced by stimulation of A2A receptors [20,24,32]. Stimulation of A2A receptor, through its coupling to Gsof proteins, can potentially stimulate adenylcyclase, with consequent activation of cAMP-PKA signaling pathway and induction of the expression of different genes, such as *c-fos* and *preproenkephalin*, by activating the constitutive transcription factor cAMP response element binding protein (CREB) [20,24,32]. A2A receptor-mediated activation of PKA can also induce phosphorylation of dopamine and cAMP-regulated phosphoprotein 32 kDa (DARPP-32) at threonine 34 (Thr34) and a phosphatase 2-mediated dephosphorylation of DARPP-32 at Thr75 [20,24,32]. Furthermore, A2A receptor-mediated activation of PKA
can phosphorylate AMPA glutamate receptors, which play a crucial role in the initial plastic changes of glutamatergic synapses, which includes synaptic recruitment of AMPA receptors [20,24,32]. However, under basal conditions, stimulation of A2A receptors can poorly activate cAMP-PKA signaling and increase gene expression, due to a strong tonic inhibitory effect of endogenous dopamine and D2 receptor stimulation on adenylly-cyclase [20,24,32]. D2 receptor blockade is then necessary to reveal the effects of a tonic activation by endogenous adenosine on A2A receptor-mediated PKA activation [20,24,32], which indicates that blockade of the A2A receptor-mediated stimulation of cAMP-PKA signaling unlikely contributes to the acute behavioral effects of caffeine, as previously suggested [36]. It is most probable that the coexistence of two apparently incompatible A2A-D2 receptor interactions in the enkephalinergic MSN depends on the existence of a pool of A2A and D2 receptors forming heteromers, and a pool of A2A and D2 receptors forming homodimers. When forming homodimers, D2 receptor couples preferentially to G1, which allows its coupling to adenyl cyclase. When forming heteromers with the A2A receptor, the D2 receptor cannot activate G1, because a key epitope involved in Gi activation (an arginine-rich epitope localized in the amino-terminus of the third intracellular loop of the D2 receptor) is also involved in A2A-D2 receptor heteromerization [37]. In this case, the D2 receptor probably couples to G11 with the consequent activation of the PLC pathway [24]. This would be a similar situation to that recently described for the D1-D2 receptor heteromer [38].

The allosteric interaction in the A2A-D2 receptor heteromer seems to play a key role in the motor-activating effects of caffeine. In fact, this A2A-D2 receptor interaction has been given a lot of attention in the literature, more recently with the application of A2A receptor antagonists as an adjuvant therapy for L-DOPA in Parkinson’s disease (for recent review, see ref. [39]). But, A1-D1 receptor interactions are also of significant functional and pharmacological importance. Thus, as mentioned before, A1 receptor antagonists potentiate the motor activating effects of D1 receptor stimulation (40,41). Similarly to what happens with A2A and D2 receptors, antagonistic interactions take place in the A1-D1 receptor heteromer and at the second messenger level. In this case, the interactions are not reciprocal, and stimulation of A1 receptors inhibits both the binding of dopamine to the D1 receptor [42,43] and the D1 receptor-mediated activation of cAMP-PKA signaling pathway and the expression of genes, such as c-fos and preprodynorphin in the dynorphinergic MSN [43,44].

Presynaptic mechanisms: Dopamine release

In addition to the postsynaptic mechanisms related to the A2A-D2 and A1-D1 receptor interactions, adenosine also acts presynaptically in the striatal spine module, modulating glutamate and dopamine release. Different studies have repeatedly shown that A1 and A2A receptors exert opposite modulatory roles on extracellular levels of glutamate and dopamine in the striatum, with activation of A1 receptors inhibiting and activation of A2A receptors stimulating glutamate and dopamine release (reviewed in ref. [3]). More recently, we found that systemic or striatal administration of caffeine or an A1 but not an A2A receptor antagonist produces a significant increase in the extracellular concentrations of glutamate and dopamine in the ventral striatum, particularly in the most medial part, the medial shell of the nucleus accumbens [45,46]. It was hypothesized that dopamine release was mostly dependent on glutamate release induced by blockade of A1 receptors localized in glutamatergic terminals and on stimulation of ionotropic glutamate receptors localized in dopaminergic terminals [46]. The ability of caffeine to release dopamine in the nucleus accumbens was questioned by another research group [47], but a recent study demonstrated the existence of subregional differences in the effect of A1 receptor blockade in different parts of the nucleus accumbens and other striatal areas, most probably related to subregional differences in the level of tonic activation by endogenous adenosine [31]. Furthermore, glutamate-independent mechanisms were also found to be involved in A1 receptor blockade-mediated striatal dopamine release, which depends on A1 receptors localized in dopaminergic nerve terminals [31].

It has been demonstrated with electron-microscopy and immunocytochemical experiments that a high proportion of glutamatergic nerve terminals contain both A1 and A2A receptors [21]. Recent experiments indicate that A2A receptors are in fact localized predominantly in glutamatergic terminals that establish contact with dynorphinergic MSNs, which constitute the so-called direct striatal efferent pathway [23]. In functional studies with striatal nerve terminal preparations, stimulation of the A1 receptor was found to decrease while stimulation of A2A receptors potentiated potassium-induced glutamate release [21]. Importantly, when both A1 and A2A receptors were stimulated, the response was not a counteractive effect, but the same as results from A2A receptor stimulation, i.e., a potentiation of glutamate release [21]. Furthermore, in the same kind of preparations, low concentrations of...
Adenosine inhibited while high concentrations stimulated glutamate release [21]. This would agree with the reported higher affinity for adenosine of the A<sub>1</sub> compared to the A<sub>2A</sub> receptor [48] and would provide a mechanism for a fine-tuned modulation of glutamate release by adenosine, with low concentrations inhibiting and high concentrations stimulating glutamatergic neurotransmission to the direct striatal efferent pathway. The mechanism by which A<sub>2A</sub> receptor stimulation shuts down the effects of A<sub>1</sub> receptor stimulation seems to be related to an allosteric interaction in A<sub>1</sub>-A<sub>2A</sub> receptor heteromers. Thus, A<sub>1</sub> and A<sub>2A</sub> receptors have been shown to form heteromers in transfected cells [21]. In membrane preparations from transfected cells and from rat striatum, stimulation of A<sub>2A</sub> receptors decreases the affinity of A<sub>1</sub> receptor for agonist binding [21].

The question arises as to which is the contribution of A<sub>1</sub> and A<sub>2A</sub> receptors and the many pre- and postsynaptic mechanisms in the psychostimulant effects of caffeine. There has been a long-running debate about the preferential involvement of A<sub>1</sub> and A<sub>2A</sub> receptors in the motor-activating effects of caffeine (reviewed in refs. [3,22]). Particularly influential were the experiments with A<sub>2A</sub> receptor knockout (A<sub>2A</sub>KO) mice, which showed a lack of motor-activating effects of caffeine [49]. However, by comparing both quantitative and qualitative aspects of the motor activity induced by caffeine and selective A<sub>1</sub> and A<sub>2A</sub> receptor antagonists, recent studies have clearly shown that caffeine, when administered acutely, shows a profile of a non-selective adenosine receptor antagonist with even a preferential A<sub>1</sub> receptor antagonism [50,51]. Importantly, chronic exposure to caffeine differentially modifies its motor effects dependent on A<sub>1</sub> and A<sub>2A</sub> receptor blockade. Thus, chronic exposure to caffeine in the drinking water of rats results in partial tolerance to the motor effects of an additional acute administration of caffeine, and total cross-tolerance to the motor effects of an A<sub>1</sub> but not an A<sub>2A</sub> receptor antagonist [50]. This indicates that tolerance to the effects of A<sub>1</sub> receptor blockade is mostly responsible for the tolerance to the motor-activating effects of caffeine, and that the residual motor-activating effects of caffeine in tolerant individuals might be largely because of A<sub>2A</sub> receptor blockade [50].

Numerous experimental findings indicate that dopamine release in the medial striatal compartments is involved in invigoration of approach and in some aspects of incentive learning (for recent review, see ref. [13]). In relation to psychostimulants, dopamine release in the very medial striatal compartments seems to be involved in both their motor-activating and reinforcing effects [13, 52]. Therefore, the pre- and postsynaptic dopaminergic mechanisms mentioned above (striatal dopamine release and adenosine-dopamine receptor-receptor interactions) taking place in the medial striatal compartments are most probably involved in the motor and reinforcing effects of caffeine. Importantly, chronic administration of caffeine in the drinking water completely counteracted the effects of caffeine or an A<sub>1</sub> receptor antagonist on dopamine and glutamate release, while the effect of an A<sub>2A</sub> receptor antagonist was not modified [53]. Thus, these biochemical changes correlate with the studies on motor activity [50], strongly suggesting the involvement of presynaptic mechanisms in the psychostimulant effects of caffeine. In relation to the dopamine-releasing effects, at least two factors could explain the weaker reinforcing properties of caffeine as compared with other psychostimulants: its specific subregional effects [31] and the development of tolerance [50,53].

In summary, caffeine produces its motor and reinforcing effects by releasing the pre- and postsynaptic brakes that adenosine imposes on dopaminergic neurotransmission in the striatal spine module. By targeting A<sub>1</sub>-A<sub>2A</sub> receptor heteromers in glutamatergic terminals and A<sub>1</sub> receptors in dopaminergic terminals (postsynaptic brake), caffeine induces glutamate-dependent and glutamate-independent release of dopamine. These presynaptic effects of caffeine are potentiated by the release of the postsynaptic brake imposed by antagonistic adenosine-dopamine receptor-receptor interactions in the A<sub>2A</sub>-D<sub>2</sub> and A<sub>1</sub>-D<sub>1</sub> receptor heteromers. However, it is important to point out the possible additional contribution coming from A<sub>1</sub> receptors localized in the nuclei of origin of the ascending dopaminergic systems [54,55].

**CAFFEINE AND THE ASCENDING AROUSAL SYSTEMS**

Arousal is a state of behavioral readiness in response to sensory stimulation, which is associated with cortical electroencephalographic (EEG) activation, which depends on the activation of ascending arousal systems localized in the pontomesencephalic tegmentum, basal forebrain and hypothalamus. The arousal-enhancing properties of caffeine are mostly dopamine-independent and they are related to its ability to antagonize the sleep-promoting effects of adenosine. To understand the mechanisms involved in caffeine-induced arousal we have to understand how adenosine modulates the function of the ascending arousal systems.
Moruzzi and Magoun, in 1949, were the first to demonstrate that activation of the pontomesencephalic tegmentum, what they called the ‘ascending reticular activating system’, is associated with arousal [56]. We know now that the ascending reticular activating system is heterogeneous and contains interconnected ascending glutamatergic, cholinergic and noradrenergic pathways [57–59] (Fig. 2). A main characteristic of these chemically defined ascending systems, as well as of the corticopetal basal forebrain system and the hypothalamic ascending systems (see below), is their ability to modulate simultaneously large areas of the brain and synchronize high-frequency cortical activity during the wakefulness period. This characteristic is related to their morphological and functional properties. First, these systems have a relatively low number of cells of origin with widespread branching terminal processes. Second, a synchronization of activity takes place between their cells of origin [60]. Third, their terminals often contain varicosities without synaptic specializations [61] which release neurotransmitters in a “volume transmission” mode [62]. The physiological activation of the ascending reticular system depends on multiple rostral and caudal inputs carrying motor, limbic, visceral and sensorial multimodal information. It even receives an important cortical input from the prefrontal cortex [63,64]. The different involvement in cortico-subcortical activation induced by the different ascending glutamatergic, cholinergic and noradrenergic pathways is determined by their preferential target innervation, as well as the different inputs to their pontomesencephalic nuclei of origin [57–59]. Glutamatergic cells of the reticular formation project mostly to the thalamus, to the midline-intralaminar and reticular nuclei, which transmit the activity of the ascending reticular activating system to extensive areas of the cerebral cortex. Most cholinergic cells originate in the laterodorsal and pedunculopontine nuclei (LDT and PPT nuclei), which through a dorsal tegmental pathway also project heavily to the thalamus (Fig. 2). A ventral tegmental pathway forms the extrathalamic cholinergic relay to the cortex and in-
nervates the cells that are the origin of the corticopetal basal forebrain system (see below) (Fig. 2). It must be pointed out that PPT nucleus is not only cholinergic, but that also contains non-cholinergic cells with extensive projections [65]. In fact, the projection from the PPT nucleus is considered to be largely non-cholinergic and, most probably glutamatergic [66]. Furthermore, some studies indicate that nitric oxide (NO) is an important neuronal messenger of the cholinergic cells of the ascending reticular activating system [67]. Noradrenergic cells originate mostly in the locus coeruleus, just lateral to the LDT nucleus, and project in a diffuse manner to many brain areas, including the thalamus, amygdala, the corticopetal basal forebrain system, and the cerebral cortex [59] (Fig. 2).

The firing of cholinergic neurons of the LDT and PPT nuclei is temporally correlated with cortical activation. The activity of the cholinergic cells decreases during slow-wave sleep and increases again during fast-wave sleep [58]. The activity of brainstem cholinergic neurons during the waking state is not stimulus-specific. This is consistent with their multiple connections with adjacent ascending fiber systems in the reticular formation, as well as with their extensive afferent connections from subcortical limbic (nucleus accumbens) and motor brain areas (substantia nigra pars reticulata, globus pallidus). Animals with PPT nuclei lesions suffer from a global impairment in attention, rather than deficits of specific attention-related tasks [68]. Similarly to the brainstem cholinergic cells, noradrenergic cells of the nucleus coeruleus are also more active during the waking state and less active during slow wave-sleep. However, their activity ceases during fast-wave sleep [58]. During the waking state the activity of locus coeruleus is decreased when the animal is engaged in automatic, consumatory, non-goal directed motor activity, where it is mostly inattentive to extrapersonal environmental stimuli [69]. Furthermore, different from the brainstem cholinergic cells, the activity of the noradrenergic cells during the waking state is stimulus-specific. Thus, they increase their activity in response to novel or stressful “alerting” stimuli, with the concomitant general increase in cortical activity. This is related to the afferent connections of the nucleus coeruleus from nucleus paragigantocellularis and nucleus prepositus (both located in the rostral medulla) and with the amygdala, brain structures that convey information from emotionally arousing stimuli [70–72]. Animals with lesions of the ascending noradrenergic pathway experience difficulties in some tasks requiring attention, particularly those involving “alerting” stimuli [59]. Furthermore, there is experimental evidence suggesting that locus coeruleus also plays an important role in attending to non-stressful motivational stimuli [60]. Motivational stimuli most probably influence locus coeruleus by means of its afferent connections with amygdala and prefrontal cortex [71,73].

The corticopetal basal forebrain system is considered as the major extrathalamic relay of the ascending reticular activating system to the cortex [58,74,75]. This system represents a continuous stream of cells that extends from the medial septum, through the diagonal band into the substantia innominata, ventral pallidum and nucleus basalis of Meynert [57]. Although it was initially suggested that in both rodents and primates most neurons from the basal forebrain projecting to the cortex were cholinergic, it is now known that in rodents a roughly equal number of basal forebrain GABAergic and cholinergic cells project to cortical areas [76,77]. Finally, there is also evidence for a glutamatergic component [78]. The corticopetal basal forebrain system innervates the entire telencephalic mantle, including the cerebral cortex, hippocampal formation and amygdala. Main inputs of this system come also from these areas, although the cortical input is restricted to the prefrontal cortex and seems to target preferentially non-cholinergic cells [76,79]. Also, important afferent connections originate in the ventral striatum (motor input), the hypothalamus, the ascending serotoninergic and dopaminergic systems and the different components of the ascending reticular activating system [80], and the ascending hypothalamic arousal systems [81,82] (Fig. 2). The corticopetal basal forebrain system plays a decisive role in cortical activation. Although the predominantly cortical excitatory role of acetylcholine is well established, GABA is basically an inhibitory neurotransmitter. Nevertheless, GABAergic cells of the corticopetal basal forebrain system induce cortical activation by disinhibition, by targeting the cortical system of GABAergic interneurons [83]. Activity of its cells correlates with cortical activation during waking and fast-wave sleep. During the waking state, the activity of the cells of the corticopetal basal forebrain system is stimulus-specific and specially associated with motivational stimuli [84–86] and motor activity [87]. This is most probably related, first, to the very marked glutamatergic afferent connections from the amygdala and prefrontal cortex, where motivational stimuli are represented [88]. Second, the motor activity-related changes in cell activity can be mediated by the direct connections from the ventral striatum and the indirect connections from the basal ganglia, through the
ascending reticular activating system [74,87]. There is an increasing number of experimental data suggesting the existence of anatomical and functional segregated subsystems in the corticopetal basal forebrain, with distinct afferent-efferent connections such as the GABAergic subsystem, that selectively receives input from the prefrontal cortex. These subsystems should afford different roles in different attention processes [76,77].

Two more ascending arousal systems originate in the hypothalamic area: the histaminergic and orexinergic systems, which originate in the caudal and dorsolateral hypothalamus, respectively [81,82,89–91]. The same as the noradrenergic (and also the serotoninergic) system, histaminergic and orexinergic cells are active during the waking state, particularly active waking, and virtually inactive during slow and fast-wave sleep [92–94]. Histaminergic cells originate in the tuberomammillary (TM) nuclei of the hypothalamus and innervate diffusely most areas of the brain, including the thalamus and cortex [81,89]. TM nuclei receive a main GABAergic-galaninergic inhibitory input from the ventrolateral-preoptic (VLPO) area of the anterior hypothalamus, considered as a main sleep center. Furthermore, TM cells reciprocally connect with LDT and PPT nuclei and project to the origin of the corticopetal basal forebrain system and to the locus coeruleus [81,92] (Fig. 2).

Orexinergic neurons are localized in the perifornical lateral hypothalamic area, extending medially to the dorsomedial hypothalamic nucleus and sending projections to the midline hypothalamic nuclei, basal forebrain, and cortex, with a very similar distribution to that of the noradrenergic system, with which it is heavily interconnected [82,91]. Their most prominent projections include many hypothalamic areas (including TM nucleus), locus coeruleus (the densest extrahypothalamic projection), basal forebrain, dorsal raphe nucleus, LDT and PPT nuclei, and substantia nigra and ventral tegmental area [82]. The connections with the histaminergic system seem to be particularly relevant and, in fact, several studies strongly suggest that the arousal effects of orexin depend on the activation of the histaminergic system [95,96]. Orexin neurons also receive direct projections from the hypothalamus, including TM nucleus, VLPO area and the suprachiasmatic nucleus, which is critically involved in the generation of circadian rhythm [97] (Fig. 2).

The ascending dopaminergic and serotoninergic pathways, which also originate in the brainstem and innervate diffusely many brain areas including the cerebral cortex, are often included as part of the ascending reticular activating system (see, for instance, ref. [59]). However, these systems are not ‘directly’ involved in arousal or attention. Nevertheless, the activity of serotonergic cells (but not dopaminergic cells) is state-dependent, and their firing is maximal during the waking state, decreases during slow-wave sleep and stops during fast-wave sleep. During the wakefulness period, the activity of the ascending serotonergic cells, which originate in the dorsal and median raphe nuclei, does not vary with any kind of sensorial stressful or non-stressful stimulation. On the other hand, just opposite to what happens with locus coeruleus cells, they increase their activity during automatic, consumatory, non-goal directed motor activity [98].

Adenosinergic modulation of the ascending arousal systems

An important amount of evidence indicates that adenosine is a mediator of sleepiness following prolonged wakefulness. Initial evidence came from pharmacological studies showing the sleep-inducing effects of systemic or intracerebral administration of adenosine and adenosine receptor agonists (reviewed in ref. [99]). These results, together with the fact that adenosine is a byproduct of energy metabolism and that energy restoration seems to be one of the main functions of sleep, led to the hypothesis that adenosine may serve as a homeostatic regulator of energy. Although it was initially thought that adenosine would accumulate in the extracellular space as a function of neuronal activity, either following its equilibrative transport or after metabolism from released ATP, recent studies indicate that sleep homeostasis depends largely upon gliotransmission. Thus, inhibiting gliotransmission, and therefore glial ATP release (ATP and glutamate are the main glial neurotransmitters), attenuated the sleep pressure following sleep deprivation and prevented the arousing effects of A$_1$ receptor antagonists [100].

After prolonged wakefulness in the cat, adenosine was found to accumulate in the basal forebrain and to a lesser extent in the cerebral cortex [101]. This accumulation was brain region-specific and adenosine was not found to increase in thalamus, preoptic hypothalamic area, PPT, or dorsal raphe [101]. The rise of adenosine levels inhibits the firing of the cells of origin of the corticopetal basal forebrain system, and experiments with selective ligands indicate that A$_1$ receptors are preferentially involved [99,102]. Nevertheless the activity of LDT/PPT cells is also under an inhibitory local tone by endogenous adenosine through the acti-
vation of $A_1$ receptors, probably localized both postsynaptically and presynaptically in glutamatergic terminals [103, 104]. Furthermore, recent experiments indicate that stimulation and blockade of $A_1$ receptors in the prefrontal cortex decreases and increases arousal, respectively, and that this modulation is mediated by the descending connection from the prefrontal cortex to the PPT/LDT [105].

However, the sleep-generating effects of adenosine entail additional and not less important modulations of the other ascending arousal systems. As mentioned before, the hypothalamic VLPO area (and the adjacent median preoptic nucleus) is an important sleep center, and anatomical and physiological evidences suggest that the preoptic area neurons promote sleep via descending inhibitory modulation of the arousal system. The GABAergic-galaninergic cells of the VLPO project heavily to the histaminergic TM nuclei, and electrical stimulation of the VLPO area inhibits neuronal excitability of the histaminergic cells [106]. Physiologically, VLPO area is activated by endogenous sleep promoting substances that accumulate during the waking state, including adenosine and prostaglandin $D_2$ (PGD$_2$) [107, 108]. Activation by adenosine is mediated by $A_{2A}$ receptors localized in a subset of VLPO neurons [109], and the sleep-enhancing effects of PGD$_2$ also seem to require activation of VLPO sleep regulatory neurons by adenosine acting at $A_{2A}$ receptors [109]. Apart from the connection to the TM nuclei, GABAergic cells from the VLPO also project to the locus ceruleus and the perifornical lateral hypothalamic area and several experimental findings suggest that deactivation of the histaminergic, noradrenergic and orexinergic arousal systems occurring at sleep onset and during the fast- and slow-wave sleep results from GABA-mediated inhibition originating in the preoptic hypothalamus [108].

In summary, it is now generally believed that the direct $A_1$-receptor mediated modulation of the corticopetal basal forebrain system and the indirect $A_{2A}$-receptor mediated modulation of the hypothalamic histaminergic and orexinergic systems are the main mechanisms by which adenosine exerts its sleep-promoting effects [102, 108]. However, it is important to point out the possible additional contribution coming from $A_1$ receptors localized in the nuclei of origin of the histaminergic, orexinergic and noradrenergic arousal systems [110–112].

The same as for the motor-activating effects of caffeine, there has been a strong debate about the role of $A_1$ and $A_{2A}$ receptors in its arousing effects. Similarly to the studies on motor activation, $A_1$ receptors (in the basal forebrain) were initially the main candidates (reviewed in ref. [102]), but again experiments performed in $A_{2A}$KO mice strongly suggested a predominant role of $A_{2A}$ receptors [113]. $A_{2A}$KO did not show slow-wave sleep rebound after sleep deprivation while $A_1$KO did. Furthermore, caffeine induced wakefulness in wild-type and $A_1$KO, but not in $A_{2A}$KO mice (reviewed in ref. [114]). However, recent studies using conditional CNS $A_1$KO mice question the validity of the apparently obvious interpretations of experiments with adenosine receptor KO mice [114], underscoring the limitations of the global absence of a gene during development. Thus, conditional CNS $A_1$KO mice showed a significant attenuation of the slow-wave EEG activity rebound response to restricted sleep and they also fail to maintain cognitive performance in a working memory task [114]. Nevertheless, recent experiments that measure c-Fos immunoreactivity to measure neuronal activation showed that behaviorally relevant doses of caffeine induce a remarkably restricted pattern of c-Fos expression in the ascending arousal systems. Those studies support a predominant activating effect of caffeine of the orexergic, histaminergic and noradrenergic systems [115]. Surprisingly, caffeine induced very little or no effect on the cholinergic neurons of the basal forebrain or mesopontine tegmentum [115], which might call into question the generally accepted validity of c-Fos as a universal marker of neuronal activation. In summary, when considering all the results obtained from different studies, there seems to be evidence for a role of an $A_1$-receptor mediated modulation of the corticopetal basal forebrain system and a complex $A_1$ and $A_{2A}$ receptor mediated modulation (direct and indirect, respectively) of the noradrenergic system and the hypothalamic histaminergic and orexinergic systems in the arousing effects of caffeine.

**CONCLUSION**

The study of the psychostimulant effects of caffeine implies the analysis of the multiple effects of endogenous adenosine on multiple ascending neurotransmitter systems. It is obvious that only an integrative view of those effects can allow us to understand the mechanisms of action of the most consumed psychoactive drug in the world. We have explicitly separated motor-activating (and reinforcing) and arousing properties by separating the dopaminergic systems from the ascending arousal systems. But even this separation can be
artificial. One of the purposes of this review is to show the tight interconnectivity between all the central ascending neurotransmitter systems. As described above, some of the most important inputs to the areas of origin of the ascending arousal systems come from motor brain areas, from the basal ganglia, including the substantia nigra and ventral tegmental area. It was also mentioned that, in fact, the activity of the cells of the corticopetal basal forebrain system is not only associated with motivational stimuli but also with motor activity [87]. That means that some of the arousing effects of caffeine may be secondary to an increased motor activation. In this respect, a recent study in mice with a selective inactivation of striatal A2A receptors showed that in these animals both motor-activating and arousing properties of caffeine were compromised [116]. An additional level of complexity arises when looking at a more molecular level, at the level of local modules, where we discover that the main targets of caffeine (A1 and A2A receptors) are localized in different parts of different neuronal and glial elements where they form different receptor heteromers. We seem to be quite advanced in our understanding of the modulatory role of adenosine in the striatal local modules (striatal spine module), but this is just one of the terminal fields of the ascending dopaminergic systems. To reach a complete understanding of the mechanisms behind the psychostimulant effects of caffeine we still need to find out about the role of adenosine, adenosine receptors, and adenosine receptor heteromers in local modules from all the other areas of origin and projection of the ascending dopaminergic and arousal systems. Furthermore, we are only starting to understand the mechanisms underlying some temporal properties of the psychostimulant effects of caffeine, such as sensitization and tolerance [22,50,117–119]. In addition to pharmacokinetic mechanisms [117], we need to establish which pharmacodynamic changes, such as modifications in the expression and function of adenosine and dopamine receptors and receptor heteromers [22,50,119], are involved. Some of these temporal effects, particularly sensitization, which is known to be a context-dependent process for classical psychostimulant [120], might be related to the ability of adenosine to modulate long-lasting, activity-dependent changes in synaptic efficacy at excitatory synapses, particularly in the striatum [20]. Nevertheless, in the meantime, caffeine shows to be a very good pharmacological tool to investigate the functions of the central ascending neurotransmitter systems.

ACKNOWLEDGMENTS

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REFERENCES


[107] Cauti O, Pinna A, Valentinii V, Morelli M (2003) Subchronic caffeine exposure induces sensitization to caffeine and cross-sensitization to amphetamine ipsilaterally turning behavior in-


Caffeine and Substance Use Disorders

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Caffeine is the most consumed psychoactive drug in the world. As a psychostimulant, it shows all the pharmacological properties of classical psychostimulants, such as cocaine and amphetamine. Those properties include arousal, motor activation, and reinforcing effects. Nevertheless, those effects are milder for caffeine, which depends on its unique mechanism of action, adenosine receptor antagonism. Classical psychostimulants produce a direct potentiation of central dopaminergic, noradrenergic, and serotoninergic neurotransmission, by acting on catecholamine and serotonin transporters. Caffeine, instead, indirectly activates those and several other ascending neurotransmitter systems (cholinergic, histaminergic, and orexinergic) by removing an inhibitory presynaptic adenosinergic tone, mediated by the effect of endogenous adenosine on adenosine A1 receptors. Caffeine-induced modulation of the dopaminergic system also depends on postsynaptic mechanisms that depend on assemblies of adenosine and dopamine receptors (A1-D1 and A2A-D2 receptor heteromers) (for review see Ref. 1).

The same as for classical psychostimulants, the effects of caffeine on the dopaminergic system are largely responsible for its relatively mild motor activating and reinforcing effects. However, it has been difficult to definitively show a psychostimulant-like biochemical profile of caffeine in the experimental animal. The hallmark of that profile is a significant increase in the striatal extracellular concentration of dopamine, and this has only been clearly seen in a distinct region of the ventral striatum. In this issue, Ferre et al. review recent results that indicate that paraxanthine, the main metabolite of caffeine in man, produces stronger motor activating effects and a more significant striatal dopamine-releasing effect than caffeine. This unique pharmacological profile of paraxanthine depends on an additional selective mechanism, other than adenosine receptor antagonism: inhibition of cGMP-preferring phosphodiesterases. These results open up the possibility that a part of the reinforcing effects of caffeine might depend on paraxanthine, which can reach significant plasma levels upon chronic caffeine intake.

The overwhelming caffeine consumption all over the world basically demonstrates its reinforcing effects. Several epidemiological studies indicate that regular caffeine intake creates dependence, which in part depends on withdrawal symptoms. But should we consider caffeine as a drug with potential abuse liability? Researchers and clinicians have been debating about the addictive potential and clinical importance of caffeine use. In this issue, Budney et al. present the results of a survey among addiction professionals (members of six clinical/scientific organizations that focus on addiction) about the clinical importance of caffeine withdrawal and dependence. The survey indicates that most professionals believe that caffeine withdrawal and dependence exist and are clinically relevant. However, there was no consensus about the need of including both clinical diagnoses in the Diagnostic and Statistical Manual of Mental Disorders (DSM), with a main reason being a perception of less severity compared to withdrawal and dependence of the already included abused drugs. The Budney et al. survey was performed before the release of DSM-5, which includes caffeine and cannabis withdrawal as diagnoses. Important changes in DSM-5 as compared to DSM-4 are the merging of previous abuse and dependence criteria as criteria for a single disorder, now named “Substance Use Disorder” (SUD). Furthermore, craving has been added and legal problems have been removed as new and old criteria, respectively. The final 11-criteria list of the DSM-5 allows providing a score of severity, with 2–3, 4–5, or more than 5 as mild, moderate, and severe, respectively. According to these criteria, tobacco has been aligned with other substances, but not yet caffeine.

Apart from analyzing the potential clinical importance of caffeine by itself, another aspect that might deserve even more consideration is its interactions with other addictive substances. One fast-growing danger in adolescents and young adults is the combined intake of alcohol and caffeinated beverages (usually in the form of high-energy drinks). Mechanistically, caffeine antagonizes the unwanted effects of alcohol by blocking the A1 receptors that mediate alcohol’s somnogenic and ataxic effects. Furthermore, the A1 receptor-mediated unwanted anxiogenic effects of caffeine may be ameliorated by alcohol-induced increases in the extracellular concentration of adenosine. Moreover, by means of interactions between A2A and D2 receptors, caffeine-mediated blockade of A2A receptors can potentiate the effects of alcohol-induced dopamine release, including its reinforcing effects. Mixing alcohol and caffeine has been found to be significantly associated with high-risk drinking behaviors and adverse alcohol-related consequences among college students. In the present issue, O’Brien et al. analyze the role of personality, in particular, the sensation-seeking trait, in the characteristics and consequences of caffeine-alcohol drinking. The study shows that among college students, sensation seeking moderates the risk of alcohol-associated injuries requiring medical attention. More epidemiological studies will
undoubtedly confirm the dangers of this popular combination. Even though alcohol is already considered as a substance of abuse, and even though we can question the addictive properties of caffeine, intake of the caffeine-alcohol combination might have to be considered as a SUD on its own.

Another popular combination used by young adults is caffeine (often as high-energy drinks) with amphetamine-related drugs, such as methamphetamine and MDMA (ecstasy). Furthermore, unintentional combined consumption of caffeine and amphetamine-related drugs is very common, since caffeine is often present as an additive. There is clear experimental evidence indicating that caffeine does not potentiate only the psychostimulant effects of amphetamine-related drugs. This is quite predictable when considering the combination of mechanisms of action mentioned above, particularly the direct and indirect effects on dopaminergic neurotransmission. However, as reviewed by Frau et al. in this issue, there is strong experimental evidence, which also indicates that caffeine potentiates the acute toxic effects of amphetamine-related drugs, such as seizures, hyperthermia, and tachycardia. The relevance of these interactions remains to be determined, but it should be alarming in view of the popularity of these drug associations and the fact that those toxic effects are an important determinant of the fatalities associated with amphetamine-related drug intake.

Finally, a less explored, but potentially significant combination is caffeine and marijuana. In this issue, Sousa et al. review recent experimental findings that indicate the existence of functionally significant interactions between the adenosine and cannabinoid systems. These interactions seem to be particularly important in the hippocampus, where A1 and cannabinoid CB1 receptors are colocalized and are involved in mnemonic and cognitive processes. Antagonistic interactions between these receptors underlie a caffeine-induced potentiation of cognitive impairment caused by THC. The reviewed interactions are relevant enough to justify the performance of epidemiological studies that could disclose the existence of particularly deleterious cognitive effects associated with the combined intake of caffeine and marijuana.

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References

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Alcohol and Caffeine: The Perfect Storm

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Although it is widely believed that caffeine antagonizes the intoxicating effects of alcohol, the molecular mechanisms underlying their interaction are incompletely understood. It is known that both caffeine and alcohol alter adenosine neurotransmission, but the relationship is complex, and may be dose dependent. In this article, we review the available literature on combining caffeine and alcohol. Ethical constraints prohibit laboratory studies that would mimic the high levels of alcohol intoxication achieved by many young people in real-world settings, with or without the addition of caffeine. We propose a possible neurochemical mechanism for the increase in alcohol consumption and alcohol-related consequences that have been observed in persons who simultaneously consume caffeine. Caffeine is a nonselective adenosine receptor antagonist. During acute alcohol intake, caffeine antagonizes the “unwanted” effects of alcohol by blocking the adenosine A₁ receptors that mediate alcohol’s somnogenic and ataxic effects. The A₁ receptor-mediated “unwanted” anxiogenic effects of caffeine may be ameliorated by alcohol-induced increase in the extracellular concentration of adenosine. Moreover, by means of interactions between adenosine A₂A and dopamine D₂ receptors, caffeine-mediated blockade of adenosine A₂A receptors can potentiate the effects of alcohol-induced dopamine release. Chronic alcohol intake decreases adenosine tone. Caffeine may provide a “treatment” for the withdrawal effects of alcohol by blocking the effects of upregulated A₁ receptors. Finally, blockade of A₂A receptors by caffeine may contribute to the reinforcing effects of alcohol.

Introduction

On October 30, 1991, the eastern seaboard of the United States was struck by “a perfect storm,” so called because its destructive power rose from an unprecedented set of circumstances: an ice-cold high-pressure system moving south from Canada, a low-pressure system over the Great Lakes moving east toward Nova Scotia, and a late season hurricane moving north from the tropics, ironically called “Grace.” The mixture of alcohol, caffeinated energy drinks, and youth has been described as another “perfect storm,” because it combines “high pressure” (the central nervous system stimulant caffeine), “low pressure” (the central nervous system depressant alcohol), and the “tropical hurricane” of youth, a period of life characterized by risk taking, independence seeking, and experimentation.¹ In this article, we review the available literature on combining caffeine and alcohol, explain the neurochemical basis for their interaction, and propose a possible mechanism for the “storm” aftermath: greater alcohol consumption and an increase in serious alcohol-related consequences.

Human Metabolism of Caffeine

Caffeine absorption is rapid and complete in humans, with 99% of orally ingested caffeine absorbed from the digestive tract within 45 minutes.² Absorption is not modified by gender, genetics, liver disease, or the ingestion of drugs or alcohol.³ Caffeine crosses all biological membranes and is distributed in all body fluids.³ Peak plasma concentrations are observed within 1–2 hours following a single oral dose of caffeine (4 mg/kg).³ Neither caffeine nor its metabolites accumulate in the organs or tissues of the body. In adults, 1%–2% of ingested caffeine is excreted directly in the urine; 98% of caffeine is metabolized by the cytochrome P450 system of the liver into three active metabolites: paraxanthine, theobromine, and theophylline.³ The isozyme CYP1A2 is responsible for 90% of caffeine clearance.³

Numerous factors modify caffeine clearance

- In infants, the immaturity of hepatic enzyme systems impairs the elimination of caffeine, in comparison to adults.

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Liver disease, especially cirrhosis, significantly reduces clearance of caffeine.

The half-life of caffeine is significantly prolonged in women taking oral contraceptives. The menstrual cycle does not significantly alter the pharmacokinetics of caffeine in healthy eumenorrheic women.

Pregnancy increases the half-life of caffeine, in part due to decreased CYP1A2 activity. Caffeine crosses the placenta, but moderate caffeine consumption (less than 200 mg/day) does not appear to increase risk for spontaneous abortion, preterm birth, low birth weight, or congenital malformations.

Cigarette smoking nearly doubles the rate of caffeine metabolism, by increasing liver enzyme activity.

Quinolone antibiotics (e.g., ciprofloxacin and norfloxacin) decrease the metabolism of caffeine in a dose-dependent manner, most likely by inhibiting the activity of CYP1A2 isozymes.

Alcohol, grapefruit juice, and cruciferous vegetables prolong the half-life of caffeine.

Human Metabolism of Alcohol

Orally ingested alcohol is rapidly absorbed into the human bloodstream from the stomach and small intestine and distributes into total body water. On an empty stomach, peak blood alcohol levels occur about 30 minutes following the oral ingestion of alcohol. For ingested liquids, the principal determinant of the gastric emptying rate is volume; large volumes empty at an exponentially faster rate than small volumes. Because alcohol absorption occurs more rapidly in the small intestine, delays in gastric emptying decrease the rate of alcohol absorption. Food in the gastrointestinal tract slows alcohol absorption; the higher the fatty fat content of a meal, the longer the absorption process takes.

A small amount of alcohol is metabolized in the stomach by the enzyme alcohol dehydrogenase (ADH). Compared with men, women have lower activity of ADH in their stomachs, causing a larger proportion of ingested alcohol to reach the bloodstream. Alcohol is primarily metabolized by the liver, at a steady rate independent of how much alcohol has been consumed. The rate of liver metabolism, typically between 15 and 20 mg% per hour, varies somewhat among individuals, depending on the amount and efficiency of one’s liver enzymes, genetic variation in the enzymes, and medication use.

Blood alcohol concentration in humans is therefore determined by a number of factors, including individual rates of absorption and metabolism, gender, body weight, percentage of body water, use of medications, the rate of drinking, and concurrent consumption of food.

Challenges in Human Research on Caffeine/Alcohol

Caffeine is the most studied drug in history, but confounding variables cause difficulty with the interpretation of research on its human health effects. The caffeine in foods and beverages may be difficult to quantify. The caffeine content of coffee cannot be easily estimated from the reported intake of coffee, because different methods of roasting, grinding, and brewing affect the amount of caffeine. A “single serving” of coffee may range from 4 to 16 ounces (120–480 mL); “an energy drink” may be an 1 ounce (30 mL) “shot” or a 23.5 ounce (705 mL) can. Different brands of energy drinks of the same size contain different amounts of caffeine.

Aversive side effects and a reduction in perceived benefits generally limit the doses of caffeine used by the general population. In human experimental studies, the amount of caffeine consumed is typically moderate, making it difficult to establish a dose-response relationship for adverse effects. Caffeinated foods and beverages may contain other pharmacologically active substances, making it difficult to isolate the effects of caffeine. Even experiments using standardized dosages with humans in laboratory settings are confounded by individual variation in the rate of caffeine metabolism, differences in sensitivity to caffeine, and lifestyle habits. Research on women has been limited by concerns about possible reproductive risks. Finally, many original studies are confounded by failure to account for withdrawal and withdrawal reversal effects in human subjects.

Laboratory studies in human subjects have not established the safety of congeestion of caffeine with high levels of alcohol. Individual expectancies, the timing of dose administration, individual variability in both alcohol tolerance and caffeine sensitivity, and differences in the types of cognitive and performance tests used as outcomes make it very difficult to interpret human health effects. Moreover, ethical constraints prohibit human experiments that would reproduce the manner of consumption and the level of intoxication typically achieved by young adults in real-world situations, making it difficult to extrapolate laboratory findings.

Research on Human Consumption of Caffeine and Alcohol: Prevalence Studies

Malinauskas et al. surveyed 492 college students regarding patterns of energy drink use. The consumption of energy drinks “with alcohol while partying” was reported by 57% of women and 50% of men. Among students who drank three or more energy drinks in a given situation, 49% stated that they did so to mix with alcohol while partying. In a survey of 450 Italian medical students by Oteri et al., 56.9% reported energy drink consumption. Of the total sample, 48.4% mixed energy drink and alcohol; 36% of those who reported ever combining energy drink and alcohol had done so on more than three occasions in the previous month. Attila and Çakir investigated the frequency of energy drink usage among 439 fourth-year college students in Turkey. Among current users, 37.2% reported that they mixed energy drinks with alcoholic beverages. In a survey of 72 male energy drink users at a Halifax university, 76% of participants reported having mixed energy drink and alcohol. Students drank significantly more alcohol on occasions when they also consumed energy drinks (8.6 drinks vs. 4.6 drinks; p = 0.016).

Research on Human Consumption of Caffeine and Alcohol: Association with Alcohol-Related Problems

Energy drink consumption was positively associated with a problem behavior syndrome in a survey of 602 undergraduates by Miller, who found that this relationship was significantly moderated by race. Frequent consumers of energy drinks reported drinking alcohol and having alcohol-related problems more than twice as often as less-frequent energy
drink consumers or nonconsumers. Frequent energy drink consumption was positively associated with alcohol problems in White students, but not in Black students. Among college students who reported past 30-day drinking, 24% consumed alcohol mixed with energy drinks, in a 2008 study by O’Brien et al. The consumption of alcohol mixed with energy drink was strongly associated with high-risk drinking behavior, including increased binge drinking and more frequent episodes of weekly drunkenness. Even after adjusting for the increased alcohol consumed, college students who drank caffeinated alcoholic beverages had significantly higher prevalence of serious alcohol-related consequences, including being sufficiently sick or injured as a result of drinking to seek medical attention, being taken advantage of sexually, and riding with a drunken driver.

Thombs et al. conducted an event-level analysis of 802 exiting patrons in an U.S. college bar district. Drinkers who reported mixing alcohol with energy drink had a threefold risk of being legally intoxicated (breath alcohol content ≥0.08 g/210 L, the legal limit for operating a motor vehicle in the United States for drivers aged 21 years or older), and a fourfold increased odds of reporting the intention to drive a motor vehicle when leaving the bar district, compared with drinkers who reported consuming alcohol alone.

In a longitudinal study conducted by Arria et al., the annual weighted prevalence of caffeinated energy drink usage among 1060 undergraduates at a large public university in the United States was 22.6% in the second year and 36.5% in the third year. Compared with nonenergy drink users, students who used energy drinks drank alcohol more frequently and in higher quantities. Prospectively, energy drink users were significantly more likely than nonenergy drink users to initiate nonmedical use of prescription stimulants and prescription analgesics, even after adjustment for demographics, sensation-seeking personality interest.

Arria et al. explored the associations between caffeinated energy drink usage, alcohol-use patterns, and alcohol-related consequences in 1097 fourth-year college students. Students who reported occasional or monthly energy drink consumption were classified as “low-frequency” users (52.5% of respondents). Students who reported consuming energy drinks on weekly, almost-daily, or daily basis were classified as “high-frequency” users (13% of respondents). Compared with low-frequency energy drink users, high-frequency energy drink users were significantly more likely to have gotten drunk at an early age. Independent of demographics, high-frequency users drank alcohol more frequently and in higher quantities, and were twice as likely to meet Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV) criteria for alcohol dependence, compared with low-frequency users.

Research on the Human Consumption of Caffeine and Alcohol: Laboratory Studies

Azcona et al. used a double-blind placebo-controlled crossover study of eight healthy male volunteers to evaluate the psychomotor and subjective effects of alcohol and caffeine. In four experimental sessions, participants were given placebo, alcohol (0.8 g/kg body weight), caffeine (400 mg), or alcohol (0.8 g/kg body weight) plus caffeine (400 mg). Plasma concentrations of alcohol and caffeine were measured at baseline and at eight intervals after drug intake. Subjects were requested to abstain from alcohol, coffee, tea, or cola for 24 hours before and throughout the experimental session. Psychomotor performance was measured by critical flicker fusion (a measure of cortical arousal), simple reaction time (SRT), and a tapping (reflex) test. Visual evoked potentials and two assessments of subjective mood were also utilized. Alcohol consumption significantly increased SRT and decreased amplitude of the evoked potentials. Caffeine decreased SRT and increased the amplitude of the evoked potentials. In this experiment, the profiles of placebo and the combination of alcohol plus caffeine were not significantly different from results of either of the two substances alone. The addition of caffeine to alcohol did not significantly change subjective feelings of depression, anxiety, or drunkenness.

Ferreira et al. tested physiologic indicators (heart rate, blood pressure, respiratory exchange rate, oxygen uptake, and ventilatory threshold), biochemical variables (glucose, lactate, insulin, cortisol, ACTH, dopamine, norepinephrine, and epinephrine), blood alcohol levels, and the performance of participants on a maximal effort on a bicycle ergometer. All subjects at baseline were “moderate” users of alcohol and “moderate” consumers of energy drinks; individual differences in consumption were not controlled. In a double-blind study over four sessions, 14 male volunteers were given weight-based doses of alcohol (1.0 g/kg), energy drink (3.57 mL/kg Red Bull), energy drink plus alcohol, and a control beverage (water). No significant differences in physiologic and biochemical parameters were observed between the alcohol and the alcohol plus energy drink sessions. The performance in the maximal effort test following the ingestion of energy drink plus alcohol was similar to that observed with alcohol alone.

Ferreira et al. gave weight-based doses of alcohol alone (either 0.6 or 1.0 g/kg), a caffeinated energy drink alone, or alcohol plus energy drink to 26 healthy male volunteers in three separate experimental sessions. Participants were “similar” in their baseline use of alcohol and energy drinks. Researchers measured breath alcohol concentrations, motor coordination (using the Grooved Peg-board test), and visual reaction time. Subjective intoxication was evaluated using a visual analog scale of somatic symptoms. Compared with the ingestion of alcohol alone, the consumption of energy drink plus alcohol significantly reduced subjective drunkenness, but did not significantly ameliorate alcohol-induced deficits in motor coordination and visual reaction time. The addition of energy drink did not alter breath alcohol concentration in persons who consumed alcohol.

Marczinski and Fillmore evaluated dual-task interference and psychologic refractory periods in 12 adult volunteers using a double-blinded within-subject design that crossed two doses of alcohol (0.0 and 0.65 g/kg) with three doses of caffeine (0.0, 2.0, and 4.0 mg/kg). Participants (six men and six women) were instructed to fast for 4 hours, abstain from caffeine for 8 hours, and abstain from alcohol for 24 hours, prior to the study session. Alcohol significantly impaired information processing, increasing the psychologic refractory period needed to complete a second task performed in close proximity to a first task. Response accuracy to the
second task was also impaired. Co-administration of caffeine antagonized alcohol-induced impairment of the psychologic refractory period, but it had no antagonizing effect on alcohol-induced impairment of accuracy. Participants in Marczinski’s study reported reduced subjective intoxication in response to caffeine co-administration, despite their performance impairment.

Curry and Stasio studied a global measure of neuropsychological functioning in 27 nonsmoking women, using a double-blinded placebo-controlled model in which participants consumed 16 ounces of a nonalcoholic, noncaffeinated beverage (Diet 7-Up), a caffeinated energy drink alone (Monster Green, 160 mg caffeine/16 ounces), or a caffeinated 6% alcoholic malt beverage (Sparks Orange, 87 mg of caffeine). Participants were asked to abstain from caffeine use for 1 hour prior to the assessments. Researchers used the Repeatable Battery for the Assessment of Neuropsychological Status to evaluate five cognitive domains: immediate memory, delayed memory, visuospatial/constructional, attention, and language. Body weight was used to calculate an estimated blood alcohol level. Compared with the energy drink group, visuospatial construction and language scores were significantly decreased in the group who consumed caffeinated alcohol. Both Monster and Sparks contained proprietary amounts of guarana.

In response to anecdotal evidence that links sudden cardiac death with the combination of energy drinks, alcohol, and exercise, Wiklund et al. examined 10 healthy volunteers who consumed energy drinks (three 250 mL cans of Red Bull®), energy drink plus alcohol (0.4 g of ethanol per kilogram of body weight), or no drink, 30 minutes prior to maximal effort on a bicycle ergometer. Researchers required “at least a 1-week washout period” between experimental sessions. No clinically significant dysrhythmias were observed, but postexercise, recovery in heart rate and heart rate variability was slower for participants who consumed energy drink plus alcohol, compared with those who consumed energy drink alone. The authors suggest that blunted cardiac autonomic modulation after exercise may increase risk of dysrhythmia for predisposed individuals.

In a randomized trial by Howland et al., nonsmoking, non-dependent, heavy-episodic adult drinkers (n = 127) received caffeinated beer, non-caffeinated beer, caffeinated nonalcoholic beer, or non-caffeinated nonalcoholic beer using a 2 x 2 between-groups model. All participants consumed ≥1 and ≤7 caffeinated beverages daily. Alcoholic beverage administration was targeted to achieve breath alcohol concentration of 0.12 g%. A 30-minute simulated driving test and the Psychomotor Vigilance Task (PVT) were administered; participants were asked to estimate their own blood alcohol concentration for predisposed individuals.

A study by El Yacoubi et al. compared the hypnotic effects of alcohol following the administration of caffeine (25 mg/kg) in A2ARKO mice. Lower doses, alcohol acts as a central nervous system depressant in mice, causing sedation and incoordination. Higher doses of alcohol significantly impair consciousness, causing a “comma-like” state that is measured by loss of the righting reflex (an animal’s failure to correct its position when lying on its back). Adenosine receptor deficient mutant mice (A2ARKO) were less sensitive to alcohol-induced loss of righting reflex, a finding that suggests that the A2A receptor is involved in mediating the behavioral effects elicited by intoxicating doses of alcohol.

Kunin et al. observed that a narrow dose range of caffeine facilitated an increase in alcohol drinking behavior in free-feeding laboratory rats. Animals that were given an intraperitoneal injection of caffeine (5 mg/kg) demonstrated a dose-related increase in the consumption of 8% and 10% alcohol, but those given 10 mg/kg of caffeine did not differ in their alcohol consumption from placebo (saline-treated) animals. A second experiment assessed the effect of caffeine on the maintenance of established alcohol consumption. In rats that had been acclimatized to increasing concentrations of alcohol, the administration of caffeine enhanced alcohol consumption at a dosage of 5 mg/kg, but animals treated

Research on the Effects of Caffeine and Alcohol: Animal Studies

Gulick and Gould used C57BL/6J mice to test the effect of caffeine on alcohol-induced changes in anxiety, locomotion, and plus-maze discriminative avoidance. The plus-maze resembles a plus sign, with two opposing enclosed arms and two opposing open arms. An aversive arm delivers 75-W light and 85-Db white noise when a mouse is in the arm. The plus-maze model measures learning (as percent time spent in aversive enclosed arm versus percent time in the nonaversive enclosed arm; increased time in the aversive arm and decreased time in the nonaversive arm = decreased learning) as well as anxiety (as percent time spent in the open arms; increased time in the open arms = decreased anxiety). In this experiment, alcohol decreased anxiety and learning in a dose-dependent fashion; caffeine increased anxiety and decreased locomotion and learning in a dose-dependent fashion. Caffeine did not reverse alcohol-induced learning deficits, but a high dose of alcohol (1.4 g/kg) was noted to block the anxiogenic effect of caffeine.

A study by El Yacoubi et al. compared the hypnotic effects of alcohol following the administration of caffeine (25 mg/kg) in A2AR WT and A2AR KO mice. At lower doses, alcohol acts as a central nervous system depressant in mice, causing sedation and incoordination. Higher doses of alcohol significantly impair consciousness, causing a “comma-like” state that is measured by loss of the righting reflex (an animal’s failure to correct its position when lying on its back). Adenosine receptor deficient mutant mice (A2ARKO) were less sensitive to alcohol-induced loss of righting reflex, a finding that suggests that the A2A receptor is involved in mediating the behavioral effects elicited by intoxicating doses of alcohol.

Kunin et al. observed that a narrow dose range of caffeine facilitated an increase in alcohol drinking behavior in free-feeding laboratory rats. Animals that were given an intraperitoneal injection of caffeine (5 mg/kg) demonstrated a dose-related increase in the consumption of 8% and 10% alcohol, but those given 10 mg/kg of caffeine did not differ in their alcohol consumption from placebo (saline-treated) animals. A second experiment assessed the effect of caffeine on the maintenance of established alcohol consumption. In rats that had been acclimatized to increasing concentrations of alcohol, the administration of caffeine enhanced alcohol consumption at a dosage of 5 mg/kg, but animals treated

and alcohol for 24 hours prior to the experiment. In a single laboratory session, participants were randomly assigned to receive alcohol (0.5 g/kg of 40% ABV vodka), energy drink (3.57 mL/kg of Red Bull®), alcohol plus energy drink, or placebo (3.57 mL/kg of a decaffeinated soft drink). Alcohol significantly impaired both response execution and response inhibition. The consumption of energy drink mixed with alcohol antagonized the alcohol-induced impairment of response execution, but not alcohol-induced impairment of response inhibition. Individuals who consumed energy drinks mixed with alcohol reported increased levels of stimulation compared with those who drank alcohol alone, but the addition of energy drink did not significantly alter the drinker’s subjective feelings of intoxication or the drinker’s perception of ability to drive.
with 2.5 and 10 mg/kg caffeine did not differ from saline-treated animals. Researchers offered two possible hypotheses to explain this phenomenon: that the stimulant effect of caffeine encouraged “self-medication” with alcohol, or that caffeine sensitized the rats to alcohol’s reinforcing effects.

Mechanisms of the Pharmacological Interactions of Alcohol and Caffeine

Both in humans and the experimental animal, caffeine produces the same qualitative pharmacological effects as classical psychostimulants, such as cocaine and amphetamine: an increase in motor activity, arousal effects, and reinforcing effects. It is important to point out that caffeine has a weaker reinforcing efficacy than classical psychostimulants. Thus, caffeine users often fulfill the criteria for drug dependence, but not for drug abuse, established by the DSM-IV. This seems to be mostly due to the fact that, different to classical psychostimulants, there is a little window between the “wanted” psychostimulant effects and the “unwanted” effects of caffeine, specially anxiety.

On the other hand, alcohol is psychodepressant and has somnogenic, anxiolytic, and motor-depressant and motor-impairing (ataxic) properties, but also experimentally proven reinforcing effects. Similarly to what happens with caffeine, there is a relatively little window between the “wanted” anxiolytic and reinforcing effects and the “unwanted” somnogenic and ataxic effects of alcohol. Just by comparing the pharmacological effects of caffeine and alcohol, and without entering into the mechanistic aspects, it becomes obvious that alcohol–caffeine could be a desired drug combination, since they could mutually counteract their unwanted effects. Thus, the arousal properties of caffeine could compensate the somnogenic effects of alcohol, while the anxiolytic effects of alcohol could compensate the anxiogenic effects of caffeine. In fact, as we will be describing with more detail in the following section, there is experimental evidence supporting these pharmacological interactions. But, what it becomes really intriguing is that, in fact, there is a common target for most “unwanted” and some of the “wanted” effects of caffeine and alcohol: adenosine neurotransmission.

Adenosine as a Mediator of the Psychostimulant Effects of Caffeine

Caffeine is a nonselective competitive adenosine receptor antagonist and produces its psychostimulant effects by countering the tonic effects of endogenous adenosine on central adenosine receptors. This depends largely on the ability of adenosine to modulate the function of multiple central ascending neurotransmitter systems, which are involved in motor activation and reward (dopaminergic systems) and arousal effects (cholinergic, noradrenergic, histaminergic, and orexinergic systems). Among the four cloned adenosine receptors (A1, A2A, A2B, and A3 receptors), A1 and A2A receptors are the ones predominantly expressed in the brain. Caffeine has similar in vitro affinities for A1, A2A, and A2B receptors and much lower affinity for A3 receptor. A1 and A2A receptors are the preferential targets for caffeine in the brain, since physiological extracellular levels of adenosine are sufficient to occupy and, therefore, stimulate A1 and A2A receptors. On the other hand, A2B receptors have a lower affinity for adenosine and they are only activated by pathologically high extracellular levels of adenosine. A1 receptors are widely expressed in the brain, including the striatum, while A2A receptors are highly concentrated in the striatum.

Striatal A1 and A2A receptors seem to underlie the motor-activating and reinforcing effects of caffeine, which depend on its ability to release the strong functional brake that adenosine imposes to the ascending dopaminergic systems. There are two ascending dopaminergic systems, which originate in the substantia nigra pars compacta and the ventral tegmental area and innervate the dorsal and ventral striatum, respectively. Striatal A2A receptors modulate dopamine neurotransmission by establishing direct interactions with dopamine D2 receptors, forming A2A-D2 receptor heteromers. In these heteromers, stimulation of A2A receptors blocks D2 receptor function. In the ventral striatum, interactions between A2A and D2 receptors seem to play an important role in the reward-related behaviors. The same as D2 receptor antagonists, A2A receptor agonists elevate brain stimulation reward threshold, indicating that adenosine, via A2A receptors, may inhibit central reward processes. Further, stimulation of striatal presynaptic A1 receptors inhibits while its blockade facilitates dopamine release by glutamate-dependent and glutamate-independent mechanisms. By targeting A1 receptors in striatal glutamatergic terminals and A1 receptors in striatal dopamine terminals (presynaptic brake), caffeine induces glutamate-dependent and glutamate-independent release of dopamine. These presynaptic effects of caffeine are potentiated by the release of the postsynaptic brake imposed by antagonistic interactions in the striatal A2A-D2 heteromers.

An important amount of evidence indicates that adenosine is a mediator of sleepiness following prolonged wakefulness. Initial evidence came from pharmacological studies that show the sleep-inducing effects of systemic or intracerebral administration of adenosine and adenosine receptor agonists. It is now generally believed that a direct A1 receptor-mediated modulation of the corticopetal basal forebrain system and an indirect A2A receptor-mediated modulation of the hypothalamic histaminergic systems are the main mechanisms by which adenosine exerts its sleep-promoting effects. However, it is important to point out that a possible additional contribution comes from A1 receptors localized in the nuclei of origin of the histaminergic, orexinergic, and noradrenergic arousal systems. Arousal effects of caffeine depend on the blockade of multiple inhibitory mechanisms that adenosine, as an endogenous sleep-promoting substance, exerts on the multiply interconnected ascending arousal systems.

Adenosine Mechanisms in the Acute Pharmacological Effects of Alcohol

The pharmacological effects of alcohol are multiple, since it targets many neurotransmitter receptors and ion channels, involving a multitude of neurotransmitter systems in widespread regions of the brain. Among those systems, alcohol is known to potentiate GABAergic neurotransmission by facilitating GABA A receptor-mediated currents by direct and indirect mechanisms as well as by promoting GABA release. In addition, alcohol inhibits glutamatergic
neurotransmission by acting on ionotropic glutamate receptors, especially by attenuating N-methyl-D-aspartate receptor function.34,47 Obviously, both the facilitation of GABAergic neurotransmission and the inhibition of glutamatergic neurotransmission are most probably involved in the central depressant effects of alcohol. Further, alcohol modulates dopamine neurotransmission by directly affecting the firing activity of dopamine neurons in the VTA.48 The mechanisms underlying this effect of alcohol are not completely elucidated, but it seems to involve a direct effect on a subtype of potassium channel that regulates the excitability of VTA neurons, as well as indirect effects through modulation of inputs to the VTA.48 It is widely believed that alcohol-induced increase in VTA neuronal firing, with the resulting increase of dopamine release in the ventral striatum, mediates the reinforcing effects of alcohol.49,50 It is important to point out that recent evidence indicates that acetaldehyde formed from alcohol in the brain or in the periphery participates in the effects of alcohol on VTA neurons.51

Apart from GABA, glutamate, and dopamine, an important amount of experimental data shows that adenosine is a main neurotransmitter involved in the acute and chronic pharmacological effects of alcohol (Fig. 1).35–37,52 Pioneering studies by Dar et al. showed that the adenosine uptake blocker dipyridamole potentiated, whereas the adenosine receptor antagonist theophylline reduced, the acute somnogenic effects and attenuate the nonwanted effects of alcohol. Under conditions of chronic alcohol consumption, there is a reduced adenosine tone, which is associated with a decreased activation of A2A receptors, which might increase dopaminergic neurotransmission and, therefore, contribute to the increased alcohol consumption, and with upregulation of A1 receptors, which might be involved in the tolerance to the acute effects of alcohol and also to the withdrawal symptoms (see text).

What are the mechanisms of increased adenosine tone after acute administration of alcohol? One mechanism is the motor-depressant effects of an A1 receptor agonist and the motor-activating effect of theophylline.54

After those initial studies, clear data have been obtained indicating that adenosine, by acting on A1 receptors, is a key mediator of the acute ataxic, somnogenic, and anxiolytic effects of alcohol (Fig. 1). The extensive work by Dar and coworkers supports the participation of adenosine and A1 receptors in the cerebellum, cortex, and striatum in the acute ataxic effects of alcohol. Thus, local infusion of A1 receptor agonists or antagonists in those brain areas increases or decreases, respectively, the motor-imparing effects of alcohol.55–59 The recent studies by Thakkar and coworkers strongly suggest that alcohol-induced increase in adenosine in the basal forebrain and stimulation of A1 receptors in the cholinergic neurons of the corticopetal basal forebrain arousal system is particularly involved in the acute somnogenic effects of alcohol.50–62 First, they showed with in vivo microdialysis that local perfusion of alcohol elevates significantly the extracellular concentration of adenosine in the basal forebrain.60 Second, systemic alcohol administration reduced the activity of cholinergic cells in the basal forebrain and the bilateral microinjection of an A1 receptor agonist in the same brain region significantly attenuated alcohol-induced sedation.61 In relation to anxiety, several studies have shown the involvement of A1 receptors. A1 receptor knockout mice display enhanced anxiety,63 and the anxiogenic effects of caffeine have been generally attributed to blockade of adenosine tone, which opposes its dopamine-mediated reinforcing effects by means of antagonistic striatal A2A-D2 receptor interactions, and which is responsible for the nonwanted effects of alcohol (such as motor incoordination and somnogenic effects). Caffeine, by antagonizing the acute effects of alcohol, provides a unique tool to enhance the reinforcing effects and attenuate the nonwanted effects of alcohol. Under conditions of chronic alcohol consumption, there is a reduced adenosine tone, which is associated with a decreased activation of A2A receptors, which might increase dopaminergic neurotransmission and, therefore, contribute to the increased alcohol consumption, and with upregulation of A1 receptors, which might be involved in the tolerance to the acute effects of alcohol and also to the withdrawal symptoms (see text).
metabolism of alcohol by the liver, which produces acetate that can be metabolized to adenosine in the brain.68 But the main mechanism seems to be a direct inhibition of the equilibrative nucleoside transporter (ENT1). This effect was first demonstrated \textit{in vitro}, in cells in culture.67 As expected, mice lacking ENT1 show reduced effects of acute administration of alcohol, such as less somnogenic and ataxic responses.68 On the other hand, transgenic overexpression of human ENT1 in mouse neurons increases sensitivity to the acute intoxicating effects of alcohol.69 In summary, the ENT1-dependent increase in the extracellular concentration of adenosine, by acting on A1 receptors localized in different brain areas, seems to play a very important role in the ataxic, somnogenic, and anxiolytic effects of the acute administration of alcohol.

Does adenosine also play any role in the reinforcing effects of alcohol? In fact, as mentioned before in relation to the interactions between A2A and D2 receptors in the ventral striatum, an increased adenosine tone should impede dopamine neurotransmission by decreasing the effects dopamine release induced by alcohol. In fact, A2A receptor activation decreases alcohol consumption.70 Therefore, alcohol-induced increase in extracellular adenosine seems to act as a brake for the reinforcing effects associated with the acute administration of alcohol. It must, however, be mentioned that another line of research suggests that A2A receptor stimulation, under certain conditions, synergizes with D2 receptor stimulation in the ventral striatum and it has been suggested that this mechanism could be involved in the reinforcing effects of alcohol.76 According to this rationale, A2A receptor antagonists could be useful in preventing alcohol abuse and some preclinical data support this possibility.71 However, another situation has to be taken into account under conditions of chronic alcohol consumption, where adenosine, adenosine receptors, and ENT1 also play a key but different role, compared with the acute situation.

Adenosine Mechanisms in the Chronic Pharmacological Effects of Alcohol

Contrary to the acute situation, in the chronic alcohol situation, we have a reduced adenosine tone. In fact, chronic alcohol exposure results in a decreased expression of ENT1 and, therefore, a decrease in alcohol-mediated inhibition of ENT1.69 The loss of reuptake of adenosine after chronic exposure to alcohol is most probably the main mechanism by which alcohol tolerance develops both in cell lines69 and animals.72 Several studies have shown that the consequent reduction in the adenosine tone is associated with an upregulation of A1 receptors, which seems to be at least partially responsible for the tolerance to the acute effects of alcohol and also to the main symptoms of alcohol withdrawal, such as insomnia, anxiety, and seizures (Fig. 1).73-77 Choi et al. showed that alcohol consumption in ENT1 knockout mice was significantly higher than that of wild-type littermates.68 The pharmacological effects of alcohol in ENT1 knockout mice were in fact associated with a decrease in adenosine tone, demonstrated as a decrease in A1 receptor-mediated modulation of striatal glutamatergic neurotransmission.69 Other experimental results also indicate that ENT1 expression is inversely correlated with alcohol consumption. Thus, ENT1 expression is significantly higher in the alcohol-avoiding CD1 outbred mouse strain than in the alcohol-preferring C57BL/67 inbred strain or in hybrid C57BL/67X CD1 mice, which also displays alcohol-preferring behavior.78

The question is then why a reduced adenosine tone is associated with increased alcohol consumption. Apart from its role in tolerance and withdrawal, a decreased adenosine tone should potentiate dopamine neurotransmission in the ventral striatum by means of A2A-D2 receptor interactions and, therefore, potentiate the effects of alcohol on dopamine release in the ventral striatum. In fact, A2A receptor activation decreases alcohol consumption.70 In line with the particular involvement of A2A and D2 receptors, D2 receptor knockout mice show a marked reduction in alcohol preference,79 while A2A receptor knockout mice drink more alcohol.70,80 About the efficacy of A2A receptor antagonists in decreasing alcohol consumption in rats,71,81 we believe that other mechanisms are involved, such as the blockade of presynaptic A2A receptors in striatal glutamatergic terminals.82,83 This mechanism has been recently suggested to be involved in the ability of an A2A receptor antagonist to counteract cannabinoid-induced self-administration in monkeys.84

Adenosine Mechanisms in the Alcohol-Caffeine Combination

During acute alcohol intake, caffeine largely antagonizes the “unwanted” effects of alcohol by blocking A1 receptors, which mediate alcohol’s somnogenic and ataxic effects. On the other hand, alcohol-induced increase in the extracellular concentration of adenosine can decrease the A1 receptor-mediated “unwanted” anxiogenic effects of caffeine.79 The mutual antagonism of “unwanted” effects gives the possibility of increasing significantly the intake of both drugs in the pursuit of the “wanted” reinforcing effects. Further, the striatal A2A-D2 receptor interactions provide a mechanism by which caffeine can potentiate the reinforcing effects of alcohol, since blockade of ventral striatal A2A receptors can potentiate postsynaptically the alcohol-mediated dopamine release. During chronic alcohol intake, in addition to providing a mechanism for counteracting tolerance to the acute effects, by blocking the effects of upregulated A1 receptors, caffeine provides a “treatment” for the withdrawal effects of alcohol. Further, blockade of A2A receptors by caffeine most likely contributes to the “wanted” reinforcing effects of alcohol, which probably depend on an already decreased inhibitory tone of adenosine on ventral striatal dopamine neurotransmission.

In summary, adenosine neurotransmission is a unique mechanistic link between caffeine and alcohol, and provides an explanation for the potentially risky effects when the two substances are combined.

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References


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Psychostimulant pharmacological profile of paraxanthine, the main metabolite of caffeine in humans

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Abstract
Caffeine induces locomotor activation by its ability to block adenosine receptors. Caffeine is metabolized to several methylxanthines, with paraxanthine being the main metabolite in humans. In this study we show that in rats paraxanthine has a stronger locomotor activating effect than caffeine or the two other main metabolites of caffeine, theophylline and theobromine. As previously described for caffeine, the locomotor activating doses of paraxanthine more efficiently counteract the locomotor depressant effects of an adenosine A1 than an adenosine A2A receptor agonist. In drug discrimination experiments in rats trained to discriminate a maximal locomotor activating dose of caffeine, paraxanthine, unlike theophylline, generalized poorly to caffeine suggesting the existence of additional mechanisms other than adenosine antagonism in the behavioral effects of paraxanthine. Pretreatment with the nitric oxide inhibitor N(G)-nitro-L-arginine methyl ester (L-NAME) reduced the locomotor activating effects of paraxanthine, but not caffeine. On the other hand, pretreatment with the selective cGMP-preferring phosphodiesterase PDE9 inhibitor BAY 73-6691, increased locomotor activity induced by caffeine, but not paraxanthine. Ex vivo experiments demonstrated that paraxanthine, but not caffeine, can induce cGMP accumulation in the rat striatum. Finally, in vivo microdialysis experiments showed that paraxanthine, but not caffeine, significantly increases extracellular levels of dopamine in the dorsolateral striatum, which was blocked by L-NAME. These findings indicate that inhibition of cGMP-preferring PDE is involved in the locomotor activating effects of the acute administration of paraxanthine. The present results demonstrate a unique psychostimulant profile of paraxanthine, which might contribute to the reinforcing effects of caffeine in humans.

1. Introduction

Caffeine is the most widely consumed psychoactive substance in the world. It is generally believed that caffeine exerts psychostimulant effects acting as a nonselective adenosine A1 receptor (A1R) and A2A receptor (A2AR) antagonist (Fredholm and Persson, 1982; Nehlig et al., 1992; Fredholm and Lindström, 1999; Fisone et al., 2004; Ferré, 2008). In rats, the trimethylxanthine caffeine is mainly demethylated to the dimethylxanthines paraxanthine, theophylline and theobromine in roughly similar amounts (Arnaud, 1985; Berthou et al., 1988). In humans, caffeine is also rapidly metabolized to the three dimethylxanthines, but with a very different metabolizing rate, with paraxanthine constituting by far the main metabolite (approximately 80% of the three dimethylxanthines) (Lelo et al., 1989; Berthou et al., 1992; for a recent review see Arnaud, 2011). The first studies which compared the pharmacological effects of caffeine and its main metabolites were reported almost 30 year ago when it was shown that caffeine, paraxanthine, theophylline, but not theobromine, were able to increase locomotor activation in mice (Seale et al., 1984). Also, in rats trained to discriminate caffeine from saline, both paraxanthine and theophylline, but not theobromine, were able to generalize to the caffeine-cue (Carney et al., 1985). Moreover, caffeine but not...
paraxanthine, was able to generalize to theophylline in rats trained to discriminate theophylline from saline (Carney et al., 1985). Therefore, those studies already suggested more similarities between caffeine and theophylline than with paraxanthine and even less with theobromine, which was consistently the least active methylxanthine. Paraxanthine has also less anxiogenic activity and toxicity in rodents than caffeine (Stavrak, 1988; Benowitz et al., 1995; Okuro et al., 2010).

It is widely assumed that the main mechanism of action involved in the behavioral effects of caffeine and paraxanthine is their antagonism of adenosine receptors, but they have little differences in their affinities for both adenosine A1Rs and A2ARs (Snyder et al., 1981), which suggests the existence of additional mechanisms to explain their pharmacological differences. A dopaminergic component for paraxanthine was proposed based on results showing displacement for a low concentration of the labeled dopamine D1 receptor (D1R) antagonist [3H]SCH-23390 in the rat striatum (Ferré et al., 1990) and the ability of SCH-23390 to partially counteract the displacement for a low concentration of the labeled dopamine D1 antagonist (K.A. Jacobson, personal communication). It is well known that methylxanthines have also modest phosphodiesterase (PDE) inhibitory activity (personal communication). It is well known that methylxanthines have also modest phosphodiesterase (PDE) inhibitory activity (personal communication).

3. Materials and methods

2.1. Animals and drugs

Male Sprague–Dawley albino rats (Charles River Laboratories, Wilmington, MA), weighing 300–350 g, were used in all the experimental procedures. Animals were housed 2 per cage and kept on a 12/12-h dark/light cycle with food and water available ad libitum. All animals used in the study were maintained in accordance with the guidelines of the National Institutes of Health Animal Care and the animal research conducted to perform this study was approved by the NIDA IRP Animal Care and Use Committee (protocol #: 09-Re08). The methylxanthines caffeine, theophylline, theobromine and paraxanthine, the adenosine A2AR agonist CPA (N7-cyclopentyladenosine), the adenosine A1R agonist CPA (8-Cyclopentyl-1, 3-dimethylxanthine) and the PDE9 inhibitor BAY 73-6991 were purchased from Sigma–Aldrich (St. Louis, MO). The adenosine A2AR antagonist KW-6002 and the adenosine A2R agonist CGS 21680 (2-p-[2-carboxyethyl] phenethylamino-5’-N-ethylcarboxamidoadenosine) were kindly provided by the CHDI Foundation Inc. (Los Angeles, CA USA). The NO synthesis inhibitor L-NMMA (N’-Nitro-L-arginine methyl ester hydrochloride) and the PDE4 inhibitor rolipram were purchased from Tocris Bioscience (Ellisville, MI), MSX-3, a soluble phosphate prodrug of MSX-2, was synthesized at the Pharmaceutical Institute, University of Bonn, Germany (Hockemeyer et al., 2004). The doses and the preparation of the adenosine receptor agonists and antagonists were selected based on previous experiments in which they elicited fully significant behavioral effects in the behavioral parameters tested, under our experimental conditions (Karcz-Kubica et al., 2003; Orrú et al., 2011b). Paraxanthine, theophylline, theobromine, CPT, BAY 73-6991 and rolipram were suspended in a solution of 3% dimethyl-sulfoxide, 5% Tween 80 and 90% ddH2O. KW-6002 was suspended in a solution of 8% TWEEN80 and 92% ddH2O. Caffeine was dissolved in saline solution. All drugs were administered i.p. in an injection volume of 2 ml/kg body weight.

2.2. Locomotor activity

Locomotor activity was measured by placing the animals individually in an open field motility soundproof chambers (50 × 50 cm) (Med Associates Inc., VT). Locomotion was measured by counting the number of breaks in the infrared beams of the chambers during consecutive periods of 10 min. Before each testing session, the animals were moved into the experimental room and allowed to habituate to the new environment for at least 2 h before being introduced in the chamber. Recording of the locomotor activity started immediately after placing the animals in the boxes (without habituation) and lasted for 90 min. Data were analyzed as the average of all transformed values (square root) of averaged counts per 10 min during the first hour. This period corresponds to the highest exploratory activity during exposure to a new test environment. All animals were tested only once.

2.3. Surgical procedures and in vivo microdialysis

Rats were deeply anesthetized with 3 ml/kg of Equithesin (NIDA Pharm, Baltimore, MD), placed in a stereotaxic apparatus and implanted with concentric dialysis probes (Eicom Corp, Tokyo) in the lateral striatum: anterior − 0.0 mm from bregma, lateral − 4.5 mm from bregma, vertical − 6.0 mm from dura (Paxinos and Watson, 2006). After surgery, rats were allowed to recover in hemispherical CMA-120 cages (CMA Microdialysis AB, Solna, Sweden) equipped with a swivel (Plastics One, Roanoke, VA). Twenty-four hours after implanting the probe, experiments were performed on freely moving rats in the same hemispherical home cages in which they recovered overnight from surgery. A Ringer solution containing (in mM): 147 NaCl, 4.0 KCl, 2.2 CaCl2 was pumped through the microdialysis probe at a flow rate of 1 μl/min. After a washout period of 90 min, dopamine microdialysate samples were collected for an additional 150 min, loaded in a refrigerated autosampler and analyzed by reverse high-performance liquid chromatography (HPLC) coupled with a coulometric detector (5200a Coulochem III, ESA, Chelmsford). Dopamine values were transformed as percentage of basal values (mean of the three values before vehicle or drug injection) and analyzed using analysis of variance (ANOVA) followed by Tukey’s multiple comparisons test (Statistica, Stat Soft, Tulsa, OK). At the end of the experiment, rats were given an overdose of equithesin, the brains were extracted, fixed in formaldehyde, and the probe place- ment was checked using cresyl violet staining.

2.4. Drug-discrimination procedure

Slightly food-restricted rats were trained as described previously (Solinas et al., 2005) under a discrete-trial schedule of food-pellet delivery to respond on one lever after an injection of a training dose of 30 mg/kg of caffeine (n = 10) and on the other lever after an injection of 1 ml/kg saline vehicle. Injectons of caffeine or saline were given i.p. 30 min before the start of the session. At the start of the session, a white house light was turned on, and in its presence, the rats were required to make 10 consecutive responses (fixed-ratio 10 schedule of food delivery) on the lever appropriate to the pre-session treatment. The completion of 10 consecutive responses on the correct lever produced delivery of a 45-g food pellet and initiated a 45-s timeout during which lever-press responses had no programmed consequences and the chamber was dark. Responses on the incorrect lever had no programmed consequences other than to reset the fixed-ratio requirement on the correct lever. After each timeout, the white house light was again turned on, and the next trial began. Each session ended after completion of 20 fixed-ratio trials or after 30 min elapsed, whichever occurred first. Discrimination-training sessions were conducted 5 days per week under a single alternation schedule (i.e., DDSDSDDS etc., where D fi rst demonstrate that paraxanthine has a main mechanism responsible for the difference in the locomotor activating properties of paraxanthine in reserpinized mice (Ferré et al., 1991). However, the binding experiments with [3H]SCH-23390 could not be replicated by other authors (K.A. Jacobson, personal communication). It is well known that methylxanthines have also modest phosphodiesterase (PDE) inhibitory activity (personal communication). It is well known that methylxanthines have also modest phosphodiesterase (PDE) inhibitory activity (personal communication).
other drugs were then initiated. The caffeine dose–response curve (3, 10, 18, 30, and 56 mg/kg) after determination was confirmed, before testing other drugs. Then, a range of doses of different methylxanthines—paraxanthine (10– 56 mg/kg; n = 8), theophylline (3–56 mg/kg; n = 10), and theobromine (10–56 mg/kg; n = 10) —were administered instead of caffeine to examine generalization to training stimulus. All methylxanthines were injected i.p. 10 min before the start of the session. Test sessions were identical to training sessions, with the exception that 10 consecutive responses on either one of the two levers ended the trial. Switching responding from one lever to the other lever reset the ratio requirement. In a test phase, a single alternation schedule was introduced, and test sessions were usually conducted on Tuesdays and Fridays. Thus, a 2-week schedule was introduced. Test sessions were conducted only if the criterion of 80% accuracy and not more than four incorrect responses during the first session was maintained in the two preceding training sessions.

2.5. Radioligand-binding experiments

Membrane suspensions from sheep striatum were processed as described previously (Casado et al., 1990; Sarrió et al., 2000). Tissue was disrupted with a Polytron homogenizer (PTA 20 TS rotor, setting 3; Kinematica, Basel, Switzerland) for three 5-s periods in 50 mM Tris–HCl buffer, pH 7.4 containing a protease inhibitor cocktail (Sigma). Cell debris was eliminated by centrifugation at 10,000 g (30 min, 4 °C) and membranes were obtained by centrifugation at 105,000 g (40 min, 4 °C). The pellet was resuspended and recentrifuged under the same conditions and was stored at −80 °C. Membranes were washed once more as described above and resuspended in 50 mM Tris–HCl buffer, pH 7.4, for immediate use. Protein was quantified by the bicinchoninic acid method (Pierce Chemical Co., Rockford, IL) using bovine serum albumin dilutions as standard. Binding experiments were performed with sheep striatal membrane suspensions at 25 °C in 50 mM Tris–HCl buffer, pH 7.4, containing 10 mM MgCl2 and 0.2 I.U./ml of ADA (EC 3.5.4.4; Roche, Basel, Switzerland). For competition experiments, we incubated membrane suspensions (0.1 mg of protein/ml) with a constant free concentration (0.8 nM) of the D8R antagonist [3H]SCH-23390 (Tocris Bioscience, Ellisville, MO) and confirmed that the value was the same as calculated by extrapolation of the competition curves. Free and membrane bound ligand were separated by rapid filtration of 500 μl aliquots in a cell harvester (Brandel, Gaithersburg, MD) through Whatman GF/C filters embedded in 0.3% polyethylenimine that were subsequently washed for 5 s with 5 ml of ice-cold Tris–HCl buffer. The filters were incubated with 10 ml of Ecoscint H scintillation cocktail (National Diagnostics, Atlanta, GA) overnight at room temperature and radioactivity counts were determined using a Tri-Carb 2800TR scintillation counter (PerkinElmer, Boston, MA, USA) with an efficiency of 62%.

2.6. Measurement of cGMP and cAMP in ex vivo rat striatal tissue

Rats were injected i.p. with vehicle or either caffeine, paraxanthine or BAY 73-6691 at different doses and killed by decapitation 30 min after the drug administration. The heads of the animals were immersed in liquid nitrogen for 6 s and the striata were rapidly dissected out on an ice-cold surface. The tissue, after the dissection, was frozen out in liquid nitrogen. The striata were homogenized in 0.1 N HCl followed by centrifugation and the supernatant was used to measure cGMP or cAMP concentrations using an enzyme immunoassay kit (Enzo Life Science, NY).

2.7. Statistical analysis

Statistical analysis for locomotor activity, drug discrimination and ex vivo tissue studies were carried out using a one-way ANOVA followed by Dunnett’s or Newman’s post-hoc tests or by a two-way ANOVA using the Bonferroni post-hoc as comparison test (GraphPAD Prism Software, San Diego, CA). For the microdialysis experiments, data were analyzed using a one-way ANOVA for repeated measures over time followed by Dunnett’s multiple comparison tests (Statistica, Stat Soft, Tulsa, OK). Statistical significance was accepted at a level of P < 0.05. All data are presented as mean ± S.E.M. Radioligand binding experiments were analyzed by non-linear regression, using the commercial Grafit software (Erithacus Software).

3. Results

3.1. Locomotor activation induced by caffeine, paraxanthine, theophylline and theobromine in non-habituated rats

Increasing doses of paraxanthine, theophylline, theobromine and caffeine were tested in these experiments (from 1 to 56 mg/kg). All four compounds but theobromine produced locomotor activity with inverted “U” shape dose–response curves with the order of potency being theophylline > caffeine > paraxanthine > theobromine (Fig. 1). These results are in correspondence with their described affinities for both adenosine A1Rs and A2ARs (Shi and Daly, 1999). Paraxanthine was the most efficient compound among the methylxanthines that were tested and it produced the strongest locomotor activation (particularly at the dose of 30 mg/kg), with the order of efficacies being: paraxanthine > caffeine > theophylline > theobromine (Fig. 1). At the peak dose of 30 mg/kg, paraxanthine produced a significantly stronger locomotor activating effect than caffeine (Fig. 1).

3.2. Counteraction of adenosine A1R and A2AR agonist-induced locomotor depression by caffeine, paraxanthine, theophylline and theobromine

Based on a previous study from our laboratory (Karcz-Kubicha et al., 2003), we used equipotent locomotor depressant doses of the A1R agonist CPA (0.1 mg/kg) and the A2AR agonist CGS 21680 (0.5 mg/kg) to evaluate the A1R and A2AR antagonist profile of caffeine, theophylline, paraxanthine and theobromine. In fact, the selective A1R antagonist CPT (1 mg/kg) significantly counteracted the locomotor depressant effect of CPA, but not CGS 21680, and the selective A2AR antagonist MSX-3 (1 mg/kg) significantly countered the locomotor depressant effect of CGS 21680, but not CPA (Fig. 2). As previously published (Karcz-Kubicha et al., 2003), locomotor activating doses of caffeine significantly counteracted the locomotor depressant effect of CPA, but not of CGS 21680 (Fig. 3a), indicating that the locomotor activating effects of an acute administration of caffeine depend mostly on A1R blockade. Similarly, locomotor activating doses of paraxanthine and theophylline but not theobromine were more potent at counteracting the effect of CPA than CGS 21680 (Fig. 3b–d). Paraxanthine was by far the most efficient at counteracting the locomotor depression induced by either CPA or CGS 21680 when compared to theophylline and theobromine. These results indicate that A1R and not A2AR antagonism is primarily involved in the locomotor activating effects of acute administration of methylxanthines. However, the previously
described similar affinity of \(A_1\)R for caffeine, theophylline and paraxanthine (see Introduction) suggests that the stronger locomotor activating effect of paraxanthine must involve an additional mechanism of action.

### 3.3. Generalization tests with caffeine, paraxanthine, theophylline and theobromine in rats discriminating caffeine (30 mg/kg) from vehicle

Caffeine produced a dose-dependent increase in drug-lever selection with maximal selection of the drug lever (96.22%) at the 30 mg/kg training dose of caffeine. Generalization to the training stimulus was significant at caffeine doses of 30 and 56 mg/kg (Fig. 4). The 56 mg/kg dose of caffeine significantly decreased rates of responding. Theophylline produced partial generalization to the caffeine-training stimulus, which was significant at doses of 30 and 56 mg/kg (maximal drug-lever selection was 68.45% and 62.65%, respectively) (Fig. 4). The 56 mg/kg dose of theophylline significantly decreased rates of responding. Paraxanthine produced a low level of partial generalization (maximal drug-lever selection 33.05%) to the training stimulus at a dose of 56 mg/kg (Fig. 4). At the same dose (56 mg/kg), theobromine also produced similar level of partial generalization as paraxanthine (maximal drug-lever selection 33.13%), but statistical significance was not reached. In the tested range of doses, neither paraxanthine nor theobromine significantly decreased rates of responding. These results indicate that theophylline shares very much the same mechanism than caffeine and again suggest that paraxanthine must involve other mechanism of action, at least responsible for the stimulus discrimination effects.

### 3.4. Displacement of \(D_1\)R antagonist binding from striatal membranes by paraxanthine and caffeine

In view of the existence of contradictory results about its previously proposed direct effects on \(D_1\)R (see Introduction), we...
reevaluated the possible ability of paraxanthine to displace the selective D₁R antagonist [³H]SCH-23390 from striatal membrane preparations (Ferré et al., 1990, 1991). Paraxanthine, and to a lesser extent caffeine, could displace the binding of [³H]SCH-23390, but only partially and at very high, non-pharmacological, concentrations. At 3 mM, paraxanthine displaced 27% and caffeine displaced 16% of the specific binding. This indicates that the effects of paraxanthine on locomotion in the doses used in the present study cannot be mediated by direct effects at D₁R.

3.5. Role of NO-cGMP signaling on the locomotor activity induced by paraxanthine

In view of the possible role of NO-cGMP signaling in the behavioral effects of psychostimulants (see Introduction) we first evaluated the effect of the NO synthase inhibitor l-NAME. The dose of l-NAME used (30 mg/kg) was based on pilot experiments and on results obtained by other research groups (Kayir and Uzbay, 2004). l-NAME, when administered alone, did not produce any significant change in locomotor activity in rats, excluding then any sedative or muscles relaxant effect. However, pretreatment of rats with l-NAME (30 mg/kg) significantly reduced the locomotor activating effect of paraxanthine (30 mg/kg) (Fig. 5a and b) but not of caffeine (30 mg/kg) (Fig. 5c and d). The degree of locomotor activation induced by paraxanthine after pretreatment with l-NAME was similar to the degree of locomotor activation induced by caffeine itself. Based on that, we then checked if the NO-dependent component of the locomotor activating effects of paraxanthine was due to a selective inhibition of cGMP-preferring PDEs. At a dose of 3 mg/kg, the PDE9 inhibitor BAY 73-6691 did not produce any

![Fig. 4. Effects of the adenosine receptor antagonists paraxanthine (parax), theophylline (theoph) and theobromine (theobr) in rats trained to discriminate 30 mg/kg caffeine (caff) from vehicle (VEH). Data represent mean ± S.E.M. (n = 10). Ordinates percentage of responses on the lever associated with caffeine administration (left panel) and overall rate of lever pressing expressed as responses per second averaged over the session (right panel). Drug doses are expressed in mg/kg (log scale). Complete generalization to the caffeine-training stimulus (80% caffeine-lever selection) is represented with dashed horizontal line. * and **: P < 0.05 and P < 0.01 compared with VEH, respectively (one-ANOVA followed by Bonferroni’s post-hoc test).](image)

![Fig. 5. Effect of the NO synthase inhibitor l-NAME treatment on locomotor activation induced by paraxanthine and caffeine. Non-habituated animals were placed in the motility cages 40 min after vehicle (VEH), paraxanthine (parax, 30 mg/kg) or caffeine (caff, 30 mg/kg) administration and 30 min after VEH or l-NAME (l-NAME, 30 mg/kg). (a) and (c) represent time course for locomotion of transformed data (square root) of accumulated counts per 10-min period (mean ± S.E.M.; n = 6–8 per group). (b) and (d) represent the average (mean ± S.E.M.) of the 10-min period transformed values during the first 60 min of recording (n = 6–8 per group). ***: P < 0.001 and #: P < 0.05 compared to paraxanthine alone (one-way ANOVA followed by Bonferroni’s post-hoc test).](image)
locomotor activation per se (Fig. 6) and did not counteract the motor depressant effects of CPA or CGS 21680 (Fig. 1), indicating that at this dose it does not act as an A1R or A2AR antagonist. Nevertheless, at 3 mg/kg, BAY 73-6691 was able to increase the locomotor activity induced by caffeine (30 mg/kg), but not paraxanthine (30 mg/kg) (Fig. 6a and b). BAY 73-6691 was then tested in combination with locomotor activating doses of the A1R and A2AR antagonists CPT or KW-6002, respectively. The doses and the preparation of KW-6002, CPT and the PDE 9 inhibitor BAY 73-6691 were selected based on previous experiments in which they elicited fully significant effects in the behavioral parameters tested and under our experimental conditions (Karcz-Kubicha et al., 2003; Orrú et al., 2011a; Van der Staay et al., 2008). Co-administration of BAY 73-6691 was able to increase the locomotor activation induced by CPT, but not by KW-6002 (Fig. 6c and d). Altogether these results strongly suggest that the locomotor activating effects of an acute administration of paraxanthine depend on the combined A1 receptor blockade and PDE9 inhibition (see Discussion).

3.6. Effects of paraxanthine on cGMP accumulation in the rat striatum

The selective inhibition of a cGMP-preferring PDE by paraxanthine was then confirmed in ex vivo experiments. As shown in Fig. 7, the administration of the PDE9 inhibitor BAY 73-6691 (3 mg/kg) produced a significant cGMP accumulation in homogenates of rat striatum. The same quantitative effect was obtained with paraxanthine at the dose of 30 mg/kg, but not 10 mg/kg. On the other hand, caffeine, at 10 and 30 mg/kg did not produce any significant effect and did not modify the effect of BAY 73-6691 (Fig. 7). Neither caffeine nor paraxanthine, at the dose of 30 mg/kg, produced a significant increase in cAMP accumulation in the same striatal preparations, with values of 102 ± 9 and 111 ± 13, respectively, versus vehicle (100 ± 4). As a positive control, the selective PDE4 inhibitor rolipram produced a significant increase in cAMP levels (125 ± 2; one-way ANOVA with Newman–Keuls’ post-hoc comparisons: P < 0.05; n = 6–8 per group).

3.7. Paraxanthine-induced elevation in dopamine extracellular levels in the lateral striatum

In vivo microdialysis experiments showed that paraxanthine (30 mg/kg), but not caffeine (30 mg/kg) was able to significantly increase extracellular levels of dopamine in the lateral striatum for about 50% when compared with basal levels (Fig. 8). The administration of the nitric oxide synthase inhibitor L-NAME (30 mg/kg) by itself did not alter dopamine levels as compared to basal levels. However, pretreatment with L-NAME (30 mg/kg) significantly reduced paraxanthine-induced extracellular dopamine levels.

4. Discussion

Previous reports have shown dose-dependent psychostimulant and locomotor activating effects of caffeine and its metabolites paraxanthine, theophylline and theobromine. Adenosine receptor antagonism has been shown to be a main mechanism of action responsible for the locomotor activating effects of caffeine, and the same mechanism has been suggested to be involved in the locomotor activating effects of its main metabolites, particularly theophylline and paraxanthine (Snyder et al., 1981; Fredholm and Persson, 1982). After some debate about the role of the adenosine receptor subtypes in the behavioral effects of caffeine (Snyder et al., 1981; Goldberg et al., 1985; Svenningsson et al., 1995; El Yacoubi et al., 1999; El Yacoubi et al., 2000; Lindskog et al., 2002), we reported evidence for a predominant role of A1R in the locomotor activating effects of an acute administration of caffeine, with a more predominant involvement of A2AR under conditions of chronic caffeine treatment (Karcz-Kubicha et al., 2003; Antoniou et al., 2005). Interestingly, A2AR seems to be preferentially involved in the caffeine-induced enhancement of operant responding in rats (Randall et al., 2011).
pretreatment (30 mg/kg; L-NAME 30). * and **: increase of dopamine extracellular levels in the lateral striatum. Results express repeated measurement with Dunnett.

and in the caffeine-induced reversal of the motor depressant effects of dopamine D2 receptor (D2R), but not D1R, antagonists (Collins et al., 2010). The latter results can be explained by the known role of A2A R in the depressant effects of D2R antagonists, recently suggested to depend on a subpopulation of striatal postsynaptic A2A R that do not form heteromers with D2R (Orrú et al., 2011b). On the other hand, the differential involvement of A1 R and A2A R in the acute activating effects of caffeine on operant responding and locomotion (exploratory activity) suggest the existence of a differential tone of endogenous adenosine on different goal-directed behaviors. In the present study we also provide evidence for a predominant A1 R antagonistic profile of acute locomotor activating doses of theophylline, paraxanthine and theobromine. An interesting and not previously reported finding is that paraxanthine is significantly the most efficient compound among methylxanthines and produced the strongest locomotor activation in rats, particularly at the dose of 30 mg/kg. At this dose paraxanthine could not generalize to the most efficient locomotor activating dose of caffeine (30 mg/kg) and, at an even higher dose (56 mg/kg), paraxanthine could only partially generalize to caffeine. Since the affinities of A1 R (and also A2A R) for caffeine and paraxanthine are similar (Shi and Daly, 1999), these results indicated the existence of an additional mechanism other than adenosine receptor antagonism that controls the locomotor- and discriminative-stimulus effects of paraxanthine.

In the present study we found a role of the NO-cGMP signaling in the locomotor activating effects of paraxanthine. First, the inhibitor of NO synthase L-NAME was able to decrease the locomotor activating-effects of paraxanthine, but not the locomotor activating effects of caffeine. Interestingly, after L-NAME administration, the levels of locomotor activity produced by paraxanthine (30 mg/kg) were comparable to those of caffeine (30 mg/kg). These results appear to be in contradiction with published results regarding a counteracting effect of L-NAME on locomotor activity induced by caffeine in mice (Kayir and Uzbay, 2004). Apart from the difference in species (mice versus rats), these discrepancies might involve other paradigm variables, such as different doses used for instance. In any case, in the present study we observe a very clear differential effect of L-NAME, indicating at the least that nitric oxide has a more relevant role in the locomotor activating effects of paraxanthine than caffeine.

We then investigated if the NO-cGMP signaling-dependent mechanism involved in the locomotor activating effects of paraxanthine would be due to a selective inhibition of cGMP-prefering PDEs. Several studies had already shown that when comparing the psychostimulant effects of methylxanthines, relatively higher concentrations (100–1000 μM) of caffeine are required to inhibit cAMP-prefering PDE activity than those doses required for the interaction with adenosine receptors (10–100 μM) (Sutherland and Rall, 1958; Butcher and Sutherland, 1962; reviewed by Francis et al., 2011). However, although this property of caffeine has been known for decades, the ability of caffeine and its main metabolites to modify cGMP-prefering PDE activity had not been investigated. We found that the cGMP-prefering PDE (PDE9) inhibitor BAY 73-6691, potentiated caffeine- but not paraxanthine-induced locomotor activation. Based on our results, we speculated that the differences in the locomotor activating effects of paraxanthine and caffeine are related to differences in their ability to inhibit cGMP-prefering PDE, most probably PDE9. In fact, in ex vivo experiments, we were able to demonstrate that at the dose with maximal differences in the locomotor activating effects (30 mg/kg), paraxanthine, but not caffeine, induced a significant increase in the striatal concentration of cGMP. Importantly, the striatum contains one of the highest PDE9 levels in the rat brain (Van Staveren and Markerink-van Ittersum, 2005; Reyes-Irisari et al., 2007).

The results seemed to indicate that simultaneous blockade of adenosine receptors and activation of NO-cGMP pathway underlies the behavioral effects paraxanthine. In fact, we found that BAY 73-6691 potentiated the locomotor activation induced by the A1 R antagonist CPT, but not by the A2A R antagonist KW-6002. The relatively low level of activation induced by CPT (as compared for instance with that of KW-6002) is related to its short duration of action, which agrees with its reported pharmacokinetic properties (Baumgold et al., 1992), and to the fact that we were using animals not habituated to the recording environment. Thus, the locomotor activating effects of CPT coincided with the initial burst of exploratory activity and there was very little, albeit significant, locomotor activation compared with vehicle-treated animals. In habituated animals, CPT produces a very significant locomotor activation (Karcz-Kubicha et al., 2003; Antoniou et al., 2005), which we have previously demonstrated it is qualitatively very similar to that of caffeine (Antoniou et al., 2005). We also reported that, on the other
hand, the locomotor activating effects of the A2AR antagonist MSX-3 are qualitatively dissimilar to that of caffeine (Antoniou et al., 2005), again underscoring a predominant role of A1R on the acute locomotor activating effects of the caffeine. Also, in our experience within the same rat strain, the effect of the dose of KW-6002 used in our experiments is far from the maximal effect (Orrù et al., 2011a), indicating that the lack of a potentiating effect of BAY 73–6691 on the locomotor activating effects of KW-6002 cannot be related to a ceiling effect. Altogether, our results strongly support the existence of an interaction of two main mechanisms, A1R antagonism and PDE9 inhibition, as being responsible for the locomotor activating effects of an acute administration of paraxanthine.

We have previously shown in experiments with in vivo microdialysis in rats that the locomotor activating effects of A1R antagonists (or for the A1R-mediated component of caffeine) correlate particularly with their ability to induce dopamine release in the striatum, particularly in ventral compartments (Solinas et al., 2002; Quarta et al., 2004; Borycz et al., 2007). This effect, however, is milder compared to classical psychostimulants, which produce larger increases in the extracellular striatal concentrations of dopamine (Di Chiara et al., 2004). In the present study, also correlating with locomotor activity, we found that paraxanthine is more efficient that caffeine at increasing the extracellular striatal concentration of dopamine. In fact, paraxanthine increased dopamine in a lateral striatal compartment, where caffeine was ineffective. It is therefore possible that the interaction between A1R and NO-cGMP signaling takes place in striatal dopaminergic nerve terminals and that A1R antagonism plus cGMP-prefering phosphodiesterase inhibition is responsible for the effect of paraxanthine on striatal extracellular concentrations of dopamine. In fact, t-NAME, at a dose that was ineffective on its own, completely counteracted the effect of paraxanthine, demonstrating the involvement of NO-cGMP signaling in this classical psychostimulant-like effect of paraxanthine.

When considering the classical psychostimulants-like pharmacological profile of paraxanthine, a series of questions arise. Does paraxanthine have reinforcing properties? Does it contribute to the reinforcing properties of caffeine? To answer these questions we should first know if after caffeine consumption, paraxanthine reaches the CNS at concentrations high enough to elicit its psychostimulants effects. At least two factors support this possibility. First, that paraxanthine is the main metabolite of caffeine in humans (see Introduction) and, second, that the plasma levels of caffeine metabolites are known to increase with its chronic consumption, due to induction of caffeine metabolism. In the experimental animal, during chronic caffeine consumption, the metabolites can even surpass caffeine plasma levels (see for instance Gasior et al., 2002). Definitively, more clinical research is needed to undoubtedly demonstrate a role of paraxanthine in the psychostimulant effects of caffeine. Finally, one more question is if paraxanthine could be used as a substitute for classical psychostimulants, such as cocaine or amphetamine. Further preclinical studies with cGMP-prefering PDE inhibitors alone and in combination with A1R antagonists could also provide a new therapeutic approach for drug addiction.

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References


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Sex differences in reinforcing value of caffeinated beverages in adolescents
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Caffeine use is increasing among children and adolescents, but the effects of caffeine use on behavior and physiology within this population remain understudied and poorly understood. The purpose of this study was to test the hypothesis that adolescents find caffeinated soda more reinforcing than noncaffeinated soda and that this would be related to the level of usual caffeine consumption and to sex. We measured operant responding for portions of caffeinated and noncaffeinated soda at baseline and after daily consumption of 32 oz of caffeinated and noncaffeinated soda for 1 week each in 12–17-year-old participants. Participants also completed a behavioral checklist, a beverage-liking questionnaire, and a 24-h dietary recall to assess the energy intake at baseline and again after each week of beverage consumption. There was no difference in reinforcing value of noncaffeinated or caffeinated soda as a function of usual caffeine consumption. However, males found the caffeinated soda significantly more reinforcing than did females after the exposure period. To our knowledge, this is the first study to show a sex difference in the reinforcing value of caffeinated soda. These data suggest that boys may be more susceptible to the reinforcing effects of caffeine.

Introduction
Caffeine is the most commonly used psychoactive substance throughout the world (Nehlig, 1999). It is classified as a stimulant drug that is typically used for its ability to arouse the central nervous system. Although moderate caffeine use is 'generally recognized as safe' by the US Food and Drug Administration and the American Medical Association, this classification is largely based on studies conducted in adults. The safety of caffeine use among children is understudied and poorly understood. Since 1977, there has been a 70% increase in caffeine consumption among children and adolescents (Harnack et al., 1999). The average daily caffeine intake in children of age 5–18 years ranges from 38 to 69.5 mg (Morgan et al., 1982; Frary et al., 2005). When corrected for body weight, this equates to approximately 1.0 mg/kg which is about 75% of what adults typically consume (Frary et al., 2005). Caffeinated beverage consumption in general, and soda consumption in particular, is of concern because of its potentially negative health effects and its established relationship with sleep dysfunction, obesity, and dental caries. For example, children of age 2–18 years who consume 9 oz or more of soda per day drink less milk and fruit juice and ingest about 200 more calories per day when compared with infrequent soda drinkers (Harnack et al., 1999). Studies have shown that soda is the preferred route of caffeine administration among adolescents, however coffee-type drinks and ‘energy drinks’, which contain significantly more caffeine per serving than soda are also becoming more popular within this population (Harnack et al., 1999; Smiciklas-Wright et al., 2003; Frary et al., 2005).

Studies in adults and children show that, in a subset of caffeine consumers, caffeine is both reliably self administered and is reinforcing. In humans, doses as low as 25 mg are self administered in caffeine consumers, but not in nonconsumers when tested using laboratory self-administration paradigms (Griffiths and Mumford, 1995; Richardson et al., 1996; Hughes and Oliveto, 1997). When given repeated exposure to coffee or capsules containing placebo and caffeine (identified by letters or colors), 47–85% of adult caffeine users reliably self administer caffeine after a period of repeated exposure to both placebo and caffeine (Evans et al., 1994; Hughes et al., 1995). In a study using a self-administration paradigm designed to mimic realistic consumption, adolescents were given bottles of noncaffeinated soda with caffeine added back on one occasion, but not the other. The bottles were labeled A one day and B the next. Then the participants were given both A and B bottles and told to drink the one they preferred. The next week, the same procedures were followed except that the bottles were labeled with C and D. After the end of 4 weeks, the data revealed that 22% of the children studied preferentially self-administered the caffeine-containing beverage (Hale et al., 1995). Although the terms self administration and

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reinforcement are often used interchangeably, to quantify reinforcing value, the amount of work performed to get access to a substance must be measured. Very few studies have examined the reinforcing value of caffeine in adults and none have done so in children. One study that did look at the level of reinforcement found that when high level, habitual caffeine consumers (>1000 mg/day) were required to ride a stationary bike to gain access to caffeinated coffee, participants worked for 10 cups of coffee per day when the amount of cycling per cup was only a few minutes, but when the amount of cycling was increased to more than 30 min per cup, participants were only willing to work for an average of two cups per day (Griffiths et al., 1989). Although the amount of caffeine consumed decreased as the work required to gain access to it increased, participants were still willing to perform a significant amount of work (cycling for >60 min) to gain access to a minimum amount of coffee.

One factor that can influence the reinforcing properties of a substance is sex. There are established sex differences in both animal and human models in drug self-administration (Lynch, 2008) and drug sensitivity (Perkins et al., 2008). These differences have been attributed, at least in part, to differences in gonadal hormones (Dreher et al., 2007; Lynch, 2008). In addition, an imaging study in humans showed that neural responses to reward-related stimuli change as a function of menstrual cycle phase, with heightened activation of the neural reward system during the mid-follicular phase, which is characterized by moderate levels of estradiol and low levels of progesterone (Dreher et al., 2007). There are few data available on sex differences in caffeine use, sensitivity, or reward response. One study showed that regular caffeine consumption is protective of cognitive decline in women, but not in men (Ritchie et al., 2007). However, the majority of studies have not found any sex differences in the effects of caffeine (Farag et al., 2006; Lopez-Garcia et al., 2006; Turley and Gerst, 2006). It is important to note that few studies have taken menstrual cycle phase into account when comparing men and women, which may increase variability and reduce the likelihood of detecting sex differences. In addition, the majority of these studies were conducted in adults. It is possible that sex differences are more pronounced during the initiation and establishment of caffeine consumption and diminish after prolonged, habitual usage.

A previous study by Hale et al. (1995) showed that 22% of caffeine consumers reliably self-administered caffeine after repeated exposure to caffeinated and noncaffeinated version of the same soda. The purpose of this study was to extend those findings to a laboratory-based paradigm aimed at quantifying the reinforcing value of caffeine among adolescents. We hypothesized that caffeine added to soda would increase its reinforcing value in adolescents. Two secondary hypotheses were that the level of reinforcement would vary as a function of typical caffeine consumption and sex, with high caffeine consumers and males finding caffeinated beverages more reinforcing than low caffeine consumers and females. We tested these hypotheses in a double blind, placebo-controlled study using laboratory-based methods for measuring reinforcement. This study contributes to the small but growing literature on the effects of caffeine in children and adolescents.

Methods
Participants
Adolescents, aged 12–17 years were recruited through a direct mailing targeted at households with at least one child who was 12–17 years of age. We chose to study 12–17-year-olds because this was similar to the age used by Hale et al. (1995) and also because children at this age are gaining autonomy in food selection and may be more likely to experiment with licit substances such as caffeine when compared with younger or older children. In addition, we posted flyers around the community, the University at Buffalo campus, and at some local middle and high schools. Individuals were considered eligible to participate if they met the following criteria: body mass index (BMI) below the 85th percentile, had previous experience with caffeine and had no adverse reactions, did not smoke cigarettes, were not using hormone-based contraceptives, and were willing to replace their typical soda and juice consumption with a beverage provided by us for 2 weeks. Usual caffeine consumption was estimated based on parental and child report of daily or weekly intake of caffeine from all major sources, including tea (40 mg/5 oz), soda (40 mg/12 oz), coffee (100 mg/5 oz), energy drinks (approximately 150 mg/12 oz), chocolate (10 mg/oz), and caffeine-containing pills (excedrin or No-Doz – 130 to 200 mg/pill). These estimates of caffeine content were based on information published by the US Department of Nutritional Services. We balanced the number of children that were recruited across each of the following caffeine consumption groups: less than 25, 25–50, 51–75, greater than 75 mg/day.

Experimental procedures
Eligible participants and their parents were asked to visit the laboratory on three occasions, each separated by 1 week, between 12.00–17.00 h. Participants were instructed not to eat or drink anything containing caffeine for 24 h before the first and third visits. They were also told not to eat or drink anything other than water for 2 h before their scheduled appointment and not to participate in vigorous physical activity sustained for more than 10 min on the day of the visit. Vigorous physical activity was defined as activity where breathing and heart rate are elevated, but talking is still possible. Participation in school-sponsored physical education did not count as vigorous physical activity.
During the first session, a parent accompanied their child to the laboratory. Parents and participants read informed consent and assent forms while the experimenter was present. They had the opportunity to ask questions and, if they decided they wanted to complete the study, they signed the forms. The child completed the behavioral checklist (Hughes et al., 1991) while the parents then completed a demographic questionnaire. Participants then sampled five different flavors of soda (green apple, black cherry, grape, cream soda, and citrus flavor) and rated each one for novelty and liking on 100 mm Visual Analog Scales. All participants rated at least one of the beverages greater than 70 on the novelty scale. The participant, with the help of a parent if necessary, was then asked to recall the foods they consumed the previous day, as well as typical caffeine use and dietary intake over the past week using a 78-item Food Frequency Questionnaire (Block). The parent was then escorted to the waiting area.

The participant then completed the triangle test (described below). After the triangle test, the participant was given another set of two, 3-oz portions labeled A (placebo) and B (caffeine, 12.4 mg) and asked to rate each one for liking (see below). This was done to give them experience with each of the beverages for which they would be working in the computer task and to establish a baseline liking. After this, participants consumed 8 oz of cold water, to control for potential differences in thirst. Participants were then instructed on the use of the computer-based reinforcement task (described below). The participant first played a practice version of the game to become familiar with the procedures and have the opportunity to ask questions. The computer on which they practiced was randomized among participants. They were then moved to a different table that was equidistant from the two computer tables and completed the thirst scale (see below). The participant was then instructed to complete the computer task for portions of soda A on one computer and B on the other. They were told that they could begin at whichever computer they chose and that the session would end when they no longer wished to earn points for soda; there was no minimum amount of points required to be earned. When participants finished playing the game, they were instructed to complete the Thirst Questionnaire again.

At the end of the testing session, the participant was given four, 64-oz bottles of the soda that was selected for them based on their taste-test ratings (the beverage rated closest to 100 mm for liking that was also greater than 70 mm on the novelty scale). These bottles had the labels removed and were relabeled with an A or a B. They were either caffeinated (33 mg/8 oz) or noncaffeinated. This concentration of caffeine was chosen because it is similar to that found in soda that is commonly consumed among adolescents. In addition, we decided to standardize the amount consumed by each participant, despite the fact that this amount was more than was typical for more than half of the participants, because we wanted to assure that each participant had the same exposure to each type of soda. For example, other studies mimicking 'real world' consumption allowed participants to consume what they normally would have in a day/week, but this means that low-consumption children may have only had one or two glasses a week, which would probably not be enough exposure for them to determine that there were differences between the A and B beverage. The purpose of the exposure period was to familiarize each participant with the different beverages and allow them adequate exposure to find one more or less reinforcing than the other. The participant was not explicitly made aware of the fact that caffeine was being manipulated. Instead, caffeine was listed as one of several things that could have been manipulated between the two sodas, including artificial sweetener, carbonation, and artificial coloring. This deception was an alteration in the usual Institutional Review Board procedure, but was acceptable because it involved no greater than minimal risk, and it did not adversely affect the welfare of the participants because they were told at the beginning that they would be debriefed as to the exact nature of the experiment at the end. This level of deception was necessary to prevent potential preconceptions of caffeine from altering experimental results. Participants were instructed that the beverage provided should be consumed in place of any other soda, coffee, energy drink, or tea. They were told to consume 32 oz per day and that the beverage was to be consumed in 8 oz portions. They were provided with an 8 oz measuring cup and were told to pour the 8 oz into a glass and drink it within 15 min. If they chose to use ice, they were instructed that they must use ice each time they drank the beverage. This helped to maintain consistency, increase reporting accuracy, and reduce waste. They were also instructed to record times and amounts of beverages and food consumed in a provided habit book.

One week later, all empty bottles were returned to the laboratory. During this visit, the participant completed the behavioral checklist, a previous day dietary recall, and a beverage-likeing questionnaire. Participants were given a new habit book and a second set of four, 64-oz bottles with the opposite caffeine condition (if they received A during week 1, they would get B during week 2) and the procedures were repeated.

The following week, 8 days after the second laboratory visit, participants returned to the laboratory for a third visit. Before this visit, participants were instructed not to eat or drink anything containing caffeine and to terminate consumption of the test beverages for 24 h prior to the visit. They were also told not to eat or drink anything other than water for 2 h before their scheduled
appointment and not to participate in vigorous physical activity on the day of the visit. A saliva sample was collected for caffeine measurement. Data from any participant who had caffeine levels indicative of recent usage were excluded. The participant then completed the same behavioral checklist and liking questionnaires from the previous week as well as a 24-h dietary recall. Participants were asked to consume an 8-oz glass of cold water and to complete the Thirst Questionnaire. Then the reinforcing value of the two beverages was determined using the reinforcement task described below. At the end of the session, participants completed another Thirst Questionnaire, had their height and weight measured, and were debriefed and compensated for their time.

The debriefing was conducted in an interview-style using the following open-ended questions. (i) What do you think the purpose of this experiment was? (ii) Did you think that the drinks you drank at home had caffeine in them? If Yes, which one(s). (iii) Did you think any of the drinks you drank at home had sugar in them? If Yes, which one(s). After the participant answered the questions, they rejoined their parent and both parent and child were told the purpose of the study. They were each provided with a written form detailing the purpose of the study along with their payment. All procedures were conducted in accordance with National Institutes of Health guidelines of the ethical conduct of research on human participants and with the approval of the University at Buffalo Social and Behavioral Sciences Institutional Review Board.

Beverage preparation
To prepare the beverages, caffeine was suspended in flattened Sprite using a heated stir plate at a concentration of 16.5 mg/ml. Sprite was chosen so that the sweetness would help to mask the bitterness of the caffeine. When 2-l bottles were prepared, 16 ml of soda were removed from each and 16 ml of either the A solution (flat Sprite only) or the B solution (flat Sprite plus caffeine) were added back into each bottle. All solutions were prepared by a research assistant who was not involved in the data collection for this study so that the experimenters could remain blind to the caffeine content of the beverages.

Measures
Demographic Questionnaire
The parent completed a questionnaire that provided information about: who currently lived in the household, the marital status of the primary caregiver of the child, which households the child spent time in and how much time was spent in them, the highest level of education completed by the primary caregiver and spouse, the occupation of the primary care giver and spouse, the employment status of the primary care giver and spouse, where the total household income was derived from, the total household income, and the parent and child’s ethnicity and race.

Behavior checklist
This questionnaire consisted of a list of 31 physical or psychological adjectives that the child rated using a Likert scale from 1 to 9 with 1 being ‘Not at all’ and 9 being ‘Extremely’ (Hughes et al., 1991). The items on the list were made up of the following words: anxious, alert, content, depressed, dizzy, drowsy, fatigued, frequent urination, headache, insomnia, irregular heartbeat, diarrhea, impatient, hungry, irritable, motivated to work, well-being, mood swings, muscle twitches, talkative, nausea, palpitation, restlessness, ringing in ears, energetic, stomach ache, vigorous, perspiration, tremor, sleepy, and tired. Participants were asked to indicate the extent to which each word described how they felt during the past week.

Food Frequency Questionnaire
To determine the typical pattern of food consumption, participants completed a 78-item Food Frequency Questionnaire (block), which included commonly consumed American foods and beverages as well as vitamins that the child may consume. The child worked on this during their first visit to the laboratory with the help of their parent if needed.

Taste tests
For this study, we used sodas that were somewhat novel to the children and not frequently consumed. To pick an appropriate soda, participants sampled five different flavors of soda (green apple, black cherry, grape, cream soda, and citrus) and rated each one for novelty and liking on a 100 mm Visual Analog Scales. Beverages were deemed sufficiently novel if they were greater than 70 mm on a 100 mm Visual Analog Scale for novelty anchored with ‘Extremely’ (100 mm) and ‘Not at all’ (0 mm). Participants were asked to rate each drink for liking using a 100 mm Visual Analog Scale anchored with ‘Like a lot’ (100 mm) and ‘Do not like at all’ (0 mm). The beverage that had the highest rating for liking that was also greater than 70 mm for novelty was used for this study.

Soda liking
To assess liking for the sodas, participants were given a Likert scale ranging from 1 to 7 with 1 being ‘Do not like at all’ and 7 being ‘Like very much’ to rate their liking of soda A (placebo) and B (caffeine). Participants were given liking questionnaires for both soda A and B at baseline after tasting a small sample of each as well as individual liking questionnaires for the soda that was consumed the previous week at visits 2 and 3.

Triangle test
Because differences in taste between the caffeinated and noncaffeinated beverage could influence liking,
reinforcement, and expectation of caffeine, we needed to determine whether or not participants could distinguish between the two beverages on the basis of taste. Therefore, a triangle test was performed. Participants were given three 3-oz portions of the selected beverage labeled A, B, or C. They were told that two of the beverages were same and one was different. They were asked to indicate which one tasted different from the other two on a paper provided and, if they could not taste the difference, they were told to guess. This was repeated three times with the different drink containing caffeine on two of the trials and placebo on the other two trials.

Thirst
Before and after the reinforcement task, participants were given a 100 mm Visual Analog Scale anchored by ‘Not thirsty at all’ (0 mm) and ‘Extremely thirsty’ (100 mm) to rate subjective thirst.

Habit books
A book was given to the participants each week to record their meals, snacks, and physical activity. Participants were instructed to record, in as much detail as possible, their daily food and beverage consumption including the time and amount consumed.

Salivary caffeine analysis
Six milliliter of saliva was taken from the participant at the beginning of the third laboratory visit by having the child spit into a 12 ml vial using a funnel while chewing on a piece of wax to enhance the production of saliva. Saliva samples were coded and stored at –20°C until the study was completed. All samples were then shipped on dry ice to LabStat (Kitchener, Ontario) for caffeine analysis. Briefly, duplicate samples underwent a multi-step partitioning involving repeated liquid-liquid extractions and cryogenic freezing. The extracted sample was then analyzed by gas chromatography and detected by a thermionic specific detector. The analytes were compared with known standards to determine the relative levels of caffeine. Data from any participant with salivary caffeine indicative of recent usage (> 850 ng/ml) were excluded from the study.

Beverage reinforcement task
Participants were instructed on the use of the computer task where they performed instrumental responses for soda A (placebo) on one computer and soda B (caffeine) on the other. On both the computer screens, there were three shapes that were different in colors and orientations. The task was similar to a slot machine where every time the mouse button was clicked, the shapes rotated and changed color. When all the shapes matched, the participant received one point. For every 5 points, they received a 3-oz portion of the soda for which they were playing. After each portion of soda was earned, it became more difficult to earn the next portion. The schedule of reinforcement was a progressive, variable (± 5%) ratio schedule of: VR4, VR8, VR16, VR32, VR64, VR128, VR256, VR512, VR1024, and VR2048. The schedules on each computer were independent, so the participant could work on one computer and complete the VR32 schedule and then switch computers and begin at VR4 for the other soda. Participants were told that they could begin at whichever computer they want and go back and forth between the computers as often as they wanted without asking. Each time 5 points were earned, the experimenter brought the 3-oz portion of soda in and placed it on a table that was separate and equidistant from the ones where the computers were. The participant was told they could stop playing and consume the soda whenever they wished without asking. We only asked that they did not drink soda and play the game at the same time. To reduce the likelihood that participants were playing the game out of boredom, they were told that they could stop at any time and that the session would end when they no longer wished to earn points for either soda A or B.

Analytic plan
Participant characteristics, including age, BMI, and self-reported caffeine consumption and measured variables, including scores on the triangle test and food intake data from the Block Food Frequency Questionnaire, were compared using a one-way analysis of variance with sex and caffeine consumption group (high vs. low) as between-subjects factors. Caffeine consumption group was determined using a median split of the data with participants consuming less than 50 mg/day considered as ‘low’ users and those consuming greater than or equal to 50 mg/day considered as ‘high’ users. Other characteristics, such as race and parental education and income, were compared across the sex and caffeine consumption group using χ² analyses.

The pattern of operant responding for portions of soda and responses on the behavioral checklist were compared using mixed effects regression models (MRM) with sex, usual caffeine consumption (continuous variable) and soda type (placebo or caffeine) as time-invariant predictors, and phase (baseline or posttest) and schedule of reinforcement (operative responding only) as time-variant predictors. In addition, order of placebo or caffeine presentation (for weekly beverage consumption), kilocalories consumed on the day of the experiment, preexperimental thirst, and score on the triangle test were included as covariates in the analysis. Ratings of thirst were compared using the same MRM, but pre/post was added as an additional time variant predictor for these analyses. Post-hoc analyses were conducted using linear contrasts.

Energy intake, beverage liking, and caffeine consumption were compared using a mixed analysis of variance with
sex and caffeine consumption group (high vs. low) as between-subjects variables and phase (baseline, postplacebo, or postcaffeine) as the within-subjects factor. SYSTAT Version 11.0 (Chicago, Illinois, USA) was used for all data analyses and the significance level was set at P value less than 0.05.

Results

Participant characteristics

There were no differences in BMI, age, race, or parental education as a function of sex or caffeine use group, or were there any interactions between sex and caffeine use for any of the above variables (Table 1). There was a significant relationship between sex and parental income with females having a higher number of parents with an annual income of less than $49,999 (P < 0.05). There was a significant difference in self-reported caffeine use as a function of caffeine use group [F(1,45) = 53.88; P < 0.001], but this was by design.

Reinforcing value of caffeinated soda and within-session caffeine consumption

The MRM analysis showed a significant interaction of sex, schedule of reinforcement, and caffeine content on the pattern of operant responding (P = 0.04). Post-hoc tests revealed that there was no interaction of sex, caffeine content, and schedule of reinforcement on responding at baseline (P = 0.08; Fig. 1a and c), but there was a significant sex x caffeine content x schedule of reinforcement interaction after posttesting (P < 0.01; Fig. 1b and d). In addition, there were no significant sex differences in responding for placebo (P > 0.1; Fig. 1b), but males responded more for caffeine than did females at posttest (P < 0.05; Fig. 1d). There were no significant main effects or interactions with caffeine consumption group for reinforcing value.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Descriptive characteristics of the participants shown by sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants</td>
<td>Male</td>
</tr>
<tr>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Age (years)</td>
<td>14.3</td>
</tr>
<tr>
<td>Average daily caffeine intake</td>
<td>71.8</td>
</tr>
<tr>
<td>BMI (kg/cm^2)</td>
<td>20.7</td>
</tr>
<tr>
<td>Race</td>
<td>N</td>
</tr>
<tr>
<td>Caucasian</td>
<td>21</td>
</tr>
<tr>
<td>African-American</td>
<td>4</td>
</tr>
<tr>
<td>Hispanic</td>
<td>1</td>
</tr>
<tr>
<td>Other/mixed race</td>
<td>0</td>
</tr>
<tr>
<td>Parental income</td>
<td></td>
</tr>
<tr>
<td>&lt; Under $49,999</td>
<td>3</td>
</tr>
<tr>
<td>$50,000-$99,999</td>
<td>19</td>
</tr>
<tr>
<td>≥ $100,000</td>
<td>4</td>
</tr>
<tr>
<td>Parental education</td>
<td></td>
</tr>
<tr>
<td>High school</td>
<td>4</td>
</tr>
<tr>
<td>Completed college</td>
<td>14</td>
</tr>
<tr>
<td>Graduate school</td>
<td>8</td>
</tr>
</tbody>
</table>

Bold values, significantly different from males (P < 0.01).

BMI, body mass index; SD, standard deviation.

There was a significant main effect of sex [F(1,44) = 4.1; P < 0.05] and a significant three-way interaction among sex, caffeine use group, and experimental phase [F(1,44) = 4.98; P < 0.05] on within-session caffeine consumption. Males consumed more caffeine than females (22.3 ± 5.4 vs. 12.8 ± 4.2). Low-consuming males and high-consuming females reduced consumption of caffeine from baseline to posttesting, but high-consuming males and low-consuming females showed no change (Fig. 2).

Energy intake, beverage liking, and thirst

Self-reported energy intake was higher in boys than in girls [F(1,43) = 10.19; P < 0.005; Table 2], but there were no significant differences as a function of caffeine consumption group [F(1,45) = 0.23; P = NS] and no significant interactions between sex and caffeine consumption group [F(1,45) = 0.23; P = NS]. Self-reported beverage liking decreased after each phase relative to baseline ratings [F(1,180) = 11.15; P < 0.005]. There were no significant differences in beverage liking by sex, group, or caffeine content, nor were there significant interactions among these factors (all P > 0.10). For ratings of thirst, there was a significant main effect of group (P < 0.05) and prepost (P < 0.001) and a significant interaction of group and phase (P < 0.02), with all consumers showing a decrease in thirst from presession to postsession, high consumers reporting more thirst than the low consumers and with low consumers showing an increase in thirst from baseline to posttesting phase, but high consumers showing no change in thirst as a function of phase.

Effects of chronic and acute caffeine use and sex on behavioral checklist data

Of the 31 items on the behavioral checklist, only 10 items had any significant differences. Of these 10, four are classified as psychological measures and six are classified as physiological measures (Table 3). For the psychological measures, there was a significant interaction of caffeine use group and phase (P < 0.02) on ratings of contentment, with low-caffeine users rating contentment higher after the week of placebo (5.2 ± 0.3) than after the week of caffeine (4.4 ± 0.6) and high-caffeine users showing the opposite pattern with lower ratings after the week of placebo (4.5 ± 0.6) than after the week of caffeine (5.0 ± 0.5). There was a significant main effect of sex on ratings of irritability (P < 0.05), with females reporting higher irritability than males (3.0 ± 0.6 vs. 2.5 ± 0.5). There was a significant main effect of sex on ratings of motivation to work (P < 0.05) with females rating their motivation to work as higher than males (4.2 ± 0.6 vs. 3.4 ± 0.6). There was also a significant main effect of sex for ratings of talkativeness (P < 0.001) with females reporting being more talkative than males (5.8 ± 0.6 vs. 4.6 ± 0.6). There were also several significant findings for physiological measures. There was a significant main effect of phase on hunger (P < 0.02) with higher reports of hunger...
during the week of placebo (4.65 ± 0.5) than the week of caffeine (4.09 ± 0.4). There was a significant main effect of caffeine use group on ratings of headache (P < 0.05), stomachache (P < 0.01), dizziness (P < 0.05), muscle twitch (P < 0.05), and tremor (P < 0.05) with all measures reported to be greater by high-caffeine users compared with low-caffeine users. There were also interactions of sex and phase (P < 0.001) and caffeine use group (P < 0.01) on ratings of stomach ache. These interactions were driven by the female low users reporting increased stomach ache after caffeine compared with placebo, but no effects of caffeine in other groups.

**Food Frequency Questionnaire data**

There were no significant differences for any of the measures from the Food Frequency Questionnaire.

**Discussion**

In this manuscript, we report the results of a double blind, placebo-controlled study to assess the reinforcing value of caffeinated and noncaffeinated beverages. Contrary to our initial hypothesis, we did not find any differences in the reinforcing value of caffeinated beverages as a function of usual caffeine consumption. Instead, we found a sex difference in caffeine reinforcement, with boys finding caffeine more reinforcing than girls after the exposure period, with no sex differences at baseline or for the noncaffeinated beverage (placebo). We found some differences in results from the behavioral checklist as a function of typical caffeine use, with high-caffeine users reporting more headache, stomachache, dizziness, muscle twitching, and tremor than low-caffeine users. Finally, we found no sex or caffeine-use differences

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**Fig. 1**

Mean ± SEM number of responses for soda containing placebo (a and b) or caffeine (c and d) at baseline (a and c) and after the exposure period (b and d) in boys (black circles) and girls (white circles) across different schedules of reinforcement. The mixed effects regression revealed a significant sex × phase × caffeine content × schedules of reinforcement interaction where boys responded more for caffeine than did girls after the exposure period (P<0.05).
in any of the measures from the Food Frequency Questionnaire. When taken together, these data suggest that, after exposure to a caffeinated and noncaffeinated version of the same soda, males find the caffeinated version more reinforcing than females. Future studies will focus on determining the mechanism for this sex difference.

There are several potential explanations for the sex difference in the reinforcing value of caffeinated beverages. First, the sex differences could be driven by differences in circulating steroid hormones. In animal studies, the acquisition of cocaine self administration occurs earlier in females than in males (Hu et al., 2004). In addition, ovariectomized rats self administer cocaine less than gonad-intact females, but when estradiol is given, self administration is increased above gonad-intact levels (Jackson et al., 2006). Studies in humans have shown that subjective responses to drugs are enhanced in women compared with men (Sofuoglu et al., 1999) and that these differences are related to circulating sex steroids, as women show enhanced subjective effects during the follicular phase of the menstrual cycle as compared with the luteal phase (Justice and deWit, 1999; Evans et al., 2002). These studies suggest that there are sex differences in drug effects and in motivation to obtain drugs that are mediated by steroid hormones. In our study, we found that females were less motivated to obtain caffeinated beverages than males. It is possible that females are more sensitive to the effects of caffeine and that the dose that we administered was causing negative subjective effects in females. If this was the case, we would predict that females would have reported more negative effects on our behavioral checklist, but there were no sex differences in mood ratings in response to caffeine. In addition, although we did not measure steroid hormones or assess menstrual cycle phase in our participants, we would expect that if the effects were varying across the menstrual cycle, we would have observed more variability in females than in males, which we did not.

Another potential reason for the sex difference in this study may be that, for girls, sugar-sweetened soda was not as reinforcing as a noncaloric or ‘diet’ soda would have been. We had several female participants expressing concern about consuming so much nondiet soda during the study, and after 2 weeks of consumption, they may not have wanted to work any more. One argument against this is that there was no sex difference in baseline beverage reinforcement and, for the posttest, there was no sex difference in the reinforcing value of noncaffeinated beverages. In fact, there was a trend for females to work harder for the placebo than the caffeinated beverage. This suggests that females were still working for the nondiet soda, just not when it contained caffeine. Future studies will address this issue by determining the differences in reinforcing value between caffeinated and noncaffeinated diet and regular sodas.

In this study, we examined both the reinforcing value of soda as well as soda liking. We showed that, while males found caffeinated soda more reinforcing than females after the exposure period, there was no sex difference in self-reported liking of the different sodas. Studies that

Table 2  Data collected from the participants during the final laboratory visit divided by sex and caffeine consumption group

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low (&lt; 50 mg/day)</td>
<td>High (≥ 50 mg/day)</td>
</tr>
<tr>
<td>Caffeine use (mg)a</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>Liking placebo</td>
<td>29.9 ± 3.5</td>
<td>120.3 ± 18.1</td>
</tr>
<tr>
<td>Liking caffeine</td>
<td>4.9 ± 0.3</td>
<td>4.9 ± 0.3</td>
</tr>
<tr>
<td>Presession thirst</td>
<td>5.1 ± 0.4</td>
<td>4.7 ± 0.4</td>
</tr>
<tr>
<td>Postsession thirst</td>
<td>39.3 ± 6.1</td>
<td>35.7 ± 6.7</td>
</tr>
<tr>
<td>Energy intakeb</td>
<td>19.1 ± 2.8</td>
<td>20.8 ± 5.8</td>
</tr>
<tr>
<td></td>
<td>2326 ± 161</td>
<td>2477 ± 193</td>
</tr>
</tbody>
</table>

SE, standard error.
aThere was a significant effect of group on caffeine use.
bPre-post on thirst.
Sex on energy intake.
have concurrently examined motivation for a stimulus (‘wanting’) and hedonic ratings of a stimulus (‘liking’) have consistently shown that for a number of stimuli, including food (Epstein et al., 2004; Finlayson et al., 2007a, b, 2008; Temple et al., 2009) and drugs (Strakowski and Sax, 1998; Strakowski et al., 2001; Robinson and Berridge, 2008) that liking can be dissociated from wanting. For example, a recent study by Finlayson et al. (2007a) showed that subjective ‘liking’ and ‘wanting’ for a savory food were similar when hungry, but diverged after consumption of a savory meal, with liking for savory foods increasing and ‘wanting’ of savory foods decreasing. This study suggests that ‘wanting’ and ‘liking’ are dissociable and are sensitive to different factors. Our study has shown a dissociation of ‘wanting’ and ‘liking’ in two ways. First, we have shown a sex difference in the reinforcing value or ‘wanting’ for caffeinated beverages, but no sex differences in self-reported ‘liking’ of these beverages. In addition, we have shown that males find caffeinated beverages more reinforcing than noncaffeinated, but males do not report a greater liking of caffeinated beverages. These results are consistent with several human and animal studies suggesting that ‘liking’ and ‘wanting’ are mediated by different neurobiological pathways (Strakowski and Sax, 1998; Strakowski et al., 2001; Finlayson et al., 2007b, 2008; Robinson and Berridge, 2008) and are, thus, modified by different stimuli and experiences.

Our initial hypothesis was that regular caffeine consumers would find the caffeinated beverage more reinforcing than the noncaffeinated beverage, regardless of sex. However, there was no difference in reinforcing value as a function of regular caffeine use. One potential explanation for this may be that the low-caffeine consumers were experiencing some positive effects of caffeine and that the high consumers were experiencing withdrawal. In both the cases, the participants would have been motivated to work for the caffeinated beverage, regardless of the fact that their reasons for doing so were different. Unfortunately, we did not ask participants to describe why they worked for one beverage over another. In addition, although we are hypothesizing that there were differences in positive versus negative effects, we did not see any differences in the effects of caffeine as a function of usual caffeine use on the behavioral checklist. This would suggest that there were no differences in the psychological and physiological responses to the caffeine as a function of caffeine use. Another potential reason is that we are studying a subpopulation of adolescents who regularly use caffeine, but are probably not reflective of the highest adolescent caffeine users. In this study, we balanced the groups by sex and our highest consumption group was greater than 75 mg/day. It is possible that our recruitment efforts failed to reach the highest caffeine consumers, who may consume more than 200 mg/day (Bernstein et al., 2002). Perhaps adolescents who consume very large amounts of caffeine (>400 mg/day) would have found the caffeinated beverage more reinforcing. If we could reach this population, however, we may discover a similar sex difference, as recent studies have shown that males are more likely than females to consume energy drinks (Miller, 2008) and engage in risk-taking behavior. This suggests that males may be more likely than females to be in the highest caffeine-consumption group.

There are several strengths to this study. First, we used a double blind, placebo-controlled design. Second, this was a within-subjects study, which strengthens our statistical power. Third, we verified caffeine abstinence using saliva samples collected before the final testing session.
However, this study also had limitations. First, we relied on self-report for caffeine use instead of an objective measure, such as repeated saliva sampling over several days. We explored this idea, but found it to be cost prohibitive and not without its own set of problems. Similarly, we could only verify study beverage consumption based on bottle returns. We had no way of knowing whether the soda was actually consumed, only that it was missing from the returned bottles. Another limitation was that we used a single ‘concentration’ of soda in all participants instead of conducting a dose–response study. For our purposes, a dose–response study would have been prohibitive in terms of time and recruitment. However, ongoing studies in our laboratories are using dose–response designs to determine whether there are acute effects of caffeine that are dose dependent. Finally, another limitation of this study is that we have no menstrual cycle or steroid hormone data of the participants. In light of our findings, it would be extremely useful to know what phase of the menstrual cycle the female participants were in during the study or, better yet, have a measurement of their steroid hormone levels. Initially, we did not expect that we would observe such a strong sex difference, as this did not occur in our pilot study. Future studies will collect data on the hormonal status of the participants.

Conclusion
This study showed that, after 2 weeks of exposure to caffeinated and noncaffeinated versions of the same soda, a significant sex difference emerged, where males found the caffeinated soda more reinforcing than females. There was no sex difference at baseline and no sex difference in responses for the noncaffeinated beverage. These results support previous findings that show that males and females respond differently to psychoactive substances. We did not, however, find a difference in reinforcing value of caffeinated and noncaffeinated beverages based on self-reported usual caffeine consumption. This was in contrast to previous studies showing that caffeinated beverages are more reinforcing and preferentially self-administered in high-caffeine consumers. Finally, high-caffeine users reported having more physiological symptoms (headaches, stomachache, dizziness, muscle twitches, and tremor) as compared with low-caffeine users, but none of the physiological symptoms varied by sex. By contrast, three of the psychological measurements (irritable, motivated to work, and talkative) were greater in females than in males. Finally, there was an interaction between sex and experimental phase on report of stomachache. Altogether, these data suggest that boys and girls may have different subjective responses to caffeine and that, for the most part, these effects are independent of usual caffeine consumption. These data are novel and add to the small, but growing, body of literature on caffeine use in children and adolescents.

Acknowledgements
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References


Effects of Acute Caffeine Administration on Adolescents

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University at Buffalo

Acute caffeine administration has physiological, behavioral, and subjective effects. Despite its widespread use, few studies have described the impact of caffeine consumption in children and adolescents. The purpose of this study was to investigate the effects of acute caffeine administration in adolescents. We measured cardiovascular responses and snack food intake after acute administration of 0 mg, 50 mg, 100 mg, and 200 mg of caffeine. We also compared usual food intake and subjective effects of caffeine between high- and low-caffeine consumers. Finally, we conducted a detailed analysis of caffeine sources and consumption levels. We found main effects of caffeine dose on heart rate (HR) and diastolic blood pressure (DBP), with HR decreasing and DBP increasing with increasing caffeine dose. There were significant interactions among gender, caffeine use, and time on DBP. High caffeine consumers (>50 mg/day) reported using caffeine to stay awake and drinking coffee, tea, soda, and energy drinks more than low consumers (<50 mg/day). Boys were more likely than girls to report using getting a rush, more energy, or improved athletic performance from caffeine. Finally, when we examined energy and macronutrient intake, we found that caffeine consumption was positively associated with laboratory energy intake, specifically from high-sugar, low-fat foods and also positively associated with protein and fat consumption outside of the laboratory. When taken together, these data suggest that acute caffeine administration has a broad range of effects in adolescents and that the magnitude of these effects is moderated by gender and chronic caffeine consumption.

Keywords: caffeine, drugs, adolescents, eating, gender differences

Caffeine is the most widely used stimulant in the world (Nehlig, 1999). While generally recognized as safe, most of the evidence supporting caffeine safety comes from studies conducted in adults. Given that children ages 12 to 17 are among the fastest-growing segment of the population for caffeine use (Frary, Johnson, & Wang, 2005; Harnack, Stang, & Story, 1997), it is imperative that more empirical data be collected in children and adolescents to understand how acute and chronic caffeine use affect the physiology and behavior of this population. In addition, given the recent attention being paid to caffeine-containing beverages, such as soda and energy drinks, and their relationship to obesity and sleep loss in children, it is important to understand the mechanisms that underlie these associations.

Acute caffeine has dose-dependent effects on subjective response, attention, and physiology in adults and children. For example, moderate doses of caffeine (200–350 mg) decrease heart rate and increase blood pressure in adults (Bender, Donnerstein, Samson, Zhu, & Goldberg, 1997; Lane & Williams, 1987; Sung et al., 1994; Waring, Goudsmit, Marwick, Webb, & Maxwell, 2003). In addition, these same doses of caffeine produce enhanced feelings of well-being, improve concentration, and increase arousal and energy (Garrett & Griffiths, 1997; Griffiths et al., 1990). High doses (>400 mg), however, lead to feelings of anxiety, nausea, jitteriness, and nervousness (Garrett & Griffiths, 1997). Studies in children and adolescents have shown that acute caffeine administration has similar cardiovascular and subjective effects to those described in adults. Doses of caffeine ranging from 100 to 400 mg led to increased reports of nervousness, jitteriness, fidgetiness, and decreased reports of sluggishness in children and adolescents (Bernstein et al., 1994; Elkins et al., 1981; Rapoport et al., 1981). Similar to adults, acute caffeine administration to children and adolescents increases ambulatory blood pressure in a dose dependent manner (Savoca, Evans, Wilson, Harshfield, & Ludwig, 2004; Savoca et al., 2005). Withdrawal from caffeine also produces similar effects in a subset of adolescent caffeine users as those seen in adults, such as headache, drowsiness, and fatigue (Bernstein, Carroll, Thuras, Cosgrove, & Roth, 2002; Hale, Hughes, Oliveto, & Higgins, 1995). However, these effects are seen in fewer children and are more inconsistent than what is typically observed in adult caffeine users (Rapoport et al., 1981). These differences may be because of the amount, type, and pattern of caffeine intake among children and adolescents compared with adults.

Consumption of caffeine-containing beverages in children and adolescents is associated with greater body mass index (BMI; Johnson & Kennedy, 2000; Ludwig, Peterson, & Gortmaker, 2001), greater intake of unhealthy foods, and...
lower intake of healthy foods, such as fruits, vegetables, and milk (Harnack et al., 1999). In children, the primary vehicle for caffeine is soda, which also contains 40 g of sugar per 12-ounce can (Frary et al., 2005; Harnack et al., 1999; Smiciklas-Wright, Mitchell, Mickle, Goldman, & Cook, 2003). Therefore, it is possible that one reason for the association between soda consumption and consumption of less healthy foods is that repeated pairings of sugar and caffeine facilitates the development of enhanced preference for foods and beverages containing added sugar. Sugar is a known “natural reward” that activates similar reward pathways as drugs of abuse, such as cocaine, amphetamine, and nicotine (Robinson & Berridge, 2000). Intermittent access to sugar in food-deprived rats leads to both behavioral and neurochemical (Avena & Hoebel, 2003; Colantuoni et al., 2001) similarities to drug addiction. Because of the well-established similarities between sugar and drugs of abuse (reviewed in Avena, Rada, & Hoebel, 2008), the possibility exists that caffeine can potentiate sensitivity to, liking of, and consumption of sugar, just as it does with nicotine (Jones & Griffiths, 2003; Puccio, McPhillips, Barrett-Connor, & Ganiats, 1990; Swanson, Lee, & Hopp, 1994). In addition, because caffeine can also activated the dopaminergic system (Fuxe et al., 2003; Kudlacek et al., 2003; Salim et al., 2000), caffeine paired with high levels of added sugar in foods and beverages may act synergistically to release dopamine and, as a consequence, increase the reinforcing properties of sweetened foods and beverages. To date, there have been no studies examining potential links between sugar consumption and caffeine use in children.

A previous study from our laboratory found that boys found caffeinated soda more reinforcing than did girls after a 2-week exposure period using a double-blind, placebo-controlled design (Temple, Bulkley, Briatico, & Dewey, 2009). To our knowledge, this was the first study to report a gender difference in response to caffeine in adolescents. In adults, some gender differences have been reported. For example, caffeine reduces the risk of Parkinson’s disease in men only (Ascherio et al., 2004; Ascherio et al., 2001; Benedetti et al., 2000; Ross et al., 2000), suggesting that there are gender differences in neurobiological responses to caffeine. Similar gender differences have been reported for subjective effects of caffeine. For example, one study examining the effects of acute caffeine administration on subjective state showed greater effects in men than women (Adan, Prat, Fabbri, & Sanchez-Turet, 2008). This was despite the fact that all participants were given the same dose of caffeine (100 mg), resulting in a higher mg/kg dose in girls. It is possible that gender differences in responses to caffeine administration are mediated by differences in circulating steroid hormones. This hypothesis is supported by studies showing that caffeine consumption (Kotsopoulos, Eliasen, Missmer, Hankinson, & Tworoger, 2009) and subjective responses to caffeine (Terner & de Wit, 2006) vary across the menstrual cycle. These gender differences in subjective and reinforcing effects of caffeine may mediate differences in intake patterns and motivations for caffeine usage.

The purpose of this study was to investigate the dose dependent effects of acute caffeine administration in relation to level of chronic caffeine use in adolescent boys and girls. We used a double-blind, placebo controlled design to test cardiovascular and subjective responses to caffeine as well as acute snack food ingestion. This is among the first studies to examine the effects of acute caffeine administration in this age group. The data from this study will help determine the effects of acute and chronic caffeine use in adolescents and add to the growing body of literature on gender differences in drug responses.

**Method**

**Participants and Recruitment**

Adolescents, ages 12 to 17 years old, were recruited through direct mailings, flyers distributed at local middle and high schools, as well as flyers posted around the University at Buffalo and the surrounding community. Eligibility criteria included the following: previous experience with caffeine with no adverse reactions, not using hormone-based contraceptives, not smoking, not on any medication that could have adverse interactions with caffeine (e.g., methylphenidate), and willing to visit the laboratory on four occasions for 90 min each time. Stratification of usual caffeine consumption was estimated based on the participant’s self-report of daily or weekly intake of caffeine from all major sources, including tea (40 mg/5 oz), soda (40 mg/12 oz), coffee (100 mg/5 oz), energy drinks (~150 mg/12 oz), chocolate (10 mg/oz), and caffeine-containing pills (Excedrin or No-Doze, 130–200 mg/pill). These estimates of caffeine content are based on information published by the U.S. Department of Nutritional Services. An equal number of children were recruited within each of the following caffeine consumption groups: 0–25 mg/day, 25–50 mg/day, 50–75 mg/day, >75 mg/day. This was done to have a sample that was balanced for typical caffeine use. We began with a total of 55 participants. We had one drop out before the study was completed and data from two individuals were removed because they had salivary caffeine levels indicative of recent usage. This left us with a total of 52 participants (26 boys, 26 girls).

**Telephone Screening**

Interested participants called our laboratory or completed an online survey to provide basic information, including names of parent and child, address, telephone number, child’s date of birth, child’s height and weight, any medications or health problems in the child, including dietary restrictions, latex allergies, and neurological disorders. Then we spoke to the child about amount and sources of caffeine consumption to attain an estimate of typical usage. If the child met the above eligibility criteria and was interested in participating, he or she was scheduled for four laboratory visits. Parents and participants were also instructed prior to each visit that the participant needed to abstain from consumption of caffeine for 24-hr and to not eat or drink anything other than water for 2 hours.
General Experimental Procedures

All questionnaires and measurement procedures are described in more detail below. Upon arrival to the laboratory, parents and participants were given consent and assent forms to read and sign. To remove subject expectancies about the effects of caffeine, participants were told that “the purpose of the study is to determine how substances commonly found in soft drinks affect mood and physiological measurements, such as heart rate and blood pressure” and that the beverage they would be consuming “may have levels of one or more of the following substances manipulated: sugar, aspartame, Splenda, caffeine, or artificial coloring. The levels of these substances will not exceed what is considered safe by the Food and Drug Administration.”

This deception was considered acceptable because it involved no greater than minimal risk, and was necessary to prevent potential preconceptions of caffeine’s effects from altering experimental results. Participants then completed a 24-hr dietary and physical activity recall while the parent completed a demographic questionnaire. Parents were then escorted from the room and participants provided a 3-ml saliva sample into a sterile tube that was analyzed for caffeine and steroid hormones. The participant then completed the Behavioral Checklist Questionnaire. He or she then had baseline blood pressure and heart rate readings taken. Then, the participants consumed a drink containing 0 mg, 50 mg, 100 mg, or 200 mg of caffeine. Each dose was administered on a separate visit and the order in which they were administered was counterbalanced. Blood pressure and heart rate measurements were then taken every 10 min for a total of 60 min. During the hour, participants viewed a National Geographic Video of their choice. After 1 hr, participants completed the Behavioral Checklist a second time and an ad libitum snack food eating session. The participant returned to the laboratory for three more visits following identical procedures. In between visits, participants were asked to complete the Block Kids Food Frequency Questionnaire (described below). After the final session, participants had their height and weight measured.

Both the participant and their parent were debriefed and compensated for participation. All study procedures were conducted in accordance with National Institutes of Health (NIH) guidelines for the use of humans in research and with the approval of the University at Buffalo Social and Behavioral Sciences Institutional Review Board.

Caffeine and Beverage Preparation

Caffeine and placebo treatments were prepared by an experimenter who was not involved in the data collection for this study. Caffeine at each concentration (50 mg, 100 mg, or 200 mg) was dissolved in flattened Sprite to facilitate masking of the bitter taste of the caffeine. Sprite was flattened by heating it to 140°C and stirring it at a speed of 50 rpm for 25 min. Flattened Sprite without the added caffeine was used as the placebo. The caffeine or placebo solutions were then aliquoted into 14-ml vials labeled A–D and frozen. On the day of the visit, the appropriate dose was thawed for 1 to 2 hr at room temperature. To remove expectancies about caffeine, participants were able to choose to drink orange juice, lemonade, or Sprite, which are all caffeine-free. On the first laboratory visit, participants were provided 2-oz samples of each beverage and were asked to rate how much they liked them using a 7-point Likert scale anchored with 1 = not at all and 7 = extremely. The drink with the highest rating was chosen. While the participant was providing a saliva sample and completing questionnaires, the researcher prepared 288 ml of the selected drink, to which 12 ml of placebo of caffeine (A, B, C, or D) were added.

Salivary Caffeine Measurement

Saliva collection was conducted at the beginning of each laboratory visit. Participants were instructed to expectorate into a tube with a funnel attached. They provided 3 ml of saliva, without air bubbles. This line was indicated for them on the outside of the sterile vile with a sticker labeled with participant number and visit letter. Participants were given a piece of wax which they could chew to facilitate saliva production. To verify 24-hr caffeine abstinence, the saliva sample provided on the visit where the participant received the placebo beverage was analyzed. Samples were stored at −20 °C until analyzed. Analyses of caffeine content was conducted by LabStat (Kitchner, Ontario) using a standard gas chromatography method with a structural analogue of caffeine used as an internal standard (Liguori, Hughes, Goldberg, & Callas, 1997). Participants were considered abstinent if caffeine levels fell below 0.85 μg/ml, which is consistent with overnight caffeine abstinence (Evans, Critchfield, & Griffiths, 1994; Griffiths & Woodson, 1988; Liguori et al., 1997).

Cardiovascular Responses

An automated heart rate and blood pressure monitor (Tango; SunTech Medical, Inc., Morrisville, NC) was used to collect cardiovascular measurements. The participants were seated in reclined position and instructed to relax. The nondominant arm was slipped into the blood pressure cuff, with the microphone placed over the brachial artery, between the bicep and tricep muscles. The cuff was wrapped around the participants arm. Electrodes were placed on each forearm and on the chest above the heart. A baseline reading was conducted prior to consumption of the test beverage. Then readings were taken every 10 min for 60 min. In between readings, participants watched a video. One minute prior to each reading, the video was shut off and the participant was instructed to sit in silence and relax. The video resumed after the reading was recorded.

Measurements

Weight, height, BMI. Participant weight was assessed by use of a digital scale (SECA; Hanover, MD). Height was assessed using a SECA stadiometer. On the basis of the height and weight data BMI was calculated according to the following formula: $\text{BMI} = \frac{\text{kg}}{\text{m}^2}$. 
Behavioral checklist. A questionnaire containing 31 adjectives describing mood and physiological symptoms was presented to the participants at the beginning and end of each session. The participants were asked to rate how they felt “right now” on a 9-point Likert-type scale anchored by 1 = “not at all” and 9 = “extremely.” This questionnaire has been used by multiple investigators (Richardson, Rogers, & Elliman, 1996; Yeomans, Pryke, & Durlach, 2002) and is sensitive to caffeine use (Richardson, Rogers, Elliman, & O’Dell, 1995). The participant rated their subjective experience of each adjective on a 9-point Likert-type anchored by 1 = “not at all” and 9 = “extremely” (Hughes et al., 1991). The items on the list were as follows: anxious, alert, content, depressed, dizzy, drowsy, fatigued, frequent urination, headache, insomnia, irregular heartbeat, diarrhea, impatient, hungry, irritable, motivated to work, well-being, mood swings, muscle twitches, talkative, nausea, palpitation, restlessness, ringing in ears, energetic, stomachache, vigorous, perspiration, tremor, sleepy, and tired. Participants were told to indicate how they felt at that exact moment. This questionnaire was completed on a lap top using Survey Monkey.

24-hr food and exercise recall. The participant (with the assistance of the parent) recalled his or her dietary intake and physical activity for the previous 24 hr. Any participant who had not complied with the study protocol (caffeine abstinence for 24 hr) would have been rescheduled, but we did not have anyone who was not compliant based on self-report.

Ad libitum snack food eating. Participants were given access to a variety of snack foods differing in fat and sugar content along with water ad libitum. The foods were provided in 300 kcal portions and were as follows: Skittles and Smarties (high sugar/low fat), potato chips and Doritos (low sugar/high fat), and M&Ms and Twix (high sugar/high fat). Participants were told that they were completing a taste test and that they needed to sample each food and rate its liking using a 7-point Likert scale anchored with 1 = “not at all” and 7 = “extremely.” They were told that they could eat as much as they wanted of each food because it would have to be discarded after the session. We have used this procedure previously in adults (Epstein et al., 2007).

Block Kids Food Frequency Questionnaire (2004). A 77-item Food Frequency Questionnaire developed for use in 8- to 17-year-olds was issued to assess food intake patterns. This questionnaire asks how many times in the past week to 17-year-olds was issued to assess food intake patterns. It has been used by multiple investigators (Richardson, Rogers, & Elliman, 1996; Yeomans, Pryke, & Durlach, 2002) and is sensitive to caffeine use (Richardson, Rogers, Elliman, & O’Dell, 1995). The participant rated their subjective experience of each adjective on a 9-point Likert-type anchored by 1 = “not at all” and 9 = “extremely” (Hughes et al., 1991). The items on the list were as follows: anxious, alert, content, depressed, dizzy, drowsy, fatigued, frequent urination, headache, insomnia, irregular heartbeat, diarrhea, impatient, hungry, irritable, motivated to work, well-being, mood swings, muscle twitches, talkative, nausea, palpitation, restlessness, ringing in ears, energetic, stomachache, vigorous, perspiration, tremor, sleepy, and tired. Participants were told to indicate how they felt at that exact moment. This questionnaire was completed on a lap top using Survey Monkey.

Demographic questionnaire. While the participant was reading over the assent form and being explained the study procedures, parents filled out a demographic questionnaire. They were informed that this was for research purposes only and if they did not feel comfortable answering some/any of the questions, they were not required to do so. Information on this sheet included: who currently lived in the household where the participant resided, the marital status of the primary caregiver of the child, which households the participant spent time in and how much time was spent in them, the highest level of education completed by the primary caregiver and their spouse, the occupation of the primary caregiver and their spouse, the employment status of the primary caregiver and their spouse, where the total household income was derived from, the amount of the total household income, and the parent and participant’s ethnicity and race.

Analytic Plan

The potential differences between subjects were analyzed using a one-way analysis of variance (ANOVA), with gender and caffeine use group (low vs. high) as the between subjects factors. Caffeine use group was determined using a median split for caffeine use with anyone consuming <50 mg/day considered a low user and anyone consuming ≥50 mg/day being considered a high user. This is consistent with our previous study (Temple et al., 2009) in which that average caffeine consumption of our sample was found to be 52 mg/day. Potential differences in categorical variables, such as race, household income, and parental education, were analyzed using χ². The pattern of diastolic and systolic blood pressure and heart rate were analyzed using linear regression with gender, caffeine use (mg/day), and BMI as time invariant predictors and energy intake as the time variant predictor and baseline blood pressure and heart rate as covariates. Answers on the behavioral checklist were analyzed using a mixed effects regression model with gender, caffeine use (mg/day), and BMI as time invariant predictors and drug dose as time variant predictors. Energy intake was analyzed using a mixed-effects regression model with usual caffeine consumption (mg/day), gender, and BMI as time invariant predictors and acute caffeine dose as the time variant predictor. All data were considered significantly different if p < .05 and data analyses were conducted using SYSTAT 11.0 (Chicago).

Results

Participants

Participants were male (n = 28) and female (n = 26) 12- to 17-year-olds. Data from two high-consuming boys were eliminated from analyses because they had salivary caffeine levels indicative of recent usage. This left us with 26 boys (9 low consumers and 17 high consumers) and 26 girls (17 low consumers and 9 high consumers) for the following analyses.
analysis. At the time of screening, participants were placed into groups based on their self-reported caffeine use during the phone interview and at this time, the groups were balanced for age and gender. After the participants completed the caffeine use questionnaire, which was considerably more detailed than what we used for our telephone screening, we recalculated their daily usage and some participants were reassigned to different groups. This is why we ended up with an unequal distribution of participants in each caffeine consumption group. When we analyzed participant characteristics by gender and caffeine consumption group, we found no relationships between caffeine use group or gender and BMI, parental education, household income, or race. There were also gender differences as a function of caffeine consumption (p = .05). There were also gender differences in average daily caffeine consumption, F(1, 50) = 5.02; d = 0.62; r = .297; p = .03, as well as amount of caffeine from energy drinks, F(1, 50) = 4.97; d = 0.62; r = .296; p = .03, with boys consuming more than girls. In terms of reasons for caffeine consumption, boys were more likely than girls to report using caffeine for energy, F(1, 48) = 6.2; d = .88; r = .40; p = .02; to get a rush, F(1, 48) = 11.06; d = 1.12; r = .49; p < .0001; or to enhance athletic performance, F(1, 48) = 12.2; d = 1.08; r = .48; p = .001 (Figure 1).

Caffeine Sources and Reasons for Consumption

We found that the majority of participants in this study reported consuming caffeine at least occasionally (96%) and that the single largest source of caffeine for the majority of participants was soda (92%; Table 2). There was a significant difference in the proportion of low- and high-caffeine consumers that drink coffee, χ²(1) = 5.13; p = .02; soda, χ²(1) = 4.0; p = .045; and energy drinks, χ²(1) = 11.9; p = .001, with a larger proportion of high consumers than low consumers drinking these beverages. There was also a difference in daily total caffeine consumption, F(1, 48) = 26.3; d = 1.59; r = .62; p < .0001; and daily caffeine consumed from coffee, F(1, 48) = 10.00; d = 0.97; r = .44; p = .003; tea, F(1, 48) = 6.3; d = 0.76; r = .36; p = .015; soda, F(1, 48) = 11.66; d = 0.96; r = .43; p = .001; and energy drinks, F(1, 48) = 5.99; d = 0.84; r = .39; p = .018, as a function of caffeine consumption group (Table 2).

There were gender differences in the proportion of participants consuming caffeine from different sources. A larger proportion of boys than girls reported consuming energy drinks, χ²(1) = 4.3; p = .04, and a larger proportion of girls consume tea than boys, χ²(1) = 6.2; p = .01. There were also gender differences in average daily caffeine consumption, F(1, 50) = 5.02; d = 0.62; r = .297; p = .03, as well as amount of caffeine from energy drinks, F(1, 50) = 4.97; d = 0.62; r = .296; p = .03, with boys consuming more than girls. In terms of reasons for caffeine consumption, boys were more likely than girls to report using caffeine for energy, F(1, 48) = 6.2; d = .88; r = .40; p = .02; to get a rush, F(1, 48) = 11.06; d = 1.12; r = .49; p < .0001; or to enhance athletic performance, F(1, 48) = 12.2; d = 1.08; r = .48; p = .001 (Figure 1).

Blood Pressure and Heart Rate

There were main effects of drug dose on HR (β = −0.015; SE = 0.005; Z = −3.34; p = .001; Figure 2a) and DBP (β = 0.04; SE = 0.005; Z = 7.2; p < .0001; Figure 2b). There were also main effects of baseline readings on HR (β = 0.82; SE = 0.04; Z = 20.71; p < .0001), DBP (β = 0.75; SE = 0.052; Z = 14.5; p < .0001), and SBP (all p < .0001). There was an interaction of gender, caffeine use (mg/day), and time on DBP (β = −0.001; SE <.0001; Z = −2.3; p = .02), but no interactions for SBP or HR (all p > .34). When this interaction was probed by examining each caffeine use group separately, we found that, in low consumers, there were main effects of time (β = 0.07; SE = 0.02; Z = 4.1; p < .0001) and baseline DBP

| Table 1 |
| Participant Characteristics Shown by Gender and Caffeine Use Group |

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low (n = 9)</td>
<td>High (n = 17)</td>
<td>Low (n = 17)</td>
<td>High (n = 9)</td>
</tr>
<tr>
<td>Age</td>
<td>13.4 ± 0.4</td>
<td>14.7 ± 0.3a</td>
<td>13.8 ± 0.3</td>
<td>14.4 ± 0.4a</td>
</tr>
<tr>
<td>Body mass index</td>
<td>21.7 ± 1.7</td>
<td>22.1 ± 0.7</td>
<td>21.3 ± 1.0</td>
<td>21.3 ± 1.8</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>6 (67)</td>
<td>12 (71)</td>
<td>15 (88)</td>
<td>7 (78)</td>
</tr>
<tr>
<td>African American</td>
<td>2 (22)</td>
<td>4 (24)</td>
<td>1 (6)</td>
<td>1 (11)</td>
</tr>
<tr>
<td>Asian</td>
<td>1 (11)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>1 (6)</td>
<td>1 (6)</td>
<td>1 (11)</td>
</tr>
<tr>
<td>Parental education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High school</td>
<td>1 (11)</td>
<td>1 (6)</td>
<td>1 (6)</td>
<td>2 (22)</td>
</tr>
<tr>
<td>College</td>
<td>7 (78)</td>
<td>11 (65)</td>
<td>10 (59)</td>
<td>6 (67)</td>
</tr>
<tr>
<td>Graduate school</td>
<td>1 (11)</td>
<td>5 (29)</td>
<td>6 (35)</td>
<td>1 (11)</td>
</tr>
<tr>
<td>Household income &lt; $30,000</td>
<td>1 (11)</td>
<td>6 (35)</td>
<td>2 (12)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>$30,000–$50,000</td>
<td>2 (23)</td>
<td>3 (18)</td>
<td>3 (18)</td>
<td>1 (11)</td>
</tr>
<tr>
<td>$50,000–$70,000</td>
<td>3 (33)</td>
<td>1 (6)</td>
<td>5 (29)</td>
<td>3 (33)</td>
</tr>
<tr>
<td>$70,000</td>
<td>3 (33)</td>
<td>7 (41)</td>
<td>7 (41)</td>
<td>5 (56)</td>
</tr>
</tbody>
</table>

a Significant differences as a function of caffeine use (p < .05).
EFFECTS OF CAFFEINE IN ADOLESCENTS

Table 2

Caffeine Usage From Different Sources in Male and Female Adolescents With Different Levels of Caffeine Use

<table>
<thead>
<tr>
<th>Source</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low (n = 9)</td>
<td>High (n = 17)</td>
</tr>
<tr>
<td>Drink caffeine</td>
<td>8 (89)</td>
<td>17 (100)</td>
</tr>
<tr>
<td>Daily consumption (mg)</td>
<td>22.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>4.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 (44)</td>
<td>5 (29)</td>
</tr>
<tr>
<td></td>
<td>9.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>7 (78)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17 (100)</td>
</tr>
<tr>
<td></td>
<td>9.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>2.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.1</td>
</tr>
</tbody>
</table>

Note. For each category, the top set of numbers is the number of participants who reported at least occasional consumption of each of the caffeine sources, n(%), and underneath the numbers is the mean and SE of the mean daily consumption (mg) of caffeine from each of these sources. Proportions of users were analyzed using χ² across caffeine consumption group and gender. Differences in the amount of daily caffeine use were analyzed using a one-way analysis of variance with gender and caffeine consumption group as the between subjects variables.

<sup>a</sup> Significant differences as a function of caffeine consumption group.  <sup>b</sup> Significant differences as a function of gender.

(β = 0.73; SE = 0.07 Z = 11.03; p < .0001), but no interactions or effects of gender. In high consumers, there were main effects of gender (β = 3.98; SE = 1.97; Z = 2.02; p = .043), drug dose (β = 0.025; SE = 0.008; Z = 3.13; p = .002), time (β = 0.07; SE = 0.025; Z = 2.63; p = .009), and baseline DBP (β = 0.774; SE = 0.08; Z = 9.73; p < .0001). There were also interactions between gender and time (β = −0.073; SE = 0.028; Z = −2.58; p = .01) and gender and drug dose (β = −0.037; SE = 0.008; Z = −4.74; p < .0001). When this interaction was probed by examining each gender separately, we found main effects of drug dose (β = 0.029; SE = 0.0089 Z = 3.15; p = .02), time (β = 0.117; SE = 0.03; Z = 4.01; p < .0001), and baseline DBP (β = 0.76; SE = 0.08; Z = 10.00; p < .0001) as well as an interaction of caffeine consumption (mg/day) and time (β < −0.0001; SE <0.0001; Z = −2.17; p = .03) in boys (Figure 3A). In girls, there was a main effect of baseline DBP (β = 0.72; SE = 0.08; Z = 9.4; p < .0001), but no other main effects or interactions were observed (all p ≥ .15; Figure 3B).

Caffeine Consumption and Laboratory Snack Food Intake

There was a relationship between usual caffeine consumption and energy from high-sugar/low-fat foods (β = 0.59; SE = 0.15; Z = 3.97; p < .0001; Figure 4), with higher caffeine consumption associated with greater intake of energy from high-sugar foods. There were no differences as a function of caffeine consumption group for intake of high-sugar/high-fat or low-sugar/low-fat foods (both p > .10). There were main effects of BMI on total energy consumption (β = 30.74; SE = 14.4; Z = 2.13; p = .03) and consumption of high-sugar/high-fat foods (β = 10.54; SE = 4.0; Z = 2.64; p = .008), but there were no interactions between BMI and any of the other predictors in the model (all p > .20). There were no effects of gender or acute caffeine dose (all p > .05) on snack food intake.

Caffeine Consumption and Usual Nutrient Intake

When we analyzed the data from the food frequency questionnaire, we found a significant main effect of caffeine consumption group on total daily intake of energy, F(1, 45) = 4.1; d = 0.67; r = .32; p = 0.049; energy intake from protein, F(1, 45) = 5.1; d = 0.71; r = .33; p = .029; and energy intake from fat, F(1, 45) = 4.1; d = 0.66; r = .31;
With high-caffeine consumers having higher consumption of total energy, protein, and fat compared with low-caffeine consumers. When we examined the percentage of energy from the various macronutrient sources, there were no differences as a function of caffeine or gender. In addition, there were no differences as a function of caffeine consumption group or gender on the number of servings of vegetables, grains, fruit, or fat. There was a significant effect of caffeine consumption group on daily servings of meat, \( F(1, 45) = 5.6; p = .02 \), with male dairy intake decreasing as caffeine consumption increased and female dairy consumption increasing as caffeine consumption increased.

\[ p = .048 \]

When we examined the percentage of energy from the various macronutrient sources, there were no differences as a function of caffeine or gender. In addition, there were no differences as a function of caffeine consumption group or gender on the number of servings of vegetables, grains, fruit, or fat. There was a significant effect of caffeine consumption group on daily servings of meat, \( F(1, 45) = 6.2; d = 0.81; r = .37; p = .017 \), with high-caffeine consumers reporting greater meat consumption than low-caffeine consumers. There was also a gender by group interaction on daily servings of dairy.

\[ p = .008 \]

When this interaction was probed by examining each gender differently, we found main effects of drug dose \( (p = .002) \), time \( (p < .0001) \), and baseline diastolic blood pressure \( (p < .0001) \), as well as an interaction of caffeine consumption (mg/day) and time \( (p = .03) \) in boys. In girls, there was a main effect of baseline diastolic blood pressure \( (p < .0001) \), but no other main effects or interactions were observed.
When we examined the behavioral checklist data, we found effects on only a subset of the behaviors we measured. Specifically, we found a main effect of pre/post on alertness (β = -0.46; SE = 0.22; Z = -2.08; p = .037), motivation to work (β = -0.48; SE = 0.182; Z = -2.64; p = .008), muscle twitches (β = 0.35; SE = 0.097; Z = 3.59; p < .001), talkativeness (β = -0.68; SE = 0.197; Z = -3.45; p = .001), and energy (β = -0.586; SE = 0.20; Z = -2.95; p = .003). There were main effects of gender on contentment (β = 1.29; SE = 0.645; Z = 2.00; p = .045) and heart palpitations (β = -0.177; SE = 0.084; Z = -2.11; p = .035). There were main effects of usual caffeine consumption on anxiety (β = 0.005; SE = 0.002; Z = 2.12; p = .034). There were interactions of caffeine use and drug dose on fatigue (β = -0.0001; SE < 0.0001; Z = -2.07; p = .038) and stomachache (β < 0.0001; SE < 0.0001; Z = 3.49; p < .0001). There were three-way interactions of sex, drug dose, and pre/post on (β = 0.006; SE = 0.002; Z = 2.59; p = .01) and on tremor (β = 0.002; SE = 0.001; Z = 1.98; p = .047). There were three-way interactions of usual caffeine consumption, drug dose and pre/post on fatigue (β < 0.0001; SE < 0.0001; Z = 2.802; p = .005), energy (β < 0.0001; SE < 0.0001; Z = 2.03; p = .043), and stomachache (β < 0.0001; SE < 0.0001; Z = -2.56; p = .011). There was also a three-way interaction of usual caffeine consumption, drug dose and pre/post on anxiety (β < -0.0001; SE < 0.0001; Z = 2.013; p = .044).

Discussion

This study investigated the relationship between acute and chronic caffeine consumption and cardiovascular effects, subjective responses, and ingestive behavior in adolescents. In order to accomplish this, we conducted a double-blind, placebo controlled, dose-response study in 12- to 17-year-olds. Consistent with previous studies, we found dose-dependent increases in DBP and decreases in HR. We found no relationship between typical caffeine consumption and DBP in response to acute caffeine consumption in girls, but in boys, high-caffeine consumers showed greater increases in DBP over time than did low-consuming boys. Boys also rated “to get energy,” “to get a rush,” and “athletic performance” as more important reasons for using caffeine compared with ratings from girls. When taken together, these findings suggest that boys and girls differ in their responses to caffeine. When we examined ingestive behavior as a function of chronic and acute caffeine use, we found that high- and low-caffeine consumers differed in macronutrient intake, with high consumers having more energy, protein, and fat in their typical diet and consuming more high-sugar snack foods in the laboratory compared with low-caffeine consumers. More studies need to be conducted to determine the mechanism that underlies these gender differences in response to caffeine.

In adults (Bender et al., 1997; Lane & Williams, 1987; Sung et al., 1994; Waring et al., 2003) and adolescents (Bernstein et al., 2002; Savoca et al., 2004; Savoca et al., 2005) acute administration of caffeine increases BP and decreases HR in a dose-dependent manner. Consistent with these findings, we demonstrated a dose-dependent decrease in HR after caffeine administration that was independent of gender and level of habitual caffeine consumption. We also showed a main effect of drug dose on DBP, but this effect was moderated by gender and level of chronic caffeine consumption. Specifically, both high- and low-consuming boys showed increases in DBP over time after caffeine administration, but the magnitude of the increase was greater in high-consuming boys than in low-consuming boys. In girls, there was an increase in DBP after 10 min, but then no further increases after that and no difference in responses as a function of chronic caffeine use. When taken together, our data demonstrate that boys and girls respond differently to acute caffeine administration.

In addition to gender differences in cardiovascular responses to acute caffeine administration, gender was associated with the types, amount, and motivations for caffeine consumption. Boys reported consuming more energy drinks and girls reported consuming more tea. Energy drinks have significantly more caffeine than tea. Perhaps boys enjoy or require larger doses of caffeine than girls. Alternatively, energy drink consumption may be elevated in boys because of energy drink marketing directly to adolescents and young adult males (Reissig, Strain, & Griffiths, 2009). Boys were also more likely than girls to report using caffeine for energy, to get a rush, or to enhance performance. These subjective effects of caffeine are more likely to occur after ingestion of higher acute doses of caffeine, which could also...
explain the differences in the type of caffeinated drinks consumed. We are not the first to report gender differences in caffeinated beverage consumption. Kathleen Miller has reported that male college students are significantly more likely to consume energy drinks than girls (Miller, 2008). The question remains, are girls consuming energy drinks less frequently because they do not experience the positive subjective effects of caffeine or are they not experiencing the positive subjective effects of caffeine because they are less likely to consume energy drinks?

Gender differences have been reported for subjective and physiological responses to other drugs of abuse. For example, work from Harriet de Wit’s laboratory has demonstrated that boys and girls have different subjective responses to drugs such as amphetamine. In addition, within girls, subjective responses to amphetamine change across the menstrual cycle (Terner & de Wit, 2006). One potential mechanism for gender differences in response to acute caffeine administration is that estradiol is known to decrease the metabolism of caffeine (Pollock et al., 1999). This affects the half-life of the caffeine and could affect the maximal blood level by lengthening the dose response curve (Granfors, Backman, Laitila, & Neuvonen, 2005). It is also possible that there are differences in caffeine use between boys and girls that lead to differential responses. For example, in our study, there was a trend for boys in the high-consuming group to consume more caffeine than girls in the same group (146 mg/day vs. 108 mg/day; \( p = .07 \)). While we might predict that if boys are consuming more caffeine than girls, they would be more likely to develop tolerance and would, therefore, have reduced effects of caffeine. Conversely, it is possible that girls consume less because they do not experience the positive, stimulating effects that the boys appear to find reinforcing (Temple et al., 2009). It is also possible that boys sensitized to the effects of acute caffeine administration. We found that the increase in DBP after acute caffeine administration was greater in high-consuming boys than in low-consuming boys. To our knowledge, no previous studies have reported sensitization to the effects of caffeine, and our results are too preliminary to draw any conclusions about sensitization. However, sensitization occurs to many other drugs and it would be important to determine if there are gender differences in sensitization to the effects of caffeine.

In contrast to gender differences in cardiovascular and subjective effects of caffeine, we did not find gender differences in the relationship between caffeine use and energy or macronutrient intake. We did, however, find a relationship between usual caffeine consumption and energy and macronutrient intake. Specifically, high-caffeine consumers ate more energy both in the laboratory (as assessed by snack food intake) as well as outside of the laboratory (as assessed by food frequency questionnaire) compared with low consumers. In addition, high consumers ate more high-sugar, low-fat foods in the laboratory and consumed more protein and fat outside of the laboratory. This suggests an association between chronic caffeine use and intake of higher energy density foods. Previous studies have reported and association between caffeine consumption and intake of other types of foods. Harnack and colleagues reported that soda consumption is inversely correlated with fruit, vegetable, milk intake and positively correlated with intake of “junk food” (Harnack et al., 1999). In addition, children who consume soda on a regular basis are at higher risk for obesity (Johnson & Kennedy, 2000) and for every additional serving of sugar-sweetened beverages consumed daily, there is a 60% increase in the odds of becoming obese (Ludwig et al., 2001). Therefore, the relationship between soda consumption and weight may be mediated by poor diet. Despite finding a relationship between caffeine consumption and energy intake, we did not find a similar relationship with BMI or body weight. It is possible that gender mediated the relationship between caffeine use and energy intake because boys consumed more caffeine and boys have greater energy needs compared with girls at this age. Another possibility is that because we limited or study population to nonoverweight adolescents that we were missing the heaviest children. If overweight children were included in the study, we may have observed a relationship between BMI and caffeine consumption.

We had several findings from the Behavioral Checklist. The most consistently observed finding was that acute caffeine administration altered responses on this questionnaire, including increases in alertness, contentment, motivation to work, talkative, and energy and decreases in muscle twitches. None of the pre/post effects were moderated by usual caffeine use, suggesting that, at least for a subset of the adjectives on this questionnaire, there was no evidence of tolerance. In addition, similar to effects of acute caffeine administration on food intake in the laboratory, there were no interactions with gender and any other factors on Behavioral Checklist responses, suggesting that subjective physiological and mood changes may be less susceptible to moderation by steroid hormones or other gender-related factors. A previous study in adolescent caffeine users reported that acute caffeine use decreased reports of depression, sleepiness, and fatigue and increased reports of irregular heartbeat and talkative (Hale et al., 1995). Although the study population in this study was 11- to 15-year-olds that consumed at least one can of soda per day, the findings are similar to ours in that, at least for some subjective measures, chronic caffeine use does not eliminate the acute effects of caffeine, suggesting that tolerance to these effects is minimal. It is, however, important to consider that in our study and in the Hale et al. study, participants were overnight withdrawn from caffeine. Therefore, the acute effects of caffeine may have been because of withdrawal reversal as opposed to positive stimulating effects. This distinction is important, as there are many studies suggesting that the majority of the positive effects of caffeine in chronic caffeine users are merely the result of removal of the negative effects of caffeine abstinence (James & Rogers, 2005; Rogers, Martin, Smith, Heatherley, & Smit, 2003; Yeomans, Ripley, Davies, Rusted, & Rogers, 2002). This study had several strengths, including a double-blind, placebo controlled, within-subjects, dose-response design, biological confirmation of caffeine abstinence, and efforts to conceal the nature of the study. In addition, children and
adolescents are an understudied population in terms of caffeine use. This study adds to the small, but growing body of literature on the effects of caffeine in this population. This study was not without limitations. First, although we stratified for caffeine consumption in order to get a wide range of caffeine consumers, we did not recruit many very high-caffeine consumers (>200 mg/day). Therefore, we may be missing the population that is the most dependent on caffeine. Second, our sample was small and very homogeneous in terms of race, income, and education. This limits the generalizability of our findings to largely white, upper-middle class populations. Third, by instructing participants to abstain from caffeine use prior to their visits to the laboratory, we may have increased their awareness that caffeine was being manipulated. Because this was a dose-response study and placebo was administered on one of the visits, we were more concerned with achieving caffeine abstinence than we were with expectancy effects. However, it would have been better if we could have controlled for both. Finally, although we recruited a sample balanced for gender and caffeine use based on a telephone interview, when participants completed our extensive caffeine use questionnaire, we did a more complete analysis of typical caffeine intake and regrouped participants. This led to a higher proportion of boys and a lower proportion of girls in the high-consuming group.

In sum, this study was the first to demonstrate gender differences in physiological response to acute caffeine administration in adolescents. In addition, we found gender differences in caffeine sources and motivations for caffeine consumption. Finally, we demonstrated an association between caffeine use and macronutrient and energy intake. Adolescents are among the fastest growing consumers of caffeine and yet very few empirical studies have focused on this population. It is imperative that we understand the impact of caffeine use on adolescents. Our data may shed light on the effects of caffeine use on adolescent physiology and behavior as well as uncover potential mechanisms that underlie gender differences in drug responses.

References


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DAY 1, SESSION 5: PANEL DISCUSSION: BEHAVIORAL EFFECTS ASSOCIATED WITH CAFFEINE CONSUMPTION

Energy Drink Consumption and Increased Risk for Alcohol Dependence

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Background: Energy drinks are highly caffeinated beverages that are increasingly consumed by young adults. Prior research has established associations between energy drink use and heavier drinking and alcohol-related problems among college students. This study investigated the extent to which energy drink use might pose additional risk for alcohol dependence over and above that from known risk factors.

Methods: Data were collected via personal interview from 1,097 fourth-year college students sampled from 1 large public university as part of an ongoing longitudinal study. Alcohol dependence was assessed according to DSM-IV criteria.

Results: After adjustment for the sampling design, 51.3% of students were classified as “low-frequency” energy drink users (1 to 51 days in the past year) and 10.1% as “high-frequency” users (‡52 days). Typical caffeine consumption varied widely depending on the brand consumed. Compared to the low-frequency group, high-frequency users drank alcohol more frequently (141.6 vs. 103.1 days) and in higher quantities (6.15 vs. 4.64 drinks/typical drinking day). High-frequency users were at significantly greater risk for alcohol dependence relative to both nonusers (AOR = 2.40, 95% CI = 1.27 to 4.56, p = 0.007) and low-frequency users (AOR = 1.86, 95% CI = 1.10, 3.14, p = 0.020), even after holding constant demographics, typical alcohol consumption, fraternity/sorority involvement, depressive symptoms, parental history of alcohol/drug problems, and childhood conduct problems. Low-frequency energy drink users did not differ from nonusers on their risk for alcohol dependence.

Conclusions: Weekly or daily energy drink consumption is strongly associated with alcohol dependence. Further research is warranted to understand the possible mechanisms underlying this association. College students who frequently consume energy drinks represent an important target population for alcohol prevention.

Key Words: Alcohol Dependence, Caffeine, College Students, Energy Drinks.

Energy drinks are beverages that contain high levels of caffeine—usually much more than a can of cola—ranging from 50 to 500 mg or more per can (Reissig et al., 2009). The surge of energy drink sales in recent years (Heckman et al., 2010) has raised concerns among public health professionals because of both the possibility of adverse effects from caffeine intoxication, in addition to the potential health effects of the high calorie content from sugars in most of these products (Clauson et al., 2008). Moreover, energy drink consumption appears to be associated with an escalation of alcohol-related problems in young adults, and college students in particular. These populations are known to have high rates of alcohol use disorders (Caldeira et al., 2009; Knight et al., 2002; Wu et al., 2007) and represent the principal target of marketing efforts by energy drink manufacturers (Heckman et al., 2010). Not surprisingly, recent studies of college student samples have documented that the past-month prevalence of energy drink use ranges from 39% to 57% (Malinauskas et al., 2007; Miller, 2008b; Oteri et al., 2007).

Several potential mechanisms are plausible to explain the link between energy drinks and excessive alcohol consumption and alcohol use disorders. First, caffeine administered immediately prior to bedtime or throughout the day has been shown to delay sleep onset, reduce total sleep time, alter the normal stages of sleep, and decrease the reported quality of sleep (Alford et al., 1996; Hindmarch et al., 2000; Snel, 1993). Thus, caffeine may prolong drinking episodes by delaying the onset of normal sleepiness resulting in an increase in overall alcohol intake. Second, caffeine’s neuropharmacologic and behavioral effects are mediated through antagonism of the neuromodulator adenosine (Fredholm et al., 1999) that has a
might arguably be even more risky than consuming energy past month (O’Brien et al., 2008). Given the mechanisms sta-
alcohol using students mixed energy drinks and alcohol in the (Miller, 2008a,b). Support for caffeine-induced increases in intention drug use, and other risky behaviors (Arria et al., 2010; related dissipation of caffeine effects.

drinks and alcohol concurrently simply because of the time-
alcohol (e.g., “Jaegerbombs” are made from Jaegermeister drinks are consumed on the same day or mixed directly with DSM-IV criteria for alcohol use disorders than individuals intended, spending a lot of time drinking or obtaining alcohol-related impairment of motor coordination or reaction time (Ferreira et al., 2006). Thus, in addition to a possible direct effect of caffeine on alcohol reinforcement, it is plausible that caffeine could reduce the subjective feelings of being drunk and therefore lead to dose escalation, with the drinker con-
suming more alcohol than they otherwise might. Third, because high levels of caffeine during a drinking session might exacerbate the normal disinhibiting effects of alcohol, co-
ingestion of alcohol and energy drinks could lead to engagement in risky behaviors and physically hazardous activity. A serious related concern is that the drinker’s reduced awareness of his or her level of impairment might lead him or her to mis-
judge his or her ability to safely engage in behaviors such as driving, with the eventual result being increases in alcohol-related injuries and deaths. Fourth, co-ingestion of “hard liquor” and sweet energy drinks might make the taste of such a mixed drink more palatable, thereby increasing the amount consumed. In summary, for these reasons, it is highly plausi-
ble that energy drink users might be more likely to endorse a greater number of dependence items (e.g., drinking more than intended, spending a lot of time drinking or obtaining alcohol, developing tolerance, and withdrawal) and alcohol-abuse items (e.g., driving after drinking, putting oneself in physical danger) and therefore have a higher likelihood of meeting DSM-IV criteria for alcohol use disorders than individuals who do not consume energy drinks. In many cases, energy drinks are consumed on the same day or mixed directly with alcohol (e.g., “Jaegerbombs” are made from Jaegermeister® and Red Bull®). In 1 large college student sample, 24% of alcohol using students mixed energy drinks and alcohol in the past month (O’Brien et al., 2008). Given the mechanisms stated earlier, the co-ingestion of alcohol with energy drinks might arguably be even more risky than consuming energy drinks and alcohol concurrently simply because of the time-
related dissipation of caffeine effects.

Indeed, survey and epidemiological studies also support the notion that energy drink users are at increased risk for heavier drinking, alcohol problems, illicit and nonmedical prescription drug use, and other risky behaviors (Arria et al., 2010; Miller, 2008a,b). Support for caffeine-induced increases in both alcohol consumption and risky behavior was recently demonstrated using event-level drinking data collected in the field from college-aged bar patrons (Thombs et al., 2010). In that study, relative to patrons who consumed only alcohol, patrons who consumed alcohol mixed with energy drinks attained higher blood alcohol concentrations during that drinking session and were more likely to intend to drive when leaving the bar. These results strongly suggest that combined alcohol-energy drink users might be more likely to meet criteria for alcohol dependence. However, it is also possible that the observed associations between energy drink use and heavy drinking could be explained by the fact that these 2 behaviors share common risk factors. Prior studies have identified 2 such potential confounding factors: sensation-seeking and fraternity/sorority involvement. Energy drink users have higher levels of sensation-seeking (Arria et al., 2010), which has long been recognized as an important risk factor for substance-use problems in adolescents as part of a broader high-risk phenotype characterized by disinhibition and undercontrol (Tarter et al., 1999). Sensation-seeking has been linked to binge drinking (Carlson et al., 2010), alcohol-related injury (Mundt et al., 2009), and alcohol-impaired driving (Zakletskaiia et al., 2009). Similarly, involvement in fraternities and sororities is a well-established risk factor for alcohol problems in college students (Baer, 2002; McCabe et al., 2005; Wechsler et al., 2000) and has recently been found to have strong associations with energy drink use (O’Brien et al., 2008).

Three other risk factors for alcohol problems have been identified, but have not been investigated as possible con-
founders in the energy drink–alcohol association, namely family history of alcoholism, depression, and conduct disor-
der. First, family history of alcoholism is regarded as one of the most robust predictors of alcohol problems, as it conveys both genetic and environmental vulnerabilities to alcohol problems. In college students, family history of alcoholism has been found to be significantly associated with greater risk of alcohol consumption and dependence symptoms (Sher et al., 1991), as well as with acceleration and continuation of problematic drinking over time (Jackson et al., 2001). Second, depression is strongly associated with alcohol use disorder in the general adult population, with, for example, 32% of alcohol-dependent individuals experiencing a major depressive episode at some point in their lifetime, often with depression preceding the onset of alcohol problems (Kessler et al., 1996). Some researchers have even speculated that depression and alcoholism might be 2 “manifestations of the same underlying disorder,” in light of strong familial associations between them (Grant et al., 1996). Third, conduct disorder is strongly correlated with alcohol use disorder in the general population (Kessler et al., 1996). In adolescents, conduct disorder appears to be the most common comorbid psychiatric disor-
der with substance-use problems and is heavily implicated in the development of alcohol use disorder in later adolescence (Armstrong and Costello, 2002).

This study aimed to advance the current understanding of the association between energy drinks and heavy drinking.

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2 Red Bull GmbH, Fuschl am See, Austria.
First, we were interested in the possible dose–response relationship between energy drink consumption and heavy drinking. We did not have a measure of co-ingestion of energy drinks with alcohol per se; we believed high-frequency users might be more likely to have occasions of co-ingestion. Second, we were interested in whether energy drink consumption is related not only to heavy drinking but also to alcohol-related problems, and in particular, alcohol dependence as defined by the DSM-IV (American Psychiatric Association, 1994). Lastly, we focused our analyses on the unique relationship, if any, of energy drink use with alcohol dependence over and above the 5 known risk factors described earlier, as well as level of alcohol and caffeine consumption. In summary, the study objectives were to (i) describe the patterns of energy drink use in a sample of fourth-year college students; (ii) explore the possible associations between frequency of energy drink use and demographic characteristics, other caffeine consumption, alcohol use patterns, and alcohol-related consequences; and (iii) develop an explanatory model predicting alcohol dependence on the basis of energy drink use, demographic characteristics, other caffeine consumption, alcohol use patterns, and other suspected risk factors for alcohol problems. We hypothesize that students who use energy drinks frequently will be at greater risk for alcohol dependence, independent of other risk factors for alcohol dependence.

MATERIALS AND METHODS

Design

Data were derived from the College Life Study, an ongoing longitudinal study of a cohort of 1,253 young adults. The sample was ascertained in 2 stages at 1 large, public university in the mid-Atlantic region. First, all incoming first-time, first-year students, ages 17 to 19, were invited to participate in a brief computer-based screening survey during new-student orientation in the summer of 2003, the last year in which participants were living with their parents. At Year 4, participants were asked a series of questions about their use of energy drinks in the past 12 months. The format of the questions was based on input from key informants and modeled after standard methods used with other substances. Interviewers asked, “In the past 12 months, what energy drinks have you consumed?” and recorded the brand names verbatim. To assist with recall, participants were shown a card with photographs depicting several popular energy drinks. Participants who used at least 1 energy drink were then asked to estimate, for each different beverage, the number of days they consumed it in the past 12 months and the quantity they typically consumed. Responses were coded verbatim (e.g., number of cans). To correct for the variability in volume and caffeine content of different energy drinks, we later converted these responses into fluid ounces and milligrams of caffeine, using industry data for each different product (Center for Science in the Public Interest, 2007). An overall measure of past-year frequency of energy drink use was computed as the sum of the number of days each different energy drink was consumed. No information about same-day use of multiple types of energy drinks was obtained; therefore, days on which multiple types might have been used would have been counted more than once. Because this approach might overestimate the actual number of days energy drinks were used in the past year (i.e., valid values > 365 were possible), results should be interpreted as the number of instances of energy drink use (rather than number of days).

Demographics. Sex was recorded as observed at baseline. Race was captured via self-report data at Year 3, and confirmed via administrative data from the university. Socioeconomic status was approximated from the mean adjusted gross income for participants' home ZIP code from publicly available Internal Revenue Service data from 2003, the last year in which participants were living with their parents.3

Caffeine Consumption. At Year 4, participants were asked about their consumption of caffeine in the form of coffee, tea, and soft drinks. For each type of beverage, responses were given as the number of fluid ounces they consumed on a typical day during the past year. Responses were later summed to produce an overall measure of typical caffeine consumption from beverages other than energy drinks.

Alcohol Use Patterns. Several standard interview questions assessed alcohol use patterns, including age at first intoxication (dichotomized for analytic purposes as 15 or younger vs. later than 15 or never). Frequency of alcohol use was derived from questions asking about the number of days they drank any alcohol during the past 12 months, and quantity was assessed from questions about the typical, maximum, and minimum number of drinks they consumed per day, for each day of the week (i.e., Monday through Sunday). These data were later consolidated to compute 2 mean scores representing the typical and maximum number of drinks per drinking day. Means were rounded up to the nearest integer, and nondrinkers were coded as zero.

DSM-IV Criteria for Alcohol Use Disorders. A series of questions were asked that were adapted from the National Survey on

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3Obtained from http://www.melissadata.com/lookups/taxzip.asp.
Drug Use and Health (Substance Abuse and Mental Health Services Administration, 2003) that corresponded to the DSM-IV criteria for alcohol abuse and dependence. Consistent with the DSM-IV-TR (American Psychiatric Association, 1994), dependence was defined by meeting 3 or more of the following 7 criteria: tolerance, withdrawal symptoms, often drinking more than intended, inability to cut down, spending a lot of time drinking or obtaining alcohol, giving up important activities because of drinking, and continuing to drink despite physical or mental health problems. Abuse was defined as nonindependent individuals endorsing 1 or more of the following 4 problems caused by drinking: serious problems at home, work or school; repeated trouble with the law; continued drinking despite problems with friends or family; and repeatedly putting oneself in physical danger (including driving after drinking).

Other Alcohol-Related Consequences. To assess the possibility of physical harm resulting from alcohol use, participants were asked how many times they had visited an emergency department because of their own alcohol use. Participants were also asked if they had experienced any personal injuries during the past year, without specifying alcohol as the cause.

Fraternity/Sorority Involvement. The level of involvement in fraternities and sororities during the past year was assessed with the following response options: “None,” “Irregular: Occasional/some of the time,” and “Regular: Most of the time/frequently/kept to a schedule.” Because very few individuals endorsed the “irregular” response (<4% of the sample), responses of irregular and regular were collapsed to create a dichotomous variable representing any involvement versus none.

Depressive Symptoms. The Beck Depression Inventory (BDI) was self-administered at baseline (Beck et al., 1979). This 21-item survey assesses cognitive, emotional, and physical symptoms of depression during the past few days. The scale had high internal consistency in the present sample (Cronbach’s α = 0.85).

Conduct Problems. Although a clinical diagnosis of conduct disorder was not made, a Conduct Disorder Screener was administered at baseline, which asks about 18 different conduct problems experienced prior to age 18 (e.g., setting fires, lying, stealing, using a weapon in a fight). It was scored following published procedures that take into account varying degrees of severity of each conduct problem (Johnson et al., 1995). Reliability of the computed index was satisfactory in the present sample (Cronbach’s α = 0.74).

Impulsive Sensation-Seeking. The Zuckerman-Kuhlman Personality Questionnaire Short Form (Zuckerman, 2002) was self-administered at baseline, and the 7-item subscale for impulsive sensation-seeking (ImpSS) subscale was used in the analyses as a measure of sensation-seeking. The items had satisfactory reliability in the present sample (Cronbach’s α = 0.74).

Parental History of Alcohol or Drug Problems. Substance-use problems among parents of participants were assessed in Year 2 via a self-administered family tree questionnaire (Mann et al., 1985). The presence of a parental history was coded when the participant reported that either of their biologic parents had a “definite” or “possible” alcohol or drug problem. Responses of “don’t know/don’t remember” were coded as missing.

Statistical Analyses

To statistically correct for our sampling design, we computed sampling weights within each race-sex-drug use cell, such that each cell in the longitudinal sample represents the corresponding number of screened students. Thus, over-sampled participants were weighted to represent relatively fewer students, whereas under-sampled participants represent a greater number of students, thereby enabling us to estimate the prevalence among the entire class (Arria et al., 2008). Descriptive statistics were tabulated for the entire sample with and without sampling weights to describe the prevalence and patterns of energy drink use. To account for the variability across different energy drink products, data on frequency, quantity, and caffeine intake were further analyzed among users of each of the most commonly used products.

To find a satisfactory cut-off value to distinguish “high-frequency” from “low-frequency” energy drink users, we conducted a series of logistic regression models on alcohol dependence with, in turn, the linear, quadratic, and logarithmic functions of the variable on frequency of energy drink use (i.e., number of days), holding constant demographics, typical number of alcohol drinks per day, and baseline scores for BDI and conduct problems. Results ruled out the quadratic function but supported the presence of a logarithmic function (p = 0.001), which was further confirmed by a moderately high correlation between the log-frequency variable and the predicted probabilities (r = 0.52). Next, by inspecting the normalized residuals in relation to the predicted probabilities, we identified 31 days as the cutpoint at which the pattern of variation from the regression line became substantial. Finally, to verify and refine this cutpoint, we replicated the logistic regression model with a series of new 3-level variables representing high-frequency, low-frequency, and nonusers, based on cutoffs of 31, 45, 52, and 61 days. While each version of the variable was significant in the model, results supported 52 days as the strongest cutpoint.

For the remaining analyses, the sample was restricted to the 975 individuals who had complete data on all variables of interest for the analyses. The cutpoint described earlier was used to divide the sample into 3 groups based on frequency of energy drink consumption: nonusers (used 0 days in the past year; n = 338), low-frequency users (used 1 to 51 times in the past year; n = 518), and high-frequency users (used 52 or more times in the past year; n = 119). We then tabulated descriptive statistics for the 3 groups with respect to demographic characteristics, caffeine consumption, alcohol use patterns, and alcohol-related consequences including DSM-IV criteria for alcohol use disorders. Statistically significant differences between the high-frequency and low-frequency users of energy drinks were evaluated using χ² tests of independence for categorical variables and analysis of variance for continuous variables.

Finally, a series of logistic regression analyses were performed to test our hypothesis that high-frequency energy drink use would be associated with greater risk for alcohol dependence, independent of other factors. Thus, in addition to the 3-level variable on energy drink use (nonuser, low-frequency, high-frequency), we included the following other explanatory variables: demographics (i.e., sex, race, and socioeconomic status), caffeine consumption, alcohol use patterns (typical number of alcohol drinks per drinking day and age of first intoxication), and the suspected risk factors for alcohol dependence (i.e., fraternity/sorority involvement, depressive symptoms at baseline, childhood conduct problems, parental history of alcohol/drug problems, impulsive sensation-seeking). Bivariate associations were evaluated for each variable, and then all explanatory variables (except energy drink use) were entered simultaneously in the model. To obtain a more parsimonious model, we adopted a model selection process in which variables were retained only if they approached statistical significance (setting α = 0.10). Nonsignificant variables were dropped from the model and then re-entered 1 at a time. Once we derived our “best” model from this process, we entered the 3-level energy drink-use variable to determine whether it could account for any additional variance in alcohol dependence. The 3 demographic variables were retained in every model, regardless of their statistical significance.
RESULTS

Figure 1 displays the prevalence and frequency of energy drink use in the sample. One-third (34.5%) did not consume energy drinks. About half (52.6%) were low-frequency energy drink users and 13.0% were high-frequency users. Statistically adjusting for our sampling design, the corresponding prevalence estimates were similar (38.6% \( \pm \) 51.3% \( \pm \) 10.1% \( \pm \) respectively). For descriptive purposes, if we assume that energy drink–use patterns were evenly distributed throughout the year, it is possible to further subdivide the sample into occasional (25.0%), monthly (27.6%), weekly (10.4%), and daily or almost daily users (2.6%). Among all 719 energy drink users, the average frequency was 35.2 times/y (SD = 51.0), with individual values ranging from 1 to 370. (The reader is reminded that frequencies were reported without regard to whether multiple types of energy drinks were consumed on the same day.)

Table 1. Patterns of Energy Drink Consumption and Caffeine Intake, by Brand of Energy Drink, Among 719 Past-Year Energy Drink Users

<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Volume (oz)</th>
<th>Caffeine content (mg/can)</th>
<th>Caffeine concentration (mg/oz)</th>
<th>Number (%) who consumed this beverage ≥1 time in past 12 months</th>
<th>Mean number of days beverage was consumed</th>
<th>Typical quantity consumed per drinking day (oz)</th>
<th>Typical caffeine consumption from this beverage per drinking day (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Bull(^a)</td>
<td>8.3</td>
<td>80</td>
<td>9.64</td>
<td>592 (82.3%)</td>
<td>24.50</td>
<td>9.67</td>
<td>93.19</td>
</tr>
<tr>
<td>Monster(^b)</td>
<td>16</td>
<td>160</td>
<td>10.00</td>
<td>242 (33.7%)</td>
<td>11.34</td>
<td>21.53</td>
<td>215.33</td>
</tr>
<tr>
<td>Amp(^c)</td>
<td>8.4</td>
<td>74</td>
<td>8.81</td>
<td>157 (21.8%)</td>
<td>9.68</td>
<td>9.73</td>
<td>85.74</td>
</tr>
<tr>
<td>Rockstar(^d)</td>
<td>8</td>
<td>80</td>
<td>10.00</td>
<td>132 (18.4%)</td>
<td>17.15</td>
<td>15.31</td>
<td>153.13</td>
</tr>
<tr>
<td>SoBe Essential Energy(^e)</td>
<td>8</td>
<td>83</td>
<td>10.38</td>
<td>84 (11.7%)</td>
<td>18.79</td>
<td>19.05</td>
<td>197.67</td>
</tr>
<tr>
<td>Other</td>
<td>n/a</td>
<td>n/a</td>
<td>–</td>
<td>138 (19.2%)</td>
<td>16.28</td>
<td>11.80</td>
<td>–</td>
</tr>
</tbody>
</table>

\(^a\)Data on caffeine content were obtained as mg/can from the Center for Science in the Public Interest (http://www.cspinet.org/new/cafchart.htm) and used to compute mg/oz.

\(^b\)Numbers do not sum to 719 (100%) because multiple responses were allowed.

\(^c\)Typical quantity consumed was missing for 1 user of Red Bull and 3 users of “other” energy drinks. Caffeine concentration per ounce and typical caffeine consumption were not computed as “other” is composed of several different types of energy drinks.

\(^d\)Red Bull GmbH, Fuschl am See, Austria.

\(^e\)Hansen Natural Corporation, Corona, California.

\(^f\)PepsiCo, Purchase, New York.

\(^g\)Rockstar, Inc., Las Vegas, Nevada.

\(^h\)PepsiCo, Purchase, New York.
### Table 2. Sample Characteristics and Comparisons Between Nonusers of Energy Drinks, Low-Frequency Users, and High-Frequency Users With Respect to Demographics, Other Caffeine Consumption, Alcohol Drinking Patterns, Alcohol-Related Consequences, and Suspected Risk Factors for Alcohol-Related Problems

<table>
<thead>
<tr>
<th>Demographic characteristics</th>
<th>Total sample (a) ((n = 975))</th>
<th>High-frequency energy drink users ((n = 119))</th>
<th>Low-frequency energy drink users ((n = 518))</th>
<th>Nonusers of energy drinks ((n = 338))</th>
</tr>
</thead>
<tbody>
<tr>
<td>% or Mean (SD)</td>
<td>% or Mean (SD)</td>
<td>% or Mean (SD)</td>
<td>% or Mean (SD)</td>
<td>% or Mean (SD)</td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td>46.1%</td>
<td>60.5%</td>
<td>53.5%</td>
<td>29.6%</td>
</tr>
<tr>
<td><strong>White</strong></td>
<td>74.2%</td>
<td>77.3%</td>
<td>75.7%</td>
<td>70.7%</td>
</tr>
<tr>
<td>Socioeconomic status(d)</td>
<td>7.36 (3.36)</td>
<td>7.60 (3.44)</td>
<td>7.33 (3.37)</td>
<td>7.33 (3.32)</td>
</tr>
<tr>
<td><strong>Caffeine consumption (typical number of ounces/d)</strong>(b,c,e)</td>
<td>19.24 (14.82)</td>
<td>25.79 (15.66)</td>
<td>19.49 (14.59)</td>
<td>16.55 (14.15)</td>
</tr>
<tr>
<td><strong>Alcohol use patterns</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age 15 or younger at first alcohol intoxication(b,c,e)</td>
<td>36.4%</td>
<td>59.7%</td>
<td>38.4%</td>
<td>25.1%</td>
</tr>
<tr>
<td><strong>Typical number of drinks/drinking day</strong>(b,c,e)</td>
<td>97.5 (71.6)</td>
<td>141.6 (70.8)</td>
<td>103.1 (69.2)</td>
<td>73.5 (66.3)</td>
</tr>
<tr>
<td><strong>Maximum number of drinks/drinking day</strong>(b,c,e)</td>
<td>4.30 (3.02)</td>
<td>6.15 (3.12)</td>
<td>4.64 (2.92)</td>
<td>3.13 (2.68)</td>
</tr>
<tr>
<td><strong>DSM-IV criteria for alcohol use disorders</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol dependence(b,e)</td>
<td>12.1%</td>
<td>26.9%</td>
<td>11.6%</td>
<td>7.7%</td>
</tr>
<tr>
<td><strong>Individual criteria for alcohol dependence</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tolerance(b,c)</td>
<td>24.7%</td>
<td>35.3%</td>
<td>26.1%</td>
<td>18.9%</td>
</tr>
<tr>
<td>Withdrawal(b)</td>
<td>2.6%</td>
<td>6.7%</td>
<td>2.5%</td>
<td>1.2%</td>
</tr>
<tr>
<td>Drank more than intended(b,e)</td>
<td>9.1%</td>
<td>21.0%</td>
<td>8.9%</td>
<td>5.3%</td>
</tr>
<tr>
<td>Unable to cut down(b,e)</td>
<td>9.8%</td>
<td>16.8%</td>
<td>9.3%</td>
<td>8.3%</td>
</tr>
<tr>
<td>Spent a lot of time drinking or obtaining alcohol(b,c,e)</td>
<td>33.6%</td>
<td>50.4%</td>
<td>36.1%</td>
<td>24.0%</td>
</tr>
<tr>
<td>Gave up important activities(b)</td>
<td>9.9%</td>
<td>14.3%</td>
<td>10.4%</td>
<td>5.5%</td>
</tr>
<tr>
<td>Continued drinking despite health problems(b)</td>
<td>11.7%</td>
<td>19.5%</td>
<td>12.0%</td>
<td>8.6%</td>
</tr>
<tr>
<td>Alcohol abuse(c)</td>
<td>34.7%</td>
<td>36.1%</td>
<td>40.3%</td>
<td>25.4%</td>
</tr>
<tr>
<td><strong>Individual criteria for alcohol abuse</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serious problems at home, work, or school</td>
<td>2.4%</td>
<td>4.2%</td>
<td>2.1%</td>
<td>2.1%</td>
</tr>
<tr>
<td>Repeatedly in trouble with the law</td>
<td>0.8%</td>
<td>0.0%</td>
<td>1.2%</td>
<td>0.6%</td>
</tr>
<tr>
<td>Continued drinking despite problems with family/friends</td>
<td>5.4%</td>
<td>7.6%</td>
<td>5.6%</td>
<td>4.4%</td>
</tr>
<tr>
<td>Drove after drinking (sometimes or regularly)<strong>b,c,e</strong></td>
<td>32.6%</td>
<td>44.5%</td>
<td>37.5%</td>
<td>21.0%</td>
</tr>
<tr>
<td>Put self in physical danger while drunk (sometimes or regularly)<strong>b,c</strong></td>
<td>17.5%</td>
<td>29.4%</td>
<td>19.5%</td>
<td>10.4%</td>
</tr>
<tr>
<td><strong>Other consequences</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER visit because of alcohol use</td>
<td>1.2%</td>
<td>3.4%</td>
<td>1.0%</td>
<td>0.9%</td>
</tr>
<tr>
<td>Personal injury (any cause)<strong>b</strong></td>
<td>19.0%</td>
<td>24.6%</td>
<td>20.6%</td>
<td>14.7%</td>
</tr>
<tr>
<td>Blacked out from drinking alcohol<strong>b,c,e</strong></td>
<td>37.7%</td>
<td>59.7%</td>
<td>40.2%</td>
<td>26.3%</td>
</tr>
<tr>
<td>Missed class because of alcohol hangover<strong>b,c,e</strong></td>
<td>35.8%</td>
<td>59.7%</td>
<td>39.2%</td>
<td>22.2%</td>
</tr>
<tr>
<td>Concentration problems because of alcohol hangover<strong>b,c</strong></td>
<td>46.4%</td>
<td>60.5%</td>
<td>50.8%</td>
<td>34.6%</td>
</tr>
<tr>
<td>Usual activities were limited because of alcohol hangover<strong>b,c,e</strong></td>
<td>51.9%</td>
<td>75.6%</td>
<td>56.8%</td>
<td>36.1%</td>
</tr>
<tr>
<td><strong>Suspected risk factors for alcohol-related problems</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fraternity/Sorority involvement (Year 4)<strong>b,c,e</strong></td>
<td>26.3%</td>
<td>45.4%</td>
<td>27.0%</td>
<td>18.3%</td>
</tr>
<tr>
<td>Beck Depression Inventory (Year 1)</td>
<td>5.29 (5.05)</td>
<td>5.30 (4.40)</td>
<td>5.11 (4.64)</td>
<td>5.56 (5.81)</td>
</tr>
<tr>
<td>Conduct problems<strong>b,c</strong></td>
<td>6.55 (4.70)</td>
<td>7.95 (4.88)</td>
<td>6.88 (4.67)</td>
<td>5.54 (4.49)</td>
</tr>
<tr>
<td>Parental history of alcohol/drug problems</td>
<td>24.5%</td>
<td>27.7%</td>
<td>23.0%</td>
<td>25.7%</td>
</tr>
<tr>
<td>Impulsive sensation-seeking<strong>b,c</strong></td>
<td>3.51 (2.16)</td>
<td>3.87 (2.12)</td>
<td>3.68 (2.12)</td>
<td>3.12 (2.2)</td>
</tr>
</tbody>
</table>

Statistical significance of overall chi-square tests (for categorical variables) and ANOVA (for continuous variables) is denoted as follows: \(*p < 0.05; **p < 0.01; ***p < 0.001.\)

\(a\)Results are presented for 975 individuals with nonmissing data, with the following exceptions: 2 cases missing "continued drinking despite health problems," 1 case missing "continued drinking despite problems with friends/family," and 9 cases missing "personal injury."

\(b\)Denotes significant difference between high-frequency and nonuser groups.

\(c\)Denotes significant difference between low-frequency and nonuser groups.

\(d\)Socioeconomic status was approximated from the mean adjusted gross income for participants' home ZIP code from publicly available IRS data from 2003, the last year in which participants were living with their parents.

\(e\)Denotes significant difference between high-frequency and low-frequency groups.
ENERGY DRINK CONSUMPTION AND INCREASED RISK FOR ALCOHOL DEPENDENCE

Red Bull GmbH, Fuschl am See, Austria.

a

0.007
0.020
0.328
2.40 (1.27, 4.56)
1.86 (1.10, 3.14)
1.29 (0.76, 2.19)
0.19

0.048
0.073
0.003
0.044
2.43)
1.08)
1.12)
2.58)
(1.00,
(1.00,
(1.02,
(1.01,
1.56
1.04
1.07
1.62
0.016
0.072
0.002
0.029
2.63)
1.08)
1.12)
2.66)
(1.11,
(1.00,
(1.02,
(1.05,

0.18

1.71
1.04
1.07
1.68
0.018
0.072
0.006
0.029
0.908
2.61)
1.08)
1.12)
2.68)
1.10)
(1.10,
(1.00,
(1.02,
(1.05,
(0.90,
<0.001
0.071
<0.001
0.108
0.017

<0.001
<0.001
0.066

3.19)
1.07)
1.13)
2.16)
1.22)

4.41 (2.50, 7.80)
2.81 (1.73, 4.57)
1.57 (0.97, 2.55)
n⁄a

(1.44,
(1.00,
(1.04,
(0.93,
(1.02,
2.14
1.03
1.08
1.41
1.12

0.18

1.69
1.04
1.07
1.68
1.00

<0.001
1.29 (1.19, 1.39)
<0.001
1.31 (1.22, 1.42)
<0.001
0.248
<0.001
<0.001
1.26 (1.19, 1.35)
2.22 (1.50, 3.27)

1.30 (1.20, 1.41)
0.25 (0.83, 2.05)

0.007
0.053
0.067
0.51 (0.31, 0.83)
0.61 (0.37, 1.01)
1.05 (1.00, 1.11)
0.015
0.045
0.071
0.55 (0.34, 0.89)
0.60 (0.37, 0.99)
1.05 (1.00, 1.11)
0.024
0.030
0.097
0.707
0.93)
0.95)
1.11)
1.02)
(0.35,
(0.35,
(0.99,
(0.99,
0.57
0.57
1.05
1.00
0.471
0.737
0.022
0.192
1.69)
1.69)
1.11)
1.02)
(0.78,
(0.69,
(1.01,
(1.00,
1.15
1.08
1.06
1.01

AOR (95% CI)
p

AOR (95% CI)

p

Model 2
Model 1

Socioeconomic status was approximated from the mean adjusted gross income for participants’ home ZIP code from publicly available IRS data from 2003, the last year in which
participants were living with their parents.

4

OR (95% CI)

Several differences existed between the high-frequency
group and nonusers, namely the high-frequency group had
signiﬁcantly more men (60.5% vs. 29.6%) and was more
involved in sororities ⁄ fraternities (45.4% vs. 18.3%), more
heavily involved in alcohol in every way measured, and
more likely to meet criteria for alcohol dependence (26.9%
vs. 7.7%), drive after drinking (44.5% vs. 21.0%), and place
themselves in physical danger while drunk (29.4% vs.
10.4%). They also consumed more other caffeinated beverages (25.79 vs. 16.55 ounces ⁄ d). Individuals in the highfrequency group were also more likely than nonusers to black
out, miss class because of hangover, report activity limitations
and concentration problems because of hangovers, and
experience personal injury. The overall proportion meeting
criteria for alcohol abuse did not differ among these groups.

Bivariate Results

Differences Between High-Frequency Energy Drink Users
and Nonusers

Table 3. Results of Logistic Regression on Alcohol Dependence (n = 975)

The high- and low-frequency groups did not differ signiﬁcantly with respect to demographic characteristics (see
Table 2). Aside from energy drinks, the high-frequency group
consumed more caffeinated beverages on average than the
low-frequency group (25.79 vs. 19.49 ounces ⁄ d). Several
signiﬁcant differences were observed regarding alcohol use
patterns and consequences. Speciﬁcally, individuals in the
high-frequency group were more likely to have gotten drunk
at an early age (59.7% vs. 38.4%), drank alcohol more frequently in the past year (141.6 vs. 103.1 days), drank more
alcohol drinks per day on both a typical drinking day (6.15 vs.
4.64) and a ‘‘maximum’’ drinking day (9.99 vs. 7.29), and were
more likely to meet criteria for alcohol dependence (26.9% vs.
11.6%, all ps < 0.001). Of all the other alcohol-related
consequences, 3 were signiﬁcantly more prevalent in the highfrequency group relative to the low-frequency group, namely
blacking out (59.7% vs. 40.2%), missing class because of hangover (59.7% vs. 39.2%), and hangover-related limitations in
usual activities (75.6% vs. 56.8%, all ps < 0.001). Finally,
with respect to the 5 suspected risk factors for alcohol dependence, compared to the low-frequency group, individuals
in the high-frequency group were more likely to have sorority ⁄
fraternity involvement (45.4% vs. 27.0%, p < 0.001), but
were similar with respect to all the other risk factors.

p

Differences Between High-Frequency and Low-Frequency
Energy Drink Consumption Groups

Demographics
Sex = Male
Race = White
Socioeconomic statusa
Caffeine consumption
Alcohol use patterns
Typical number of drinks ⁄ drinking day
Age 15 or younger at first alcohol intoxication
Suspected risk factors for alcohol-related problems
Fraternity ⁄ sorority involvement (Year 4)
Beck Depression Inventory (Year 1)
Conduct problems
Parental history of alcohol ⁄ drug problems
Impulsive sensation-seeking
Energy drink consumption (Year 4)
High frequency vs. none
High frequency vs. low frequency
Low frequency vs. none
Nagelkerke R2

AOR (95% CI)

Model 3

p

As seen in Table 1, Red Bull,4 was the brand most commonly and frequently consumed (82.3% of users; mean
24.5 d ⁄ y). Typical caffeine consumption from Red Bull
(mean 93.2 mg per drinking day) was considerably lower on
average relative to other products. More than half (57.3%) of
users drank more than 1 type of energy drink in the past
year.

7


With respect to the suspected risk factors for alcohol dependence, the high-frequency group scored significantly higher than nonusers on both childhood conduct problems (7.95 vs. 5.54) and impulsive sensation-seeking (3.87 vs. 3.12).

**Differences Between Low-Frequency Energy Drink Users and Nonusers**

Not surprisingly, in most comparisons, the low-frequency group generally occupied an intermediate position between the high-frequency and nonuser groups, and were therefore significantly different from nonusers on several (but not all) of the same variables as in the previous comparison of high-frequency users with nonusers. These differences are shown in Table 2. The only variable that was uniquely associated with low-frequency but not high-frequency energy drink consumption was meeting criteria for alcohol abuse, which was significantly more prevalent in the low-frequency group than nonusers (40.3% vs. 25.4%).

Lastly, it is noteworthy that race, socioeconomic status, depressive symptoms, and parental history of alcohol/drug problems were not associated with energy drink consumption in any of the aforementioned comparisons. Several other variables with extremely low sample prevalence also showed no association (i.e., problems at home, work, or school; trouble with the law; continued drinking despite problems with family/friends; and emergency department visits).

**Association Between Energy Drink Consumption and Alcohol Dependence**

As is evident from Table 3, 3 of the 5 suspected risk factors were significantly associated with alcohol dependence at the bivariate level (i.e., fraternity/sorority involvement, childhood conduct problems, impulsive sensation-seeking), but caffeine consumption was not. After the model selection process described earlier, Model 2 was deemed the “best” model to explain the relationship between all possible explanatory variables (with the exception of energy drink consumption) and alcohol dependence. Finally, Model 3 resulted when energy drink consumption was added to Model 2. In Model 3, high-frequency users were more than twice as likely as nonusers (AOR = 2.40, 95% CI = 1.27 to 4.56, \( p = 0.007 \)) and almost twice as likely as low-frequency users (AOR = 1.86, 95% CI = 1.10, 3.14, \( p = 0.020 \)) to meet criteria for alcohol dependence, after holding constant other suspected risk factors. Low-frequency energy drink consumption (relative to nonuse) was not independently associated with alcohol dependence (\( p > 0.3 \)).

**DISCUSSION**

In this study of fourth-year college students at 1 large public university, energy drinks were consumed by nearly two-thirds of students (61.4% of all college students) at some time in the past year, and 10.1% of these consumers admitted drinking energy drinks on a weekly or daily basis. These high-frequency energy drink users had significantly heavier alcohol involvement, including drinking more often, drinking more heavily on days they drank, and having greater risk for alcohol-related problems such as blackouts, hangover-related impairments, and meeting DSM-IV criteria for alcohol dependence. Furthermore, multivariate analyses revealed that high-frequency energy drink users were twice as likely as low-frequency users—and more than twice as likely as nonusers—to meet criteria for alcohol dependence, independent of demographics, typical quantity of alcohol consumed, fraternity/sorority involvement, depressive symptoms, parental history of alcohol/drug problems, and childhood conduct problems.

The present findings support and extend prior evidence that energy drink users are at increased risk for substance-use problems (Miller, 2008a). To our knowledge, this study contributes new information regarding the association between energy drink consumption and alcohol dependence, and results support the expected dose–response relationship between energy drink consumption and heavy drinking. Specifically, high-frequency energy drink users were at increased risk for alcohol dependence, independent of several other risk factors. While low-frequency consumption was not independently associated with increased risk for alcohol dependence, low-frequency users experienced several more alcohol-related problems than nonusers.

While this study confirmed the observations of other investigators (Miller, 2008a,b; O’Brien et al., 2008) that energy drink users are different from nonusers in a number of respects (i.e., higher risk-taking tendencies and substance involvement), it appears that these characteristics do not fully explain the increased risk for alcohol-related problems. Our multivariate analyses confirmed that high-frequency energy drink consumption confers a risk over and above that of these confounding risk factors for alcohol dependence.

However, because the study was cross-sectional, the possibility cannot be ruled out that heavy drinkers rely on energy drinks to help them function normally throughout the day, as a way of compensating for alcohol-related hangover effects. For example, a college student might use energy drinks to get through classes on the day after a drinking binge, and if chronic partying interferes with their study habits they might consume energy drinks to pull “all-nighters” before exams. Future research is needed to clarify the mechanisms by which energy drink consumption might be related to increased risk for alcohol-related problems, for example whether energy drink use increases the reinforcing effects of alcohol or the disinhibition typically associated with alcohol remains to be seen. Unfortunately, experimental studies might be limited in the extent to which they can model the extremely high levels of alcohol consumption that occur in naturalistic settings. Moreover, it will be important to understand the variety of contexts in which energy drinks are consumed and the differential risks between simultaneous versus concurrent ingestion of alcohol and energy drinks.
The findings must be interpreted in light of several limitations. Self-report studies are always subject to response bias, and while we have no indication that over- or under-reporting has occurred, we cannot rule out this possibility. Second, our measure of caffeine consumption relies on secondary data, albeit from a source we regard as reliable, but we did not conduct our own testing to confirm the caffeine concentrations of different products. Third, although we did not explicitly ask whether or not participants were co-ingesting energy drinks with alcohol, prior research evidence (Malinauskas et al., 2007; O'Brien et al., 2008) as well as anecdotal evidence from our participants indicates that consuming mixed drinks containing both alcohol and energy drinks is quite common, especially among high-frequency consumers. Lastly, the study ascertained young adults from 1 large public university, and thus we cannot generalize the findings to other settings.

This study’s strengths include a large sample size and the breadth of domains assessed in this study, which provides a rare opportunity to compare the statistical effect of energy drink use with that of several other important indicators of risk. In many ways, college students are an ideal population in which to study the association of energy drinks and alcohol use, because of the popularity therein of both beverages and the high prevalence of alcohol-related problems. Moreover, this study demonstrates a new methodology for measuring energy drink consumption in a more fine-grained manner than prior studies. One unique contribution of this study is the use of an empirically derived cutpoint characterizing a high-risk pattern of energy drink consumption, on the basis of risk for alcohol dependence. Interestingly, certain other alcohol-related consequences did not correlate as well with this definition of high-risk energy drink consumption, notably drunk driving and personal injury, raising the possibility that lower thresholds might better distinguish energy drink consumption patterns that confer high risk for these consequences.

In this study, we focused our analyses on alcohol dependence rather than alcohol use disorders and chose not to consider alcohol abuse as a main outcome. This decision was made in light of the body of evidence cited by the Substance Use Disorders Workgroup for the development of the DSM-5, which has concluded that the diagnostic distinction between abuse and dependence is questionable and recommends the transition to a single diagnosis of substance-use disorder that would be graded according to levels of severity (American Psychiatric Association, 2010). Not surprisingly, in our sample, there is considerable overlap between individuals meeting criteria for abuse and dependence over time, for example, with 57.5% (data not presented in a table) of our alcohol dependence cases in Year 4 having already met criteria for alcohol abuse in any of the 3 prior interviews. An examination of all 4 annual assessments revealed that 41.8% of abuse cases also met dependence criteria at some point. Therefore, we regard alcohol dependence as defined in the DSM-IV (American Psychiatric Association, 1994) as the more severe manifestation of alcohol use disorder and a more reliable indicator of serious alcohol problems in our sample.

The present findings have important implications for researchers, policymakers, and the general public. Researchers should be aware of the fact that many young adults consume alcohol in the context of energy drinks and should add questions about energy drink consumption to their assessment instruments. Given the mounting public health concern regarding how co-ingestion of energy drinks with alcohol might exacerbate aggressive and dangerous behaviors by creating a state of “wide-awake drunkenness” (Arria and O’Brien, 2009), more research is needed to understand the nature and extent of this problem.

The present finding that frequent consumption of energy drinks—but not other caffeinated beverages—contributes to increased risk for alcohol dependence adds more urgency for policymakers to adopt and enforce measures that would separate the consumption of these 2 beverages. If our findings are replicated, labeling of energy drink products that caution against mixing alcohol and energy drinks might be warranted, and vendors could be required to limit sales of energy drinks and cocktails made with them to patrons who are intoxicated.

It is troubling that there are no requirements for disclosing the caffeine content of energy drinks on the product label. We observed considerable variability in how much caffeine users are consuming from different energy drinks, and therefore find it plausible that, when consuming one of the more concentrated energy drinks, some users might be ingesting much more caffeine than they realize. Individuals who typically consume an energy drink with lower caffeine content might inadvertently ingest more caffeine than intended if under the incorrect assumption that they are “all the same.” We strongly encourage policymakers to require explicit labeling of energy drinks, so that consumers can have accurate information regarding caffeine content.

Lastly, with respect to the general public, parents and peers could play a valuable role in monitoring risk for alcohol-related consequences among energy drink users. Parents should regard frequent energy drink consumption as a red flag for heavy drinking in their college-aged children, and discourage mixing alcohol and energy drinks. Young adults should be educated about the risks of this behavior and encouraged to exercise vigilance and intervene appropriately when they observe their peers consuming energy drinks in risky situations. For example, they should be educated to understand the difference between someone who is impaired but wide awake and someone who is safe to drive. This could be a natural extension of highly successful past campaigns that have made the concept of a “designated driver” second nature for many young adults.

Further study is certainly warranted to understand how patterns of co-ingestion of alcohol and energy drinks relate to the risk for serious alcohol problems. Strong evidence now exists supporting the notion that mixing energy drinks with alcohol leads to greater alcohol consumption and therefore more dangerous blood alcohol levels (Thombs et al., 2010).
Moreover, in light of the commonalities between the characteristics of energy drink users and heavy drinkers, future studies should strive to account for multiple risk factors and any multicollinearity between them.

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REFERENCES


The "High" Risk of Energy Drinks

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The Food and Drug Administration (FDA) announced on November 17, 2010, that caffeine is an unsafe food additive to alcoholic beverages will effectively make several “premixed” alcoholic energy drinks prohibited for sale in the United States. Additionally, the Federal Trade Commission has notified manufacturers that they are engaged in the potential illegal marketing of unsafe alcoholic drinks. These rulings have been regarded by some as a welcome response to an increasing public health risk. Scientists and health professionals assisted in the FDA action by arguing that, on the basis of evidence from an increasing number of scientific studies, the direct addition of caffeine to alcoholic beverages does not meet the “generally recognized as safe” standard.1 Nevertheless, these premixed alcoholic energy drinks are only a fraction of the true public health risk.

In this Commentary, we outline why regular (nonalcoholic) energy drinks might pose just as great a threat to individual and public health and safety. More research that can guide actions of regulatory agencies is needed. Until results from such research are available, the following should be seriously considered: health care professionals should inform their patients of the risks associated with the use of highly caffeinated energy drinks; the public should educate themselves about the risks of energy drink use, in particular the danger associated with mixing energy drinks and alcohol; and the alcohol and energy drink industries should voluntarily and actively caution consumers against mixing energy drinks with alcohol, both on their product labels and in their advertising materials.

Energy drinks are beverages that contain modest to relatively high levels and concentrations of caffeine (range: 50-505 mg caffeine/serving; 2.5-35.7 mg caffeine/oz) compared with other caffeinated beverages such as a 12-ounce cola (range 34-54 mg; 2.9-4.5 mg caffeine/oz) or a 6-ounce cup of coffee (range 77-150 mg; 12.8-25 mg caffeine/oz).2 In contrast, energy “shots” are low-volume (1-2 oz) beverages and therefore have an even higher concentration of caffeine than other energy drinks (range 100-350 mg; 90-171 mg caffeine/oz).2 Although the actual caffeine concentration in some types of coffee varies substantially, with some levels comparable to that of some energy drinks, coffee is usually consumed hot and therefore more slowly. A major challenge for health professionals and researchers is the heterogeneity of the numerous energy drink products available; also the industry is largely unregulated.

Energy drink use is highly prevalent, constituting a $5.4 billion market in 2006 in the United States alone.2 A trip to any college campus would reveal that energy drinks have become enmeshed in the subculture of partying on US college campuses because of the simultaneous consumption of energy drinks with alcoholic beverages.3-6 Energy drinks are also popular among non–college-attending adults. Research shows that energy drink consumption is potentially harmful for 3 reasons. First, caffeine has been clearly associated with adverse health effects in susceptible individuals. Among adolescents, caffeine consumption has been linked to elevated blood pressure7 and sleep disturbances.8 Among pregnant women, high caffeine intake is associated with risk for late miscarriages, stillbirths,9 and small-for-gestational-age infants. Therefore, continued public health awareness regarding high levels of caffeine consumption, no matter what the beverage source, in sensitive individuals is certainly warranted.

Second, the practice of mixing energy drinks with alcohol—which is more widespread than generally recognized—has been linked consistently to drinking high volumes of alcohol per drinking session and subsequent serious alcohol-related consequences such as sexual assault and driving while intoxicated.6 Although consumers might be under the impression that caffeine counteracts the adverse effects of alcohol, research has demonstrated that individuals who combine energy drinks with alcohol underestimate their true level of impairment.10 Although any type of caffeine consumption after a drinking session might reduce sleepiness, it does not alleviate alcohol-related impairment. The state of being less likely to accurately appraise the true level of impairment has been labeled “wide-awake drunkenness” and can lead to engage...
ing in risky behavior. Recent regulatory actions to encourage removal of caffeine from some premixed alcoholic energy drinks should alert consumers that mixing alcohol with highly caffeinated energy drinks carries similar health risks and that this practice is not comparable to consuming mixed drinks such as rum and Coke, which have much lower caffeine concentrations. A second major concern is that simultaneously consuming alcohol and energy drinks can prolong the drinking session by keeping the individual awake longer and therefore may lead to drinking much more alcohol than intended. More data are needed to clarify the association between combining energy drinks with alcohol and the risk of alcohol poisoning.

Third, regardless of whether energy drinks are mixed with alcohol, recent research suggests that, even after adjustment for potential confounders such as heavier drinking patterns, energy drink use might confer a risk for alcohol dependence and perhaps nonmedical prescription drug use. The mechanisms underlying these associations are unclear. The link between energy drink use and alcohol dependence might be attributable to the popularity of mixing alcohol with energy drinks or it might be that alcohol-dependent individuals rely on highly caffeinated beverages to manage hangover effects.

Probably most concerning is the possibility that caffeine’s neuropsychopharmacologic effects might play a role in the propensity for addiction. More research is needed to understand the mechanism and to clarify the temporal association of these effects; but taken together with previous research, the message to health professionals, consumers, and the energy drink and alcohol industries should be clear: the consumption of energy drinks mixed with alcohol may have adverse health and safety consequences.

The National Institutes of Health must recognize the lack of systematic research on the health and safety effects of energy drink consumption, especially among adolescents. More research is needed in particular to guide the decision making of regulatory agencies related to placing a scientifically validated upper limit on the amount of caffeine a manufacturer can include in a single serving of any beverage. Currently, the maximum allowable caffeine limit set by the FDA for cola-like drinks is 0.02%, or 71 mg per 12-oz serving. It is unclear why this limit does not apply to energy drinks; some claim that because energy drinks contain herbal extracts and some vitamins, they are not subject to the same caffeine limit. Although more research is necessary, so are proactive steps to protect public health. To promote informed consumer choices, regulatory agencies should require specific labeling regarding caffeine content, with warnings about the risks associated with caffeine consumption in adolescents and in pregnant women as well as with explicit information about the potential risks associated with mixing energy drinks with alcohol. Scientists and health professionals cannot wait for further FDA action—available scientific evidence indicates that action is needed now. The collective priority of health professionals should be to educate the public about known risks, and industry officials and servers should caution consumers about the risks of mixing alcohol with energy drinks.


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REFERENCES

Caffeine: behavioral effects of withdrawal and related issues

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Abstract

Acquired tolerance to some behavioral effects of caffeine in humans is widely assumed to occur but is poorly documented and appears, at most, to be of low magnitude. Withdrawal from regular consumption of caffeine has been reported to result in a variety of symptoms, including: irritability, sleepiness, dysphoria, delirium, nausea, vomiting, rhinorrhea, nervousness, restlessness, anxiety, muscle tension, muscle pains and flushed face. Some of these same symptoms have been reported following excess intake of caffeine. The prevalence of symptoms reported on withdrawal in different studies also covers a wide range from 11% or less to 100%. It is suggested that the evidence leads to the conclusion that non pharmacological factors related to knowledge and expectation are the prime determinants of symptoms and their reported prevalence on withdrawal of caffeine after regular consumption.

Keywords: Acquired tolerance; Adaptive changes; Alcohol; ‘Binge’ mode; Cocaine; Double-blind; Heroin; Locomotor activity; Non-pharmacological factors; Observer rating; Prevalence of symptoms; Prospective clinical type study; Questionnaires; Retrospective survey study; Tolerance; Withdrawal symptoms

1. Introduction

Tolerance, withdrawal symptoms and dependence will be discussed. They are related phenomena of wide occurrence in pharmacology. Tolerance is the reduced effect of an agent that often results from regular administration of the agent over a period of time, a few days or a week or two, depending on the agent, so that larger doses are needed to produce the original effect. Withdrawal symptoms are symptoms that can occur when such regular administration is discontinued. Withdrawal symptoms often occur when tolerance has developed, but the quantitative relationship between degree of tolerance and consistency of occurrence and intensity of withdrawal symptoms is usually not strong and, again, depends on the agent. There is no universally accepted definition of dependence, but a widely accepted definition is that dependence on an agent is present during regular administration of an agent if discontinuance precipitates withdrawal symptoms (O’Brien, 1995). Dependence is thus defined in terms of withdrawal symptoms.

Reasons for the poor relationship between tolerance and withdrawal are clear if tolerance is recognized as an adaptive phenomenon. Adaptive changes are usual when physiological systems are subjected to continuing external influence. As an example, consider the adaptive changes that take place when a subject moves from sea level to an altitude of 5000 m and stays there. The partial pressure of oxygen at 5000 m is about 80 mmHg, down from 150 mmHg at sea level. A partial pressure of 80 mmHg is too low for full oxygenation of blood as it passes through the lungs. Adaptive changes occur. For example, respiration increases, cardiac output increases and the concentration of red cells in the blood is increased by cellular proliferation in bone marrow. The first two changes can take place quite quickly, but the last is slower. When the subject returns to sea level the changes in respiration and circulation can regress rather quickly, although if the stay at 5000 m has been prolonged and the heart has hypertrophied that takes some time to regress. The return of the concentration of red cells to normal takes many days. During that time, the subject has blood with a higher viscosity than normal, making circulation more difficult: withdrawal symptoms...
from being withdrawn from high altitude if you will. The homology to adaptation to regularly administered chemical agents is obvious. The regular administration of the agent produces changes in the physiological systems affected by the agent. Adaptive changes take place to reduce the changes caused by the agent. When administration of the agent is discontinued the countervailing adaptations are unopposed and the result can be symptoms. Agents modify, directly and indirectly, more than one physiological system, and the adaptive changes in the different systems will differ in kind and effectiveness and in the rate at which they disappear when administration of the agent is discontinued. As with altitude, the degree of tolerance seen for the different systems can vary, and the frequency, nature and intensity of withdrawal symptoms related to the different physiological systems can likewise vary. It is thus unreasonable to expect to be able to make generally applicable quantitative statements about tolerance to an agent.

Even when attention is focused on a single effect of an agent, it is logically difficult to generate a pharmacologically satisfactory quantitative account of tolerance. In classical studies of drug antagonisms (and tolerance is a special case of antagonism, self-antagonism) quantitative assessments often could be made on isolated tissues at the rate of one determination every 2 or 3 min. To obtain dose–effect curves for tolerance, first tolerance must be developed by exposure to agent, a matter commonly of 1 week or more, and then the determination of changes in effects of acute doses to assess tolerance can be made usually only at the rate of one per day, at best. The completion of the matrix of different levels of exposures and dose–effect curves at each level thus is a formidable undertaking, certainly for a drug with the pharmacokinetics of caffeine: and does not appear to have been done even in outline. For completeness, all the determinations should be repeated for different initial periods of developed tolerance: say, a week, a month, a year, 3 years. ... While the demonstration that tolerance can develop to a particular effect of an agent is relatively easy and has been done innumerable times, the demonstration lacks a quantitative context.

It should be noted that although the phenomenon of tolerance has fascinated many pharmacologists and others, and much fine work has been done, the pressure of the clinic for quantitative information has been lacking. Clinicians are aware of the existence of tolerance, but dosage of regularly taken therapeutically important drugs is determined by assessing the desired effects and adjusting dosage to optimize. Whether the adjustment of dosage is made necessary by development of tolerance or by other changes in the patient may be of importance in indicating changes in progress of the disease, a quantitative account of tolerance to the agent would not be much help in diagnosis.

2. Tolerance to caffeine

That tolerance to caffeine can develop in some species and under some regimes is beyond doubt. As an example, Finn and Holtzman (1986) exposed rats to more than 50 mg/kg per day of caffeine, obtained from the drinking water. After a week, they started giving the rats a series of doses of caffeine from 3 to 100 mg/kg assessing the effects on locomotor activity. While in rats that had not been drinking caffeine, 3 mg/kg caused about a 50% increase in locomotor activity, no dose caused an increase of more than about 25% in the caffeine drinking rats. Thus the tolerance to caffeine was great and was not surmountable by increasing the dose. Intake of 50 mg/kg per day is about half a log unit above what the heaviest human users obtain from dietary sources. Tolerance to some behavioral effects of caffeine in human users in the range that people consume caffeine is widely assumed to occur but has been surprisingly little documented. It was shown many years ago that 150 mg caffeine taken 30–40 min before retiring delayed sleep in non-coffee drinkers but not in coffee drinkers, but the difference between the groups was small (Colten et al., 1968). The difference could be related to factors determining whether an individual chose to be a coffee drinker and not to tolerance to caffeine due to the regular consumption of coffee. In any case, there seem to have been no subsequent studies that challenge the conclusion of the authors of the study that “tolerance to [behavioral effects of] caffeine in man appears to be of low magnitude”. For a more recent account of the subject, see Fredholm et al. (1999).

3. Withdrawal symptoms

A review of caffeine dependence studies by Griffiths and Woodson (1988) listed a wide variety of withdrawal symptoms that had been reported when consumers abruptly discontinued caffeine consumption, such as headache, irritability, sleepiness, lethargy, mental confusion, insomnia, psychomotor impairment, hand or limb tremor, weakness, dysphoria, delirium, nausea, vomiting, rhinorrhea, nervousness, restlessness, anxiety, muscle tension, muscle pains and flushed face. It is interesting that some of these same symptoms have also been reported in the opposite situation, that of excess consumption of caffeine, namely mental confusion, insomnia, tremor, nausea, nervousness, restlessness and flushed face (DSM IV). The symptoms are reported by the subject, and often little or nothing can be confirmed by physical examination by a physician.

Not only are reported withdrawal symptoms varied but the frequency of occurrence reported by subjects in different studies also varies widely. There are two general ways of investigating withdrawal phenomena.
One way is to survey a sample of individuals who regularly drink caffeinated beverages and ask them whether they have ever omitted their beverage and, if so, whether they had symptoms and, if so, what they were. This is a retrospective survey study. The second way is to select a sample of regular drinkers and switch them to a caffeine-free diet, usually striving to make the switch double-blind; that is, such that neither subject nor rater know when the switch is made. This is a prospective clinical type study. Both approaches have been used to assess caffeine withdrawal. The following paragraphs discuss representative studies and do not constitute an exhaustive review.

A large survey of daily caffeine consumers was conducted by telephone by Harris Laboratories of Lincoln, NE (Dews et al., 1999). Subjects were asked “do you consume caffeinated beverages (e.g. coffee, tea, colas) on a regular daily basis?”. Some 6839 of 11,211 subjects queried answered affirmatively. These subjects were further asked: “Have you had problems or symptoms on stopping caffeine in the past?” Some 752 (11%) self reported withdrawal symptoms. In a questionnaire study by Greden et al. (1978) on 83 hospitalized subjects, 18 were considered high consumers of caffeine, 14 of 67 (21%) reported headache. Among regular consumers of 2 or more cups of coffee per day, 14 of 67 (21%) reported headache after lactose the night before but only four out of 74 (5%) after caffeine the night before. The survey study previously mentioned on 11,211 subjects was followed by a controlled experiment, also conducted by Harris, on some 55 of the subjects who had reported symptoms on withdrawal from caffeine. When decaffeinated coffee was substituted for caffeinated coffee, blindly, only five of 18 (28%) actually reported symptoms on withdrawal in spite of the fact that all 18 had reported that they had withdrawal symptoms in the past. The self-reported withdrawal symptoms included sleepy, drowsy, no energy, shaky, nervous, insomnia, irritable, stressed, nausea, diarrhea, stomach upset and headache. van Dusseldorp and Katan (1990) report that 38 of 45 (84%) subjects in a double-blind withdrawal study did not realize when their coffee was switched to decaffeinated, but 19 (42%) reported more headache while only five (11%) reported less when on decaffeinated coffee. Of 99 subjects self-diagnosed as “caffeine-dependent” 11 were selected and completed a 2-day study, being given caffeine on one day and not the other, blindly (Strain et al., 1994). Nine of the 11 (82%) gave descriptions of symptoms that were diagnosed as due to withdrawal. Of the 11 subjects, seven had coffee and four had soft drinks as their usual source of caffeine. The two subjects who had no symptoms on withdrawal were coffee drinkers, despite the fact that coffee is a richer source of caffeine than soft drinks. For a study of experimental headache, Dreishbach and Pfeiffer (1943) recruited 22 subjects, including five who “had had typical periodic migraine headaches for at least 2 years”. They were asked to discontinue coffee beverages and were given capsules containing coffee or lactose. They took the same number of capsules daily but the proportion containing caffeine was progressively increased until after 6 or 7 days they were taking around 780 mg/day caffeine. When the caffeine capsules were replaced by lactose only capsules, 14 (82%) reported headache. Other symptoms reported were nausea, vomiting, rhinorrhea, mental depression, drowsiness, yawning and disinclination to work. Some of the headache, nausea and vomiting could have been due to migraine. All seven subjects in a study by Griffiths et al. (1986) reported symptoms on withdrawal (100%), as did all 62 subjects in a study by Silverman et al. (1992). In this study, 32 (52%) reported headache compared with 2% under baseline conditions. In a non-blind study by Naismith et al. (1970) all 20 subjects (100%) reported withdrawal symptoms.

In studies of post-operative headache in subjects withdrawn from caffeine as part of pre-operative fasting, of 233 patients 22% of coffee drinkers reported symptoms but only 7% of non-caffeine drinkers (Weber et al., 1993). Finally, in a couple of studies that involved regular dosing of caffeine and then abrupt withdrawal, but where the object of the study was physiological...
measures, for example, cardiovascular, rather than reported symptoms both Robertson et al. (1981) in 18 subjects and Ammon et al. (1983) in 10 subjects reported no complaints on withdrawal.

Thus the incidence of withdrawal symptoms in the clinical studies ranged from 22% (0% in studies not on symptoms of withdrawal) to 100% in a study when subjects knew when they were withdrawn.

Studies have been made to obtain more objective assessment of performance decline in caffeine withdrawal, for example on psychomotor tests (Phillips-Bute and Lane, 1997; Lane and Phillips-Bute, 1998). In general, the declines have been surprisingly small and non-significant, even at times when rating scales showed the changes noted above. One test that has shown significant declines is a vigilance task, in which the proportion of targets detected declined and reaction time increased (Lane and Phillips-Bute, 1998). Unfortunately, the results are presented as a composite of the 30 subjects so it is not possible to see how many subjects were measurably affected or how large the effect was in individual subjects.

There are many differences between these different studies. The subjects ranged from hospitalized psychiatric patients to healthy people going about their daily business. Reports on symptoms were obtained by questionnaires completed by subject, by observer rating and by telephone. The focus of the studies ranged from withdrawal symptoms in general to particular interest in headache. The subjects in some studies were recruited without regard to reported history of withdrawal symptoms; other studies recruited subjects who reported a history of withdrawal symptoms, even dependence. All these factors must contribute to the variability of symptoms and their prevalence. Even more striking, however, is the high prevalence reported, 100%, in the non-blind study compared to the low prevalence (0%) in studies not directed towards withdrawal. Less extreme, but still striking, are the differences between prevalence reported from studies conducted in laboratories identified as concerned with caffeine withdrawal problems in the literature and by the institutional press office, the studies of Griffiths et al. (1986) (100%), Silverman et al. (1992), (100%) and Strain et al. (1994) (82%), and the report from the Harris study (Dews et al., 1999) (28%), within the range of the ‘noise level’.

Harris Laboratory had not been linked to caffeine withdrawal studies and so subjects had no reason to expect that such a study was being conducted. Workers agree that coffee with caffeine has discernible effects and therefore can be distinguished from the non-coffeinated state, so that double-blind cannot be maintained in knowledgeable subjects with some focus on withdrawal. The most parsimonious explanation of much of the variability is that caffeine withdrawal causes a subtle syndrome, close to ‘background noise’ with symptoms and prevalence primarily determined by non-pharmacological factors. Such a conclusion is by no means novel, see for example, Smith et al. (1993).

Consider the following example. In the review of caffeine dependence by Griffiths and Woodson (1988), only one of eight reports published prior to 1943 mentioned headache. Following Dreisbach and Pfiefer’s 1943 description of severe headache on withdrawal, some 10 of 16 studies reported headaches on withdrawal. Once headache had been highlighted as a consequence of withdrawal, reports of headache on withdrawal became more frequent.

There are anecdotal accounts of severe caffeine withdrawal symptoms with prostration. Until a reasonable number of individuals have been documented as exhibiting severe caffeine withdrawal symptoms under controlled conditions, the evidence will remain anecdotal. Severe reactions were reported to ingestion of aspartame, including epileptiform convulsions, blindness and incapacitating nervousness. In the light of the vast experience of aspartame since, the anecdotal reports no longer command credence. The demographics of reports of caffeine withdrawal symptoms are interesting. In the Harris survey of 11,211 subjects (Dews et al., 1999) 5.5% of women, but only 0.9% of men complained of symptoms upon withdrawal that interfered with their normal daily activities (although, as was subsequently found, as many as 2/3 of even this population may have failed to report symptoms in a clinical trial). Such a high ratio of women to men is not generally seen for complaints not related to physical sex characteristics, nor is it seen for withdrawal of other agents such as heroin or phenytoin, which are prone to cause withdrawal symptoms. It has been seen, however, in complaints of “noxebo” effects after consumption of behaviorally inert substances such as aspartame. (Noxebo is a neologism coined to designate the opposite of placebo. A placebo pleases, a noxebo indisposes, both without pharmacological basis). Of the 4000 complaints of symptoms after consumption of aspartame received by the FDA between 1985 and 1993, 76% were made by women.

The reports of caffeine withdrawal symptoms continuing for days, weeks or even months raise the possibility that some individuals may miss the enhancement of performance they customarily receive at appropriate times from consumption of caffeine (Smith et al., 1993). They report their lower (non-caffeine) level of performance at times as continuance of “withdrawal symptoms”. Obviously, such a state of plain normalcy scarcely qualifies as a withdrawal syndrome.

The foregoing discussion is not intended to make the case that caffeine withdrawal symptoms do not occur but rather to put them in the context of symptoms and complaints by people in the course of their everyday lives.
4. Caffeine dependence

According to the definition of dependence given in the Introduction, the acceptance that there can be caffeine withdrawal symptoms signifies that there can be dependence on caffeine. In contemporary consideration of maintenance of abuse of drugs, withdrawal symptoms are not accorded the importance they once were. Formerly it was believed that the forestalling of withdrawal symptoms was a prime mover in sustaining abuse of drugs. There are drugs of abuse, such as heroin, that produce a clear withdrawal syndrome, which can be avoided by regular use. In contrast, many cocaine or alcohol abusers use their drug compulsively, but intermittently in a ‘binge’ mode. That is, intensive abuse over a day or few days followed by ‘voluntary’ abstention. They typically experience only minimal withdrawal symptoms consisting of tiredness when they abstain from cocaine, or insomnia and ‘nervousness’ when the alcohol binge is over. Consideration of the abuse of such drugs leads to the conclusion that a pattern of abuse can be just as strongly maintained when abstinence symptoms are inconsequential, as with cocaine, or relatively rare, as with alcohol. Forestalling delirium tremens is not believed to be a prime factor in maintaining the pattern of drinking of most alcoholics. Conversely, there are many therapeutic agents such as antihypertensive medications that produce tolerance and, if stopped abruptly, lead to withdrawal symptoms, but are not abused. So, dependence, as defined, and abuse should not be associated, and discussing caffeine in terms of drugs of abuse trivializes the dangers of such drugs as cocaine.

References


Laura M. Juliano · Roland R. Griffiths

A critical review of caffeine withdrawal: empirical validation of symptoms and signs, incidence, severity, and associated features

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Abstract Rationale: Although reports of caffeine withdrawal in the medical literature date back more than 170 years, the most rigorous experimental investigations of the phenomenon have been conducted only recently. Objectives: The purpose of this paper is to provide a comprehensive review and analysis of the literature regarding human caffeine withdrawal to empirically validate specific symptoms and signs, and to appraise important features of the syndrome. Methods: A literature search identified 57 experimental and 9 survey studies on caffeine withdrawal that met inclusion criteria. The methodological features of each study were examined to assess the validity of the effects. Results: Of 49 symptom categories identified, the following 10 fulfilled validity criteria: headache, fatigue, decreased energy/activeness, decreased alertness, drowsiness, decreased contentedness, depressed mood, difficulty concentrating, irritability, and foggy/not clearheaded. In addition, flu-like symptoms, nausea/vomiting, and muscle pain/stiffness were judged likely to represent valid symptom categories. In experimental studies, the incidence of headache was 50% and the incidence of clinically significant distress or functional impairment was 13%. Typically, onset of symptoms occurred 12–24 h after abstinence, with peak intensity at 20–51 h, and for a duration of 2–9 days. In general, the incidence or severity of symptoms increased with increases in daily dose; abstinence from doses as low as 100 mg/day produced symptoms. Research is reviewed indicating that expectancies are not a prime determinant of caffeine withdrawal and that avoidance of withdrawal symptoms plays a central role in habitual caffeine consumption. Conclusions: The caffeine-withdrawal syndrome has been well characterized and there is sufficient empirical evidence to warrant inclusion of caffeine withdrawal as a disorder in the DSM and revision of diagnostic criteria in the ICD.

Keywords Caffeine · Abstinence · Cessation · Deprivation · Withdrawal · Headache · Physical dependence · DSM · ICD · Humans

Introduction

Caffeine is the most widely used behaviorally active drug in the world (Gilbert 1984). In North America, 80–90% of adults report regular use of caffeine (Gilbert 1984; Hughes and Oliveto 1997). Mean daily intake of caffeine among caffeine consumers in the United States is about 280 mg, with higher intakes estimated in some European countries (Gilbert 1984; Barone and Roberts 1996). In the United States, coffee and soft drinks are the most common sources of caffeine, with almost half of caffeine consumers ingesting caffeine from multiple sources, including tea (Hughes and Oliveto 1997).

After oral ingestion, caffeine is rapidly and completely absorbed, with peak blood levels generally reached in 30–45 min (Denaro and Benowitz 1991; Mumford et al. 1996; Liguori et al. 1997a), and is quickly eliminated, with a typical half-life of 4–6 h (Denaro and Benowitz 1991). The primary mechanism of action of caffeine is competitive antagonism at A1 and A2A adenosine receptors (Fredholm et al. 1999). Caffeine produces a variety of physiological effects, including effects on the cerebral vascular system, blood pressure, respiratory functioning, gastric and colonic activity, urine volume, and exercise performance (James 1997). Low to moderate doses of caffeine (20–200 mg) produce reports of increased well-being, happiness, energy, alertness, and sociability,
whereas higher doses are more likely to produce reports of anxiety, jitteriness, and upset stomach (Griffiths et al. 2003). Chronic administration of caffeine results in tolerance to a number of its physiological, subjective, and behavioral effects (Griffiths and Mumford 1996). Caffeine has been shown to function as a reinforcer in humans (e.g. Hughes et al. 1991; Evans et al. 1994), and some individuals become clinically dependent on caffeine as indicated by being unable to quit and continuing use despite having medical problems made worse by caffeine (Strain et al. 1994; Hughes et al. 1998).

Regular use of caffeine also produces physical dependence, evidenced as time-limited withdrawal symptoms upon the termination or reduction of one’s usual caffeine dose. Physical dependence on caffeine has been documented in both pre-clinical and clinical research, and the biological basis has been hypothesized to be increased functional sensitivity to endogenous adenosine (Griffiths and Mumford 1996). Symptoms of caffeine withdrawal have been described in the medical literature for more than 170 years. In 1988, the first comprehensive review of clinical reports and experimental studies on caffeine withdrawal was published (Griffiths and Woodson 1988), which provided evidence for caffeine withdrawal as a discrete clinical syndrome. Since that time, the research literature on caffeine withdrawal has increased substantially. For example, of 48 blind caffeine-withdrawal studies identified for this review, only five were published before 1988.

The present review was inspired by this emergent research literature, which has not been comprehensively reviewed, as well as by the practical need to develop empirically based diagnostic criteria for caffeine withdrawal. In 1994, a tentative research diagnosis of caffeine withdrawal was proposed in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) to encourage research on diagnostic criteria and the utility of the diagnosis (American Psychiatric Association 1994). Only one-quarter of the blind studies identified for this review were available to the DSM-IV Work Group (Hughes 1994). The review also addresses the previous suggestions (Rubin and Smith 1999; Dew et al. 2002) that caffeine withdrawal is not clinically significant and is primarily determined by expectancies.

### Methods for searching and categorizing the literature

#### Search strategy and inclusion criteria

The following strategies were used to identify possible studies for this review: (1) PubMed (1950–2004) and PsycInfo (1872–2004) searches were conducted with the keywords “caffeine” in conjunction with “withdrawal,” “dependence,” “deprivation,” or “abstinence”; (2) the authors searched their personal journal article collections on caffeine withdrawal; and (3) relevant references cited in papers obtained through the first two strategies and major reviews of caffeine and caffeine withdrawal (Griffiths and Woodson 1988; Fredholm et al. 1999; Nehlig 1999; Griffiths et al. 2003) were examined to identify additional relevant papers. To be included, experimental studies had to require a caffeine abstinence period of 12 h or greater because withdrawal onset generally occurs after 12 h of abstinence (Griffiths and Woodson 1988).

#### Excluded studies

Although possibly relevant to caffeine withdrawal, several types of studies were excluded from detailed summarization and analysis (Tables 1, 2, 3, 4): (1) several studies were excluded because they were not clearly interpretable (Horst et al. 1934; Mackenzie et al. 1981; Ammon et al. 1983; Smith 1996; Reeves et al. 1999; Watson et al. 2000); (2) a series of carefully conducted studies comparing placebo and caffeine conditions after overnight abstinence were not presented in a way that was clearly interpretable as caffeine-withdrawal effects (Yeomans et al. 2000a,b 2001); (3) several studies that purported to demonstrate caffeine effects after a period of abstinence (e.g., Lieberman et al. 1987; Brice and Smith 2002) were excluded, although some of these studies acknowledged that the effects may represent a reversal of caffeine withdrawal (e.g., Bruce et al. 1986; Hindmarch et al. 1998; Kenemans et al. 1999; Smit and Rogers 2000); (4) studies exploring caffeine-withdrawal headache following surgical anesthesia were excluded because of the possible confounding effects of anesthesia (Galletly et al. 1989; Fennelly et al. 1991; Weber et al. 1993; Nikolajsen et al. 1994; Hampl et al. 1995); (5) case studies of neonatal caffeine withdrawal were excluded (McGowan et al. 1988; Thomas 1988); and (6) a survey study purporting to assess the prevalence of caffeine-withdrawal headache was excluded because the methods were ambiguous and it was not clear whether the subjects ever experienced periods of caffeine abstinence (Sjaastad and Bakketeig 2004).

#### Categorization of withdrawal studies

The inclusion strategy resulted in the identification of 42 double-blind experiments (Table 1), 15 non-blind and single-blind experiments (Table 2), and 9 survey studies (Table 3). Table 4 provides a summary of the individual withdrawal symptoms that are documented in Tables 1, 2, and 3 (for completeness, Appendix A shows which of the experimental studies in Tables 1 and 2 failed to document each of the evaluated symptoms and signs). In constructing Table 4, all symptoms documented in Tables 1, 2, and 3 were first catalogued, and then phenomenologically similar descriptors were combined (e.g., drowsiness, sleepy, drowsy/sleepy/tired, sedated, feel half awake, and decreased wakefulness were combined in a single category called “drowsiness/sleepiness”). Categories and descriptors for each category are presented below. It is recognized that the resulting 49 symptom categories may not represent fully independent constructs. In the absence of empirical
<table>
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<th>Reference</th>
<th>Design</th>
<th>Withdrawal signs and symptoms$^{b,c}$</th>
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<tr>
<td>1. Goldstein (1964) [experiments c and d]</td>
<td>N = approximately 16 heavy coffee drinkers (5 or more cups/day), 25 moderate drinkers (2–4 cups/day), and 37 light drinkers (0–1 cup/day); within-subjects design; subjects abstained from caffeine after lunch and received 150-mg caffeine or placebo in decaffeinated coffee at bedtime over four consecutive nights, with both treatments given twice; abstinence not biologically verified</td>
<td>In heavy coffee drinkers morning headache occurred significantly more frequently after placebo (25% of trials) than after caffeine (3% of trials), in moderate drinkers the frequency of headache was non-significantly higher after placebo (12% of trials) than after caffeine (7% of trials); in light drinkers the frequency of headache was &lt;1% after both caffeine and placebo.</td>
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<td>2. Goldstein et al. (1969)</td>
<td>N=38 female daily coffee drinkers (5 or more cups/day) and 18 female coffee abstainers; within subjects and across group design; subjects abstained from caffeine after dinner and received placebo, 150-mg or 300-mg caffeine in decaffeinated coffee the following morning (about 0900 hours); treatments were repeated three times; abstinence not biologically verified</td>
<td>Compared with coffee abstainers, coffee users were less alert, active/energetic, and content, and more sleepy, irritable, and nervous after caffeine abstinence; among coffee users placebo produced increased ratings of headache, lightheaded, irritable, sleepy, and decreased ratings of talkativeness, contentedness, and energy/active compared with caffeine; caffeine generally produced a dose-related suppression of withdrawal symptoms in the coffee users.</td>
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<td>3. Robertson et al. (1981)</td>
<td>N=18 adults who were not habitual coffee consumers were caffeine abstainers for 3 weeks preceding the study; within subjects and across group design; approximately half the subjects received 750 mg caffeine/day in flavored drinks for 7 days, followed by substitution of placebo flavored drinks for 4 days; abstinence was biologically verified</td>
<td>Substitution of placebo did not result in detectable effects on blood pressure, urinary noradrenaline, or urinary epinephrine.</td>
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<td>4. Griffiths et al. (1986)</td>
<td>N=7 males who were heavy coffee drinkers (mean 12 cups/day); within-subjects design; after approximately 10 consecutive days of zero caffeine consumption (mean 1.25-g/day caffeine for the last 5 days), subjects were switched to decaffeinated coffee for at least 10 days; abstinence not biologically verified but study was conducted in a residential laboratory</td>
<td>Compared with caffeinated coffee consumption, decaffeinated coffee substitution resulted in increased subject ratings of headache, sleepy, lightheaded (POMS), and decrease a alert, active, vigor (POMS), and friendliness (POMS), observation of subjects' behavior also showed significant withdrawal based on a composite withdrawal score; cigarette smoking decreased during decaffeinated coffee consumption; 100% of subjects reported withdrawal headache.</td>
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<td>5. Griffiths et al. (1990) [Phase 1]</td>
<td>N=7 investigator-subjects with histories of regular caffeine consumption; within-subjects design; subjects given capsules containing 100 mg caffeine/day for 9–14 days, then placebo capsules for 12 days, and then capsules containing 100 mg caffeine/day for 7–12 days; abstinence was biologically verified</td>
<td>Compared with caffeine consumption, placebo substitution resulted in increased headache, cerebral fullness, irritable/cross/grumpy, depression, muscle pain/stiffness, lethargy/fatigue/tired/sluggish, craving, and flu-like feelings, and decreased alert/attentive/servant, well-being, social disposition, motivation for work, concentration, energy/active, urge to do tasks/work, content/satisfied, and self-confidence; these withdrawal symptoms peaked on days 1 or 2 and progressively decreased toward pre-withdrawal levels over about a week.</td>
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<td>6. Griffiths et al. (1990) [Phase 2]</td>
<td>N=7 investigator-subjects with histories of regular caffeine consumption; within-subjects design; subjects given capsules containing 100 mg caffeine/day for 6 weeks; on five occasions placebo capsules were substituted for caffeine for 1 day (separated by an average of 9 days); abstinence was biologically verified</td>
<td>Compared with caffeine days, intermittent placebo resulted in increased fatigue (POMS$^c$), confusion-bewilderment (POMS), and total mood disturbance (POMS), and decreased vigor (POMS) and friendliness (POMS); in addition, compared with caffeine days, intermittent placebo also resulted in all of the withdrawal symptoms observed in phase 1 (see above) as well as increased muzzy, drowsy/sleepy, yawning, blurred vision, lightheaded/dizzy, impaired work/thought related activities and impaired verbal ability; all of those withdrawal symptoms plus decreased anxious/nervous were also significant when intermittent placebo was compared with a chronic placebo period (days 8–12 of placebo from phase 1) [exceptions: lightheaded/dizzy, impaired verbal ability, urge to do tasks/work and motivation for work were not significant]; within-subjects analyses of both intermittent placebo vs caffeine and caffeine vs chronic placebo demonstrated that more than 57% of subjects showed increased headache, muzzy, drowsy/sleepy, impaired work/thought related activities, and decreased social disposition, concentration, and self-confidence.</td>
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<td>7. van Dusseldorp and Katan (1990)</td>
<td>N=45 daily coffee drinkers (4–6 cups/day); within-subjects design; subjects received 5-cups/day caffeinated coffee (415 mg caffeine/day) or decaffeinated coffee, each for 6 weeks; abstinence not biologically verified</td>
<td>Withdrawal symptoms and dysphoric mood were greater for subjects who received decaffeinated coffee.</td>
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<td>8. Bruce et al. (1991)</td>
<td>N=21 habitual caffeine users (mean 389 mg/day); within subjects and across group design; subjects in one group abstained from caffeine for 24-h prior to three sessions conducted at weekly intervals; subjects in another group abstained from caffeine for 7 days before as well as between three sessions conducted at 48-h intervals; subjects in both groups received capsules containing placebo, 250-mg or 300-mg caffeine in random order; abstinence was biologically verified</td>
<td>Withdrawal symptoms were significantly greater for subjects who abstained from caffeine for 24-h prior to three sessions conducted at weekly intervals compared to the 7-day abstinence group.</td>
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<td>9. Silverman et al. (1992)</td>
<td>N=62 daily caffeine consumers (mean 235 mg/day); within-subjects design; subjects were assessed while consuming their normal caffeine intake (baseline) and after two 2-day periods during which they consumed caffeine-free diets and were administered capsules containing caffeine (matched to their usual intake) or placebo; abstinence was biologically verified</td>
<td>Compared with baseline and caffeine, placebo increased irritable/cross/grumpy, blurred vision, drowsy/sleepy, yawning, lethargy/fatigue/tired/sluggish, muzzy/foggy/not clearheaded, headache, flu-like feelings, feeling heavy in arms and legs, hot and cold spells, Beck Depression Inventory scores, somatization (SCL-90-R), state and trait scales of State-Trait Anxiety Inventory, fatigue (POMS), confusion-bewilderment (POMS), total mood disturbance (POMS), and decreased well-being, desire to socialize, talkativeness, urge to do tasks/work related activities, energy/active, content/satisfied, self-confidence, vigor (POMS), friendliness (POMS), psychomotor performance (tapping speed); compared with baseline and caffeine, during the placebo period more subjects reported moderate or severe headache (25%), used analgesics despite no aggravation (13%) and showed abnormal scores on trait-anxiety (4%), vigor (11%), and fatigue (8%), and the Beck Depression Inventory (1%).</td>
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<td>10. Evans and Griffiths (1992)</td>
<td>N=32 daily caffeine consumers (mean 343 mg/day); within subjects and across group design; subjects received capsules throughout the study; subjects in the chronic placebo condition (N=16) received placebo for 18 days, followed by a 3-day caffeine versus placebo choice phase, followed by another 7 days of placebo; subjects in the chronic caffeine condition (N=16) received caffeine for 18 days (the dose progressed from 100-mg t.i.d. to 300-mg t.i.d.); followed by a 3-day caffeine versus placebo choice phase, followed by 2 days of progressively lower doses of caffeine (approximately 180-mg t.i.d. on the 2nd day), followed by 5 days of placebo; abstinence was biologically verified</td>
<td>Compared with subjects in the chronic placebo condition, subjects in the chronic caffeine condition reported higher ratings of headache and lower ratings of talkativeness in the final withdrawal period; within the caffeine group, no effects occurred between the placebo and caffeine challenge days which was interpreted as indicating that 24 h of abstinence was insufficient to produce withdrawal symptoms after termination of very high caffeine doses (i.e., 900 mg/day).</td>
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| Reference | Design | Withdrawal signs and symptoms
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<td>Hughes et al. (1993) [summarizes results from Hughes et al. (1991, 1992); Oliveto et al. (1992a,b)]</td>
<td>37 daily coffee drinkers (mean 352 mg/day); who reported using coffee for caffeine effects or showed withdrawal or preference for caffeine in a screening test; within-subjects design; six trials at weekly intervals on one day subjects consumed decaffeinated coffee and on another day consumed caffeinated coffee (100 mg/g serving); assessment occurred after about 24-h abstinence; absence not biologically verified in all subjects</td>
<td>Within-subjects statistical analyses showed that 30%, 24%, 24%, and 49% of subjects, respectively, reported significant increases in headache, drowsiness, fatigue, or one or more of these symptoms on decaffeinated days compared with caffeinated days Overall, 82% showed evidence of caffeine withdrawal during placebo; 64% reported maximal ratings of headache; compared with standardized norms, 27% showed extreme increases in fatigue (POMS); 48% showed extreme decreases in vigor (POMS); and 36% showed elevations in depression (Beck Depression Inventory); compared with caffeine, after placebo 55% of subjects showed significant decreases in psychomotor performance (tapping speed); 45% reported analytic use despite disorientation; 73% reported functional impairment in normal daily activities</td>
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<td>Strain et al. (1994)</td>
<td>11 daily caffeine users (median 357 mg/day) who met DSM-IV diagnostic criteria for substance dependence on caffeine; within-subjects design; subjects were assessed after 2-2.5 days of abstinence; subjects consumed caffeine-free diets and were administered capsules containing caffeine (matched to their usual intake) or placebo; absence was biologically verified</td>
<td>Over all, 82% showed evidence of caffeine withdrawal during placebo; 64% reported maximal ratings of headache; compared with standardized norms, 27% showed extreme increases in fatigue (POMS); 48% showed extreme decreases in vigor (POMS); and 36% showed elevations in depression (Beck Depression Inventory); compared with caffeine, after placebo 55% of subjects showed significant decreases in psychomotor performance (tapping speed); 45% reported analytic use despite disorientation; 73% reported functional impairment in normal daily activities</td>
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<td>Brauer et al. (1994)</td>
<td>11 daily coffee consumers (mean 480 mg/day); within-subjects design; over a 3-h period subjects consumed a caffeine-free diet and were administered either capsules containing placebo or 300-mg caffeine; absence was biologically verified</td>
<td>Compared with the caffeine, placebo increased depressed, drowsy-sleepy, and fatigue/tired, and decreased irregular heartbeat and more talkative; within-subjects analyses revealed that 33% of subjects showed significant effects on one or more symptom ratings</td>
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<td>Hale et al. (1995)</td>
<td>18 adolescents (ages 11-15); daily cola drinkers (mean 2.3, 12 oz caffeinated cola/day); within-subjects design; six trials at weekly intervals on one day subjects consumed caffeine-free cola ad libitum and on another day consumed caffeinated cola ad libitum (33 mg/8 oz); assessment occurred after about 24-h abstinence; absence not biologically verified</td>
<td>Compared with the caffeine, placebo increased drowsy-sleepy-tired, headaches, fatigue/tired, lazy/sluggish, and depressed and decreased stimulated-active-energetic-excited, alert-attentive-able to concentrate, and vigor; within-subjects analyses revealed that 73% of subjects showed significant effects on one or more symptom ratings, with 45% reporting increases in drowsy-sleepy-tired; 27% reporting decreases in stimulated-active-energetic-excited, alert-attentive-able to concentrate, and vigor; 38% reporting increased fatigued/tired, depressed, and decreased content-related satisfied, and 9% reporting increased headache, irritable-frustrated-angry-cross, lazy-sluggish, and decreased talkative</td>
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<td>Hughes et al. (1995)</td>
<td>11 daily coffee drinkers (mean 5.6 cups/day) selected for showing caffeine abstinence symptoms or caffeine preference in a screening test; within-subjects design; six trials at weekly intervals; on one day subjects consumed decaffeinated coffee ad libitum and on another day consumed caffeinated coffee ad libitum (either 25 mg or 50 mg/serving); assessment occurred after about 24-h abstinence; absence not biologically verified</td>
<td>Compared with the caffeine, placebo increased drowsy-sleepy-tired, headaches, fatigue/tired, lazy/sluggish, and depressed and decreased stimulated-active-energetic-excited, alert-attentive-able to concentrate, and vigor; within-subjects analyses revealed that 73% of subjects showed significant effects on one or more symptom ratings, with 45% reporting increases in drowsy-sleepy-tired; 27% reporting decreases in stimulated-active-energetic-excited, alert-attentive-able to concentrate, and vigor; 38% reporting increased fatigued/tired, depressed, and decreased content-related satisfied, and 9% reporting increased headache, irritable-frustrated-angry-cross, lazy-sluggish, and decreased talkative</td>
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<td>Mitchell et al. (1995)</td>
<td>9 daily coffee consumers (mean 686 mg/day); within-subjects design; subjects consumed a caffeine-free diet and were administered capsules containing placebo or caffeine (50% or 100% of their usual amounts) on the day before and the day of assessment; absence was biologically verified</td>
<td>Compared with the 50% and 100% caffeine conditions, placebo (33.5-h abstinence) increased headache, sluggish, and tired-sleepy; absence did not affect amount of responding on an eponym task reformed by coffee delivery</td>
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<td>Richardson et al. (1995)</td>
<td>10 regular caffeine consumers (mean 252 mg/day) and 18 non-consumers; four groups: non-consumers and three consumer groups who abstained from caffeine for either 90 min, overnight, or 7 days; subjects in each group received capsules containing placebo, 70 mg or 250 mg in a counterbalanced order; absence not biologically verified</td>
<td>Overnight abstinence (1.5-15 h) compared with 90-min abstinence produced increased ratings of angry, tired, drowsy, dejected and decreased ratings of cheerful, friendly, and cheerful; overnight abstinence compared with 7 days of abstinence produced increased ratings of angry, tired, drowsy and dejected; overnight abstinence compared with non-consumers produced increased headache, angry, tired, drowsy, dejected and decreased cheerful</td>
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<td>Rogers et al. (1995)</td>
<td>24 low caffeine users (mean 47 mg/day) and 25 moderate caffeine users (mean 205 mg/day); ten experimental sessions; after overnight abstinence half the subjects consumed a drink containing 70 mg caffeine and half consumed a non-caffeinated drink; assessment occurred 1 h later; absence not biologically verified</td>
<td>In moderate caffeine users, compared with subjects receiving caffeine, subjects receiving placebo reported increases in tired and decreases in cheerful, cheerfulness, energetic, and lively; in contrast, no significant differences were found between caffeine and placebo in low caffeine users</td>
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<td>Streufert et al. (1995)</td>
<td>25 caffeine users who held managerial positions (mean 565 mg/day); within-subjects design; subjects were instructed to abstain from caffeine for 16-h prior to each of 2 assessment days; the day prior to the assessment day and morning of the assessment day subjects were given either capsules containing caffeine (matched to their usual intake) or placebo; absence was biologically verified</td>
<td>Compared with baseline, placebo increased tiredness, lethargy, sedation, and decreased alertness and concentration; these effects were reversed upon caffeine re-administration; compared with baseline, placebo increased EEG alpha peak magnitude and ratings of sleep quality, and decreased skin conductance, systolic blood pressure, and ratings of onset to sleep; 68% of subjects reported increased tired and lethargy; 45% reported diffuse throbbing headache, with 28% of these also reporting nausea and sickness</td>
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<td>Lader et al. (1996)</td>
<td>40 habitual caffeine consumers (mean 360 mg/day); within and across groups staggered cohort design; after baseline assessment during usual caffeine consumption, subjects received capsules containing either placebo or caffeine (matched to their usual intake); subjects received placebo capsules for 2 consecutive days followed by caffeine capsules for 2 days or 3 days; absence was biologically verified</td>
<td>Compared with baseline, placebo increased tiredness, lethargy, sedation, and decreased alertness and concentration; these effects were reversed upon caffeine re-administration; compared with baseline, placebo increased EEG alpha peak magnitude and ratings of sleep quality, and decreased skin conductance, systolic blood pressure, and ratings of onset to sleep; 68% of subjects reported increased tired and lethargy; 45% reported diffuse throbbing headache, with 28% of these also reporting nausea and sickness</td>
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<td>Comer et al. (1997)</td>
<td>12 daily caffeine users; within-subjects design; 17-day implant study; subjects given capsules containing 300-mg caffeine daily except for 2-2.5 day placebo substitutions on days 5 and 6 and 12-13; absence not biologically verified but study was conducted in a residential laboratory</td>
<td>On placebo days subjects reported increased ratings of headache, miserable, sedated, sleepy, tired, unmotivated, and yawning, and decreased ratings of alert, anxious, energetic, and self-confident</td>
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<td>Liguori et al. (1997b) [Exp 1]</td>
<td>8 daily caffeinated cola consumers (mean 157 mg/day); within-subjects design; six trials at weekly intervals; on one day subjects consumed caffeine-free colas ad libitum and on another day consumed caffeinated colas ad libitum (either 33 mg or 17 mg/mg/8 oz); assessment occurred after about 24-h abstinence; absence not biologically verified</td>
<td>Compared with the 33-mg condition, placebo decreased active/energetic-excited, more talkative, motivated to work, and well-being; compared with the 17-mg condition, placebo increased anxious/tense/nervous, amity (POMS), confusion (POMS), irritable-frustrated-angry-cross, and stomachache/upset stomach, and decreased well-being, friendliness (POMS), and vigor (POMS); within-subject analysis revealed that 50% of the subjects in the 33-mg condition and 25% of the subjects in the 17-mg condition showed headache, drowsiness; or fatigue (63% showed drowsiness and/or fatigue in one or both of the conditions)</td>
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Table 1 (continued)

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<th>Reference</th>
<th>Design</th>
<th>Withdrawal signs and symptoms$^{b,c}$</th>
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<td>23. Liguori et al. (1997b) [Exp 2]</td>
<td>N=16 daily coffee and cola consumers (mean 579 mg/day); within-subjects design; six trials at weekly intervals; on one day subjects consumed caffeine-free colas ad libitum and another day consumed caffeinated colas ad libitum (33 mg/8 oz); assessment occurred after about 24-h abstinence; abstinence not biologically verified</td>
<td>Compared with the caffeine, placebo increased drowsy/sleepy, fatigue/tired, headache, laryngitis/slow-moving, and stomachache/upset stomach, and decreased active/energetic/excited, confident, motivated to work, well-being, and performance on a psychomotor/cognitive task; withdrawal or headache or drowsiness and fatigue reliably occurred in 2 of 16 participants</td>
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<td>24. Liguori and Hughes (1997)</td>
<td>N=11 daily coffee consumers (mean 612 mg/day); within-subjects design; subjects were tested in four caffeine versus no caffeine conditions using procedures similar to Liguori et al. (1997b); the four caffeine conditions were cola 33 mg serving, cola 3 mg/serving, cola 100 mg/serving, cola 100 mg/serving; assessment occurred after about 24-h abstinence; abstinence was biologically verified</td>
<td>Compared with the caffeine, placebo increased drowsy/sleepy, fatigue/tired, laryngitis/slow-moving, and nausea/vomiting, and decreased active/energetic/excited, alert/attentive/able to concentrate, confident, frequent urination, impatience, motivated to work, stronger/more vigorous/more energy, well-being, and performance on a psychomotor/cognitive task</td>
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<td>25. Schuh and Griffiths (1997)</td>
<td>N=20 regular caffeine consumers (mean 379 mg/day); within-subjects design; subjects were assessed after two 19-h periods during which they consumed caffeine-free diets and were administered capsules containing caffeine (matched to their usual intake) or placebo; abstinence was biologically verified</td>
<td>Compared with caffeine, placebo increased headache, work-out, and flu-like feelings, and decreased alert, well-being, helpful, and upset stomach; on a drug versus money choice procedure, subjects chose to forfeit money to avoid receiving placebo—this was significantly different from the caffeine condition</td>
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<td>26. Garrett and Griffiths (1998)</td>
<td>N=28 adults; within-subjects design; each subject was exposed to four conditions each of which involved a 9-12 day exposure (capsules containing 300 mg/70 kg caffeine or placebo) followed by a 2-day challenge (caffeine or placebo): (1) acute abstinence (caffeine followed by placebo), (2) chronic abstinence (placebo followed by placebo), (3) acute caffeine (placebo followed by caffeine), (4) chronic caffeine (placebo followed by caffeine); abstinence was biologically verified</td>
<td>Placebo challenge after caffeine (acute abstinence) resulted in increased confusion-bewildement (POMS), fatigue (POMS), total mood disturbance (POMS) and decreased vigor (POMS) compared with each of the other three conditions; acute abstinence resulted in decreased alert/attentive/observant, well-being, ability to concentrate, and energy/active compared with the caffeine challenge after chronic abstinence and chronic caffeine, and increased irritable/irritable/grumpy, depressed, and drowsy/sleepy relative to placebo challenge after chronic abstinence; subjects were willing to forfeit more money to avoid receiving placebo when maintained on caffeine (acute abstinence) compared with chronic caffeine and chronic placebo</td>
</tr>
<tr>
<td>27. James (1998)</td>
<td>N=36 habitual caffeine consumers (about 170 mg/day); within-subjects design; subjects received capsules throughout the study; four conditions: 6 days of caffeine followed by 1 day of placebo (acute abstinence), 6 days of caffeine followed by 1 day of caffeine (habitual use), 6 days of placebo followed by 1 day of caffeine (chronic abstinence); abstinence was biologically verified</td>
<td>Subjects in the acute abstinence condition reported more frequent headaches (47%) than subjects in the habitual use condition (14%) and in the chronic abstinence condition (31%); subjects in acute abstinence condition also experienced more severe and longer headaches, less alertness, better sleep quality and duration, and worsened performance on a character recognition task than the average of the three other conditions</td>
</tr>
<tr>
<td>28. Phillips-Blute and Lane (1998)</td>
<td>N=31 daily coffee consumers (mean 603 mg/day); within-subjects design; subjects abstained from caffeine overnight and then consumed either capsules containing 230 mg caffeine or placebo 4-h prior to assessment; thus 4-h abstinence was compared with an abstinent 12-28 h abstinence</td>
<td>Compared with 4-h abstinence, overnight abstinence increased fatigue (POMS), sleepiness, and yawning, and decreased vigor (POMS) and systolic and diastolic blood pressure</td>
</tr>
<tr>
<td>29. Robelin and Rogers (1998)</td>
<td>N=64 daily caffeine consumers (mean 453 mg/day); between groups design; after overnight abstinence, four groups of subjects consumed three fruit-flavored drinks spaced at 75-90 min intervals; conditions were three placebo drinks or 1, 2, or 3 drinks containing caffeine (1.2-mg/kg or about 86-mg caffeine); final assessment occurred after ≥17 h of abstinence; abstinence not biologically verified</td>
<td>Compared with subjects who received 1, 2, or 3 drinks of caffeine, subjects who received placebo showed decreased energetic mood and slower reaction time performance</td>
</tr>
<tr>
<td>30. Van Soeren and Graham (1998)</td>
<td>N=6 male athletes who were daily caffeine consumers (mean 761 mg/day); within-subjects design; subjects were tested after receiving capsules containing caffeine or placebo after 0, 2, or 4 days of abstinence from dietary caffeine; six trials were separated by ≥10 days; abstinence was biologically verified</td>
<td>Although not statistically analyzed, the authors reported that all subjects reported withdrawal symptoms lasting from 2 days to 4 days including severe headaches, fatigue, lightheaded, and flu-like symptoms; time to exhaustion on a cycling task was not affected by the duration of dietary caffeine abstinence in either the placebo or caffeine conditions</td>
</tr>
<tr>
<td>31. Yomans et al. (1998)$^f$</td>
<td>N=36 caffeine consumers (mean 129 mg/day); within subject and across group design; on 4 days subjects abstained from caffeine overnight and were given beverages (herbal tea and fruit-flavored drinks, respectively) containing either caffeine (100 mg) or placebo; abstinence was biologically verified</td>
<td>Mood data were collapsed across the 4 days; compared with subjects who received caffeine, subjects who received placebo with breakfast had lower ratings of lively and energetic 2 h later, compared with subjects who received placebo at breakfast and caffeine misnomering, subjects who received placebo at both breakfast and midmorning had lower ratings of energy 30 min after the midmorning beverage; the authors interpreted the difference between caffeine and placebo as reflecting caffeine’s withdrawal effects</td>
</tr>
<tr>
<td>32. Dew’s et al. (1999)</td>
<td>N=57 daily coffee drinkers (mean 200-300 mg/day caffeine immediately before abstinence); three groups of subjects given instant coffee for 14 days; the chronic caffeine group received instant caffeine-coffee throughout the study; the abrupt abstinence group received instant decaffeinated coffee for 7 consecutive days, the gradual abstinence group received gradual reductions in caffeine over 5 days; abstinence was biologically verified</td>
<td>No statistical analyses were reported, the authors concluded that 39% of subjects showed withdrawal in the first 2 days of abstinence in the abrupt abstinence group based on a composite withdrawal measure derived from a mood and attitude questionnaire and spontaneously reported withdrawal symptoms (e.g., headache and tiredness). 22% showed substantial decreases (≥1.5 points on a 4 point scale) in their ratings of daily functioning; on the first 2 days of abstinence 28% of the abrupt abstinence group reported headache, while 17% of the chronic caffeine group reported headache</td>
</tr>
<tr>
<td>33. Evans and Griffiths (1999) [Exp 1]</td>
<td>N=15 daily caffeine consumers (mean 241 mg/day); within-subjects design; subjects were maintained on capsules containing 300 mg caffeine/day; 2-day placebo period was repeated six times with 5-9 days of caffeine between periods; abstinence was biologically verified</td>
<td>Compared with caffeine days, on placebo days subjects reported increased headache, headache/poor mood, tiredness, flu-like symptoms, fatigue (POMS), and decreased vigor (POMS), Friendliness (POMS), and activity/alertness</td>
</tr>
<tr>
<td>34. Evans and Griffiths (1999) [Exp 2]</td>
<td>N=17 daily caffeine consumers (mean 277 mg/day); within-subjects design; 2-day placebo period after subjects were maintained on capsules containing 100, 300, or 600 mg of caffeine/day, each subject was exposed to two 2-day placebo periods at each caffeine dose; abstinence was biologically verified</td>
<td>Compared with caffeine days, on placebo days after each maintenance dose subjects reported increased headache, fatigue (POMS), confusion-bewildement (POMS), and tiredness; the range and magnitude of withdrawal symptoms increased with maintenance dose, with subjects reporting the following additional symptoms after the highest dose: increased headache/poor mood, total mood disturbance (POMS), and flu-like symptoms, and decreased Friendliness (POMS), vigor (POMS), and activity/alertness; specificity of withdrawal effects can be confounded because higher doses of caffeine produced greater response to placebo with no change in baseline response to caffeine</td>
</tr>
</tbody>
</table>
Table 1 (continued)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Design</th>
<th>Withdrawal signs and symptoms$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>35. Evans and Griffiths (1999) [Exp 3]</td>
<td>N=19 daily caffeine consumers (mean 294 mg/day); within-subjects design; subjects were maintained on capsules containing 300 mg caffeine/day; approximately every 7 days a lower dose of caffeine (200, 100, 50, 25 mg) or placebo was substituted for 2 days; abstinence was biologically verified</td>
<td>Compared with the caffeine maintenance dose of 300 mg, placebo substitution produced increased headache, headache/poor mood, tiredness, fatigue (POMS), and total mood disturbance (POMS) and decreased vigor (POMS) and activity/alertness; the range and magnitude of withdrawal symptoms increased as a function of decreasing doses of caffeine, with no withdrawal reported at 200 mg and fatigue (POMS) and tiredness reported at 100 mg.</td>
</tr>
<tr>
<td>36. Evans and Griffiths (1999) [Exp 4]</td>
<td>N=25 daily caffeine consumers (263 mg/day); within-subjects design; subjects maintained on capsules containing 300 mg/day for either 1, 3, 7, or 14 consecutive days; each duration of caffeine exposure was followed by 7 days of placebo; abstinence was biologically verified</td>
<td>No withdrawal symptoms were shown in response to placebo after 1 day of caffeine; placebo after 3 days of caffeine resulted in increase headache/poor mood, fatigue (POMS), tiredness, flu-like symptoms, and total mood disturbance (POMS) and decrease activity/alertness; placebo after 7 days or 14 days of caffeine produced headache in addition to most of the symptoms that occurred in the 3-day condition; specificity of withdrawal effects can be concluded because longer durations of caffeine exposure produced greater response to placebo.</td>
</tr>
<tr>
<td>37. Jones et al. (2000)</td>
<td>N=10 daily caffeine consumers (mean 333 mg/day); within-subjects design; before two sessions subjects abstained from caffeine for 21 h and then received capsules containing caffeine (matched to their usual intake) or placebo; subjects were also evaluated while consuming their normal caffeine intake (baseline); abstinence was biologically verified</td>
<td>Compared with caffeine, placebo decreased alert/attentive/observant, ability to concentrate, energy/active, vigor (POMS), and friendliness (POMS) and increased heavy feelings in arms and legs; placebo also increased velocities in middle and anterior cerebral arteries and increased EEG theta power; caffeine and baseline conditions were not significantly different on any measure.</td>
</tr>
<tr>
<td>38. Svedin et al. (2000)</td>
<td>N=12 males (mean 173 mg/day) in the caffeine abstinence study and 18 males (mean 147 mg/day caffeine) in the non-abstinence study; in the abstinence study subjects abstained for &gt;14 h prior to testing; in both studies subjects received either a pill containing 200 mg caffeine or placebo; abstinence not biologically verified</td>
<td>Compared with caffeine administration, placebo administration to high caffeine consumers (as determined by median split) produced significant elevations in a composite somatic symptom scale (feel sick, queasy, dizzy, and perspiring). Similar effects were shown for the individual symptoms.</td>
</tr>
<tr>
<td>39. Svedin and et al. (2002b)</td>
<td>N=30 caffeine consumers (mean 330 mg/day), within-subjects and across-group design; after overnight abstinence three groups of subjects received placebo, 1-mg/kg, or 2-mg/kg caffeine in a flavored drink at breakfast on 2 test days; 60 min after the first dose subjects received a drink containing either placebo or 1-mg/kg caffeine in counterbalanced order across the 2 test days; abstinence not biologically verified</td>
<td>Compared with subjects given caffeine, subjects given placebo at breakfast had slower reaction times and lower alertness 45 min later; relative to placebo, caffeine given 60 min after breakfast decreased reaction time and increased alertness in subjects who received placebo at breakfast, but not in subjects who received caffeine at breakfast; the authors interpreted these results as suggesting that reversal of caffeine withdrawal is a major component of the effects of caffeine on mood and performance.</td>
</tr>
<tr>
<td>40. Rogers et al. (2003f) [Exp 1]</td>
<td>N=10 caffeine consumers (mean 335 mg/day) and 10 non-consumers; between groups design; before four sessions subjects abstained from caffeine for approximately 15 h; abstinence not biologically verified</td>
<td>After abstinence, caffeine consumers were less alert and more tense than non-consumers.</td>
</tr>
<tr>
<td>41. Rogers et al. (2003f) [Exp 2]</td>
<td>N=22 caffeine consumers (mean 372 mg/day) and 20 non-consumers; between groups design; after overnight abstinence subjects received either 100 mg caffeine or placebo in a flavored drink; abstinence not biologically verified</td>
<td>After abstinence, caffeine consumers were less alert than non-consumers.</td>
</tr>
<tr>
<td>42. Tinley et al. (2003f)</td>
<td>N=45 caffeine consumers (mean 324 mg/day); within-subjects and across-groups design; for 2-weeks subjects abstained from their normal sources of caffeine and consumed tea and coffee provided by the experimenters; in one group the tea and coffee were caffeinated and in the other group they were caffeine-free; subjects abstained from tea and coffee overnight before each of four test sessions conducted over the 2-week period; subjects reported mood before and after receiving a single serving of either a caffeine beverage (caffeine maintained group) or caffeine-free beverage (caffeine-free group); abstinence was biologically verified</td>
<td>Compared with the group that was chronically maintained on caffeine-free beverages, the group that was maintained on caffeine reported higher levels of headache after overnight abstinence; headache was alleviated after caffeine administration.</td>
</tr>
</tbody>
</table>

$^a$All studies had a caffeine-abstinence period of 12 h or greater

$^b$All effects are statistically significant at $P \leq 0.05$ unless otherwise noted

$^c$For consistency, when placebo and caffeine conditions are compared, the effects are described as the effects of placebo relative to caffeine

$^d$POMS indicates Profile of mood states questionnaire

$^e$Only those design elements that are relevant to the analysis of caffeine abstinence effects are described

$^f$Although abstinence was not biologically verified, saliva samples were taken and subjects were led to believe that samples would be analyzed for compliance

$^g$Likely a direct effect of caffeine rather than an effect of caffeine withdrawal

$^h$Some of the results are based on authors’ conclusions because not all significant comparisons were presented (Lader, personal communication, August 2002)
Table 2 Summary of single-blind and non-blind experimental studies of caffeine withdrawal

| Reference | Design | Withdrawal signs and symptoms
|-----------|--------|------------------------------|
| **Single-blind** | | Placebo substitution after caffeine resulted in lethargy in morning, cerebral fullness at noon, and headache in early afternoon, reaching peak intensity 3–6 h later; 82% of subjects reported definite to severe headache; 33% of these subjects were accompanied by nausea and sometimes vomiting and serious rhinorrhea; in 55% of trials headache was reported to be as severe as the subject had ever experienced; headache was alleviated by re-administration of caffeine; other withdrawal symptoms noted but not statistically analyzed (i.e., mental depression, drowsiness, yawning, and disinclination to work; serum protein, serum calcium, and hemocrit significantly lower during headache and serum inorganic phosphorus was slightly higher

| Driesbach and Pfiffer (1943) | N=22 adults; within-subjects design; caffeine administered in capsules in increasing doses over 6–7 days to 650–700 mg/day; on the 7th or 8th day placebo was substituted for caffeine capsules; abstinence not biologically verified |
| Höfer and Bird (1994a) | N=120 habitual coffee users (mean 5.7 cups/day); between groups design; after a 3-day baseline period, subjects received either 9 days of caffeinated instant coffee, 9 days of decaffeinated instant coffee or 9 days of intermittent caffeinated and decaffeinated instant coffee; abstinence was biologically verified |
| Höfer and Bird (1994b) | N=42 female habitual coffee drinkers (mean 6 cups/day); between groups design; after a 3-day phase of usual coffee consumption (baseline), subjects entered a second phase consisting of either 3 days of decaffeinated instant coffee or 3 days of caffeine tablets; abstinence was biologically verified |
| Lane (1999) | N=14 habitual coffee drinkers (3.4 cups/day); within-subjects design; after overnight abstinence subjects were given water containing 300 mg caffeine or placebo; abstinence not biologically verified |
| Ruben and Smith (1999) | N=43 regular coffee or tea consumers (mean 175 mg/day); within-subjects design; after a 2-day baseline period of normal caffeine consumption, subjects consumed caffeinated coffee (or tea) for 2 days and decaffeinated coffee (or tea) for 2 days in a counterbalanced order; abstinence not biologically verified |
| Field et al. (2003) | N=10 low caffeine consumers (mean 41 mg/day) and 10 high caffeine consumers (mean 684 mg/day); within subjects and across groups design; before two sessions, subjects abstained from caffeine for 30 h; cerebral blood flow was assessed with perfusion magnetic resonance imaging 60–90 min after receiving capsules containing caffeine (250 mg) or placebo; abstinence not biologically verified |
| **Non-blind** | | Caffeine withdrawal was defined as a statistical interaction between group and phase, with the decaffeinated coffee group showing increased headache, muscle/joint ache, sleep duration and decreased well-being, weightlessness, and motor activity; stomach/belly ache increased in the caffeine tablet group and decreased in the decaffeinated coffee group; desire for coffee was increased in the caffeine tablet group but not changed in the decaffeinated coffee group

| Naismith et al. (1970) | N=20 caffeine consumers (560 mg/day); within-subjects design; after a 10-day baseline period, subjects switched to decaffeinated coffee and abstained from all other sources of caffeine for 14 days; abstinence not biologically verified |
| Rollin (1981) | N=1 male habitual coffee user (900–1100 mg/day); within-subjects design; after a 72-h period of coffee consumption and after 24-h abstinence food was administered; at the end of 72 h either caffeinated coffee (approx. 150 mg caffeine) or decaffeinated coffee was ingested in a blinded fashion |
| Edelstein et al. (1983) | N=18 psychiatric patients who consumed three or more caffeinated beverages per day; within-subjects design; decaffeinated beverages were substituted for caffeinated beverages; abstinence not biologically verified |
| Mathew and Wilson (1988) | N=9 heavy (mean 986 mg/day) and 8 light (mean 126 mg/day) caffeine users; within-subjects design; after baseline assessment subjects abstained from caffeine for 24 h; abstinence not biologically verified |
| Rizzo et al. (1988) | N=20 daily caffeine users (mean 606 mg/day) and N=20 caffeine non-users; within-subjects design; two experimental sessions separated by 7 days; users abstained from caffeine for 2 days prior to the second session; abstinence not biologically verified |
| Courtarier et al. (1997) | N=20 adults presumed to be daily caffeine users; within-subjects design; subjects were assessed at baseline; 24 h later after complete abstinence, and 30 min and 120 min after receiving capsules containing 150 mg caffeine; abstinence was biologically verified |
| Lane (1997) | N=16 daily caffeine consumers (mean 612 mg/day); within-subjects design; subjects were assessed on two occasions; after usual caffeine consumption and after overnight abstinence (estimated 12–28 h abstinence); abstinence not biologically verified |

Compared with caffeine, placebo produced increased drowsy/sleepy, lethargy/fatigue/tired/sleepy, headache, self-confidence, and heavy feelings in arms and legs, and decreased desire to socialize/talkativeness; 9 of 14 subjects reported more headache after caffeine and 2 subjects reported more headache after placebo

Compared with caffeine, placebo produced greater cerebral blood flow velocities in white matter, anterior gray matter, and posterior gray matter regions; high caffeine users showed a greater caffeine-placebo difference in the anterior gray matter than low users; across all subjects, cerebral blood flow in the placebo condition increased linearly with daily caffeine intake
Table 2 (continued)

| Reference | Design | Withdrawal signs and symptoms
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>56. Lane and Phillips-Bute (1998)</td>
<td>N=30 daily coffee consumers (mean 569 mg/day caffeine); within-subjects design; subjects were assessed on two occasions: after usual caffeine consumption and after overnight abstinence (estimated 12-28 h abstinence); abstinence not biologically verified</td>
<td>Compared with ad libitum caffeine consumption, caffeine abstinence increased headache, drowsy/sleepy, irritable/cross/grumpy; fatigue (POMS), lethargy/tired/weak/foggy/not clearheaded, yawning, anger-hostility (POMS), confusion-bewilderment (POMS), depression-dejection (POMS), and decreased vigor (POMS), well-being, desire to socialize/withdrawal, ability to concentrate, energy/active, alert/distracted/observant; urge to do task/work or related activities; hot or cold spells, need to pass water; on a visual vigilance task abstinence decreased hit-rate and increased response time, and abstinence increased subjects' ratings of perceived difficulty and decreased perceived importance of doing well and perceived success; after abstinence 47% reported any headache and 27% reported moderate or severe headache</td>
</tr>
<tr>
<td>57. Reeves et al. (1995, 1997, 2002), Patrick et al. (1996)</td>
<td>N=13 or 14 daily caffeine users (&gt;300 mg/day); within-subjects design; subjects evaluated while consuming their normal caffeine diet (baseline) and again after 1, 2, and 4 days of abstinence; evaluations included topographic quantitative EEG, physician ratings of caffeine withdrawal severity as assessed during a structured interview, and a Minnesota/Sudol examination of the skin; abstinence was biologically verified</td>
<td>Compared with baseline, significant quantitative EEG changes were: increase in theta absolute power, increase in delta absolute power over the frontal cortical areas, decrease in the mean frequency of both alpha and beta rhythm, increase in theta relative power, and change in interhemispheric coherence; resolution of caffeine following abstinence returned altered EEG values to baseline levels; all subjects reported caffeine withdrawal symptoms and 77% had moderate or severe withdrawal severity as rated by a physician; with baseline, the number of somatic dysfunctions from the musculoskeletal examination was significantly greater on days 1, 2, and 4 of caffeine abstinence; the greatest number of somatic dysfunctions was reported on day 2; six subjects who had baseline EEGs containing diffuse paroxysmal slowing (DPS, a minor EEG dysrhythmia) showed significant increases in DPS firing during withdrawal and a return to baseline firing levels following caffeine rechallenge</td>
</tr>
</tbody>
</table>

*a All studies had a caffeine abstinence period of 12 h or greater
*b All effects are statistically significant at $P<0.05$ unless otherwise noted
*c For consistency, when placebo and caffeine conditions are compared, the effects are described as the effects of placebo relative to caffeine
*d Likely a direct effect of caffeine rather than an effect of caffeine withdrawal
*e POMS indicates Profile of mood states questionnaire

Table 3 Summary of survey studies of caffeine withdrawal

| Reference | Design | Withdrawal signs and symptoms
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>58. Goldstein and Kaizer (1969)</td>
<td>N=183 female coffee consumers surveyed about the effects they would experience if morning coffee was omitted</td>
<td>Endorsement of symptoms increased as the number of cups of coffee consumed increased; among heavy coffee users (5-10 cups/day) the percentages reporting specific symptoms were 58% feel half awake, 24% lethargic, 21% irritable, 18% sleepy, 16% unable to work effectively, 15% restlessness, 8% headache</td>
</tr>
<tr>
<td>59. Winstead (1976)</td>
<td>N=135 mostly adult inpatients on psychiatric ward; survey of occurrence of caffeine-withdrawal symptoms</td>
<td>26% of heavy caffeine users (&gt;500 mg/day) reported experiencing &quot;anxiety withdrawal symptoms&quot;</td>
</tr>
<tr>
<td>60. Groden et al. (1978)</td>
<td>N=83 psychiatric inpatients; surveyed about the occurrence of headache on omission of morning coffee</td>
<td>11% endorsed experiencing headache</td>
</tr>
<tr>
<td>61. Groden et al. (1980)</td>
<td>N=206 medical inpatients questioned about the occurrence of headache upon stopping caffeine</td>
<td>20% of total sample (28% of the sample of 152 after those who answered &quot;don't know&quot; were excluded) reported caffeine-withdrawal headache; those reporting headache had higher mean caffeine intake (616 mg/day) than those not reporting headache (435 mg/day) 24% of subjects endorsed having experienced caffeine-withdrawal headache</td>
</tr>
<tr>
<td>62. Victor et al. (1981)</td>
<td>N=124 general medical inpatients; survey of the occurrence of caffeine-withdrawal headache</td>
<td>44% of caffeine consumers reported having abstained from caffeine for ≥24 h in the past year of those, 28% reported headache, 27% drowsiness, 21% fatigue, 10% anxiety, 8% depression, 3% nausea or vomiting, 28% strong desire to use, 20% irritability, 21% yawning, 11% difficulty concentrating, 18% less motivated to work</td>
</tr>
<tr>
<td>63. Hughes et al. (1998)</td>
<td>N=162 caffeine consumers; surveyed via telephone about caffeine abstinence and withdrawal symptoms</td>
<td>11% reported withdrawal symptoms upon stopping caffeine; of those, 25% reported symptoms severe enough to interfere with normal daily activities (e.g., lost time from work); headache was the most common symptom reported; it is unknown what percentage of daily users had actually abstained from caffeine</td>
</tr>
<tr>
<td>64. Dews et al. (1999)</td>
<td>N=6,815 adults who reported daily caffeine use were asked if they had &quot;problems or symptoms on stopping caffeine in the past&quot;</td>
<td>24% of subjects who reported stopping or cutting down their caffeine consumption were asked about caffeine-withdrawal symptoms</td>
</tr>
<tr>
<td>65. Kendler and Prescott (1999)</td>
<td>N=1,642 women in a population-based twin registry interviewed about their caffeine use; individuals who reported stopping or cutting down their caffeine consumption were asked about caffeine-withdrawal symptoms</td>
<td>Based on both interviews, 81% met the withdrawal criterion; 100% of subjects who met criteria for DSM-IV caffeine dependence reported withdrawal symptoms; the percentage of subjects reporting specific symptoms were 59% drowsiness, 56% fatigue, 56% sluggish/slowed down, 56% headache, 32% restless/cannot sit still, 29% nervous/anxious, 21% sick/nauseated/vomiting, 21% drink caffeine so you do not feel bad, and 9% sad/depressed</td>
</tr>
<tr>
<td>66. Bernstein et al. (2002), Ounsted et al. (2002)</td>
<td>N=36 adolescent daily caffeine consumers (mean 244 mg/day) who endorsed two or more DSM-IV caffeine dependence criteria during telephone prescreening; interviewed and assessed for caffeine dependence based on DSM-IV substance dependence criteria; 21 of these subjects were reassessed 1 year later</td>
<td>24% of subjects who reported stopping or cutting down their caffeine consumption reported having experienced headache plus one or more of the following: marked fatigue or drowsiness, marked anxiety or depression, and nausea or vomiting</td>
</tr>
</tbody>
</table>
### Table 4  Summary of withdrawal symptoms reported in experimental and survey studies described in Tables 1, 2, 3a

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Acute abstinence versus baselineb</th>
<th>Acute abstinence versus caffeineb</th>
<th>Acute abstinence in caffeine consumers versus non-consumersb</th>
<th>Acute abstinence versus chronic abstinenceb</th>
<th>Time-limited abstinence effectsb</th>
<th>Percentage of subjects showing effect in experimental studiesc</th>
<th>Percentage of subjects reporting symptom in survey studiesd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>4, 9, 20, 44, 45, 47, 49, 50, 51</td>
<td>1, 2, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 21, 23, 25, 27, 30, 33, 34, 35, 36, 43, 46, 47, 55, 56</td>
<td>6, 8, 27, 42</td>
<td>4, 5, 7, 44, 49</td>
<td>4, 5</td>
<td>1: 25% of trials (heavy users) [control 3%] 4: 100% headache (control 14%) 6: 79% headache e 7: 42% headache (control 10%) 9: 52% moderate or severe headache (control 2%) 11: 30% headache e 12: 64% maximum headache (control 0%) 15: 9% headache</td>
<td>58: 8% headache 60: 11% headache 61: 20% headache 62: 24% headache 63: 24% headache 65: 24% headache plus additional symptom 66: 56% headache</td>
</tr>
<tr>
<td>Tiredness/fatigue</td>
<td>4, 9, 20, 49, 50</td>
<td>2, 5, 6, 8, 9, 11, 12, 14, 15, 16, 17, 18, 19, 21, 23, 24, 25, 26, 28, 30, 33, 34, 35, 36, 43, 46, 55, 56</td>
<td>6, 8, 17, 26</td>
<td>4, 5, 17, 20, 49</td>
<td>6: 57% lethargy/fatigue e 9: 8% abnormal high fatigue (control 0%) 11: 24% fatigue e 12: 27% extreme fatigue (control 9%) 15: 18% fatigued/tired e 20: 68% tired and lethargy 49: 100% lassitude</td>
<td>58: 24% lethargy 63: 23% fatigue 66: 56% fatigued</td>
<td></td>
</tr>
<tr>
<td>Decreased energy/act-</td>
<td>4, 9</td>
<td>2, 5, 6, 8, 9, 12, 15, 18, 19, 21, 22, 23, 24, 26, 28, 29, 31, 33, 34, 35, 37, 55, 56</td>
<td>6, 26</td>
<td>4, 5</td>
<td>6: 64% ↓ energy/active e 9: 11% abnormal scores (control 0%) 12: 45% extreme ↓ in vigor (control 0%) 15: 27% ↓ stimulated/active/energy/excited e</td>
<td>58: 14% depressed 63: 18% sleep 65: 58% feel-half awake</td>
<td></td>
</tr>
<tr>
<td>Decreased alertness/</td>
<td>4, 20</td>
<td>2, 5, 6, 8, 15, 19, 21, 24, 25, 26, 27, 33, 34, 35, 36, 37, 39, 56</td>
<td>6, 26</td>
<td>4, 5</td>
<td>6: 50% ↓ alert/attentive e 15: 27% ↓ alert/attentive/able to concentrate e</td>
<td>58: 24% lethargy 63: 23% fatigue 66: 56% fatigued</td>
<td></td>
</tr>
<tr>
<td>Drowsiness/sleepiness</td>
<td>4, 9, 20, 44, 45</td>
<td>2, 6, 8, 9, 11, 14, 15, 17, 19, 21, 23, 24, 28, 46, 55, 56</td>
<td>6, 17, 26</td>
<td>4, 44</td>
<td>6: 64% drowsy–sleepy e 11: 24% drowsiness e 15: 45% drowsy/sleepy/tired 22: 63% drowsiness/fatigue e 23: 13% drowsiness e</td>
<td>58: 18% sleepy 58: 58% feel-half awake 63: 27% drowsiness 65: 59% drowsy/tired</td>
<td></td>
</tr>
<tr>
<td>Decreased contented-</td>
<td>9, 44, 45</td>
<td>2, 5, 6, 9, 17, 18, 19, 21, 22, 23, 24, 25, 26, 55, 56</td>
<td>6, 26</td>
<td>5, 44</td>
<td>6: 64% ↓ well-being e 50% ↓ content/ satisfied e 15: 18% ↓ content/relaxed/satisfied e</td>
<td>58: 14% depressed 63: 11% abnormal scores BDI (control 2%) 12: 36% elevations on BDI (control 0%) 15: 18% depressed e</td>
<td></td>
</tr>
<tr>
<td>Decreased desire to</td>
<td>4, 9</td>
<td>2, 5, 6, 9, 10, 14, 17, 22, 33, 34, 37, 46, 55, 56</td>
<td>6</td>
<td>5</td>
<td>6: 79% ↓ social disposition e 15: 9% ↓ talkative e</td>
<td>58: 14% depressed 63: 11% abnormal scores BDI (control 2%) 12: 36% elevations on BDI (control 0%) 15: 18% depressed e</td>
<td></td>
</tr>
<tr>
<td>“Flu-like” symptoms</td>
<td>9</td>
<td>5, 6, 9, 25, 30, 33, 34, 36, 55</td>
<td>6</td>
<td>5</td>
<td>55: 31% flu-like symptoms (control 0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depressed mood</td>
<td>9</td>
<td>5, 6, 9, 12, 14, 15, 17, 50</td>
<td>6, 17, 26</td>
<td>5</td>
<td>6: 14% depressed e 9: 11% abnormal scores BDI (control 2%) 12: 36% elevations on BDI (control 0%) 15: 18% depressed e</td>
<td>63: 4% depression 66: 9% sad/depressed</td>
<td></td>
</tr>
</tbody>
</table>
data on the independence of specific caffeine-withdrawal symptoms (e.g., factor analysis), the listing provides a useful framework for characterizing the results of caffeine-withdrawal studies. In Table 4, symptoms are sequenced from those documented (i.e., shown in columns 2–6) in the greatest number of experimental studies to those documented in the fewest. Table 4 also differentiates experiments according to five of the most frequently used experimental methodologies that have been used to draw inferences about caffeine-withdrawal symptoms and signs. Finally, Table 4 shows the percentages of individuals reporting withdrawal symptoms in both experimental and survey studies.

### Table 4 (continued)

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Acute abstinence versus baseline</th>
<th>Acute abstinence versus caffeine</th>
<th>Acute abstinence in caffeine consumers versus non-consumers</th>
<th>Time-limited abstinence effects</th>
<th>Percentage of subjects showing effect in experimental studies</th>
<th>Percentage of subjects reporting symptom in survey studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difficulty concentrating</td>
<td>20</td>
<td>5, 6, 19, 26, 37, 55, 56</td>
<td>6, 26</td>
<td>5</td>
<td>6: 79% ↓ able to concentrate*</td>
<td>63: 11% difficulty concentrating</td>
</tr>
<tr>
<td>Irritability</td>
<td>9</td>
<td>2, 5, 6, 9, 19, 22, 56</td>
<td>2, 6, 26</td>
<td>5</td>
<td>6: 29% irritable/cross/grumpy/*</td>
<td>58: 21% irritable</td>
</tr>
<tr>
<td>Unmotivated for work</td>
<td>9</td>
<td>5, 6, 9, 21, 22, 23, 24, 56</td>
<td>5</td>
<td>6</td>
<td>6: 57% ↓ motivation for work*</td>
<td>58: 16% unable to work effectively</td>
</tr>
<tr>
<td>Müzy/loggy/not clearheaded</td>
<td>9, 20</td>
<td>6, 9, 17, 18, 55, 56</td>
<td>17</td>
<td>6</td>
<td>6: 71% müzy/*</td>
<td></td>
</tr>
<tr>
<td>Yawning</td>
<td>9</td>
<td>6, 9, 19, 21, 28, 55, 56</td>
<td>6</td>
<td>6</td>
<td>6: 43% yawning*</td>
<td>63: 21% yawning</td>
</tr>
<tr>
<td>Decreased self-confidence</td>
<td>9</td>
<td>5, 6, 9, 21, 23, 24, 46</td>
<td>6</td>
<td>5</td>
<td>6: 64% ↓ self-confidence*</td>
<td></td>
</tr>
<tr>
<td>Confusion-bewilderment</td>
<td>9</td>
<td>6, 9, 22, 26, 34, 56</td>
<td>26</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total mood disturbance</td>
<td>9</td>
<td>6, 9, 26, 34, 35, 36</td>
<td>26</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea/vomiting/upset stomach</td>
<td>22, 23, 24, 38, 43, 44</td>
<td></td>
<td>6: 29% upset stomach*</td>
<td>63: 3% nausea or vomiting</td>
<td>20: 13% nausea and sickness with headache</td>
<td>66: 21% sick/nauseated/vomiting</td>
</tr>
<tr>
<td>Muscle pain/stiffness</td>
<td>45, 50</td>
<td>5, 6</td>
<td>6</td>
<td>5</td>
<td>6: 43% muscle pain*</td>
<td></td>
</tr>
<tr>
<td>Anxiety/nervousness</td>
<td>9, 22</td>
<td>9, 22</td>
<td>2, 40</td>
<td></td>
<td>9: 8% abnormal scores anxiety (control 0%)</td>
<td>59: 26% anxiety withdrawal symptoms</td>
</tr>
<tr>
<td>Heavy feelings in arms and legs</td>
<td>9</td>
<td>9, 37, 46, 55</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased sleep duration/quality</td>
<td>20, 44, 45</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Analgesics use</td>
<td>44</td>
<td>9, 12</td>
<td>44</td>
<td></td>
<td>9: 13% analgesics use (control 2%)</td>
<td>12: 45% analgesics use</td>
</tr>
<tr>
<td>Craving</td>
<td>5, 6</td>
<td>5, 6</td>
<td>6</td>
<td>5</td>
<td>6: 43% craving for caffeine*</td>
<td>63: 28% strong desire to use</td>
</tr>
<tr>
<td>Lightheaded/dizzy</td>
<td>6, 38, 55</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blurred vision</td>
<td>9</td>
<td>6, 9</td>
<td>6</td>
<td></td>
<td>6: 14% blurred vision*</td>
<td></td>
</tr>
<tr>
<td>Anger/hostility</td>
<td>17, 56</td>
<td></td>
<td>17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hot and cold spells</td>
<td>9</td>
<td>9, 56</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*aNumbers in table refer to the entry number in Tables 1, 2, 3
*bThe strengths and weaknesses of each of the types of comparisons presented in columns 2–6 are described in the text. Studies in columns 4–6 are more rigorous because the effects are not confounded by the direct effects of caffeine
*cThis column (column 7) shows the percentage of subjects reporting the symptom in experimental studies (as summarized in columns 2–6). For comparison, when available, the percentage of subjects reporting the symptom in the control condition (i.e., baseline or caffeine condition) is shown in brackets. For studies that conducted within-subject analyses of differences between the abstinence and control conditions, an asterisk (*) indicates the percentage of subjects who showed a statistically significant difference at $P<0.05$
*dPercentage of subjects reporting symptom in survey studies: this column shows the percentage of individuals reporting the occurrence of a symptom presumably based on their previous experience in the natural environment
*eIncidence data for this study were collapsed across two conditions: acute abstinence versus caffeine and acute abstinence versus chronic abstinence
Several different experimental methodologies have been used to draw inferences about the occurrence of caffeine withdrawal. As understanding the strengths and weaknesses of each of these methodologies is critical to drawing meaningful conclusions about the validity of the findings, this section will briefly list and critique the most commonly used experimental methodologies. The first five experimental methodologies (corresponding to columns 2–6 in Table 4) involve different comparisons with acute caffeine abstinence.

**Acute abstinence versus preceding caffeine baseline (not counterbalanced)**

In this within-subjects comparison, symptoms or signs during acute caffeine abstinence are compared with those during a preceding baseline of ad libitum caffeine consumption. This comparison may have good ecological validity because it can involve a naturally occurring pattern of caffeine consumption followed by abrupt abstinence. A limitation of this comparison is that baseline versus abstinence differences may be due to the simple absence of the direct effects of caffeine during abstinence (i.e., a caffeine offset effect rather than a time-limited withdrawal effect). Another limitation is that observed differences could be confounded by order effects (i.e., conditions are not counterbalanced).

**Acute abstinence versus caffeine**

In this comparison, which could involve within subjects or across groups designs, symptoms or signs during acute caffeine abstinence are compared with those during caffeine consumption, with conditions counterbalanced or randomized across subjects. As with the comparison with a preceding baseline caffeine condition, this comparison may have good ecological validity in modeling naturally occurring effects of caffeine abstinence. Although not confounded by order effects, this comparison has the limitation that caffeine versus abstinence differences may be due to the simple absence of the direct effects of caffeine during abstinence.

**Acute abstinence in caffeine consumers versus non-consumers**

In this between-groups comparison, symptoms or signs during acute abstinence in caffeine consumers are compared with those in non-consumers. As both groups are caffeine abstinent, it can be concluded that any difference observed is not confounded by the direct effects of caffeine. However, the possible confounding effects of population differences between caffeine consumers and non-consumers cannot be ruled out because of the self-selected nature of these groups.

**Acute abstinence versus chronic abstinence**

In this comparison, which could be within subjects or across groups, symptoms or signs during acute abstinence are compared with those during chronic abstinence (e.g., 1 week or more of caffeine abstinence). As both conditions involve caffeine abstinence, it can be concluded that any difference observed is not confounded by the direct effects of caffeine. Although this comparison provides more conclusive evidence of withdrawal effects than the preceding comparisons, the approach is conservative because it may underestimate the incidence or magnitude of withdrawal effects if symptoms or signs persist in the chronic abstinence condition.

**Time-limited abstinence effects**

By definition, a drug withdrawal symptom or sign should increase upon acute abstinence and then decrease over time with continued drug abstinence. A demonstration of such time-limited effects is not confounded by the direct effects of caffeine and is important for confirming that the effects observed are withdrawal related rather than reflecting caffeine offset effects (i.e., a return to the normal drug-free state).

**Other methodologies**

In addition to the five approaches described above, several other types of experimental methodologies can help to inform the interpretation of caffeine-withdrawal effects. Although not included in Table 4, studies using these other methodologies are discussed in the following section on symptoms and signs of caffeine withdrawal.

*Variation in caffeine maintenance dose* Demonstration that the severity or incidence of a symptom or sign increases with increases in the daily caffeine maintenance dose before abstinence helps to confirm that the withdrawal effect reflects a pharmacological process.

*Acute decreases in caffeine maintenance dose* When lower caffeine doses are substituted for the usual caffeine maintenance dose, the demonstration that the severity or incidence of a symptom or sign increases as the substituted dose decreases helps to confirm that the withdrawal effect reflects a pharmacological process.

*Manipulation of duration of caffeine maintenance* Demonstration that the severity or incidence of a symptom or sign increases with increases in the duration of daily caffeine maintenance dose before abstinence helps to confirm that the withdrawal effect reflects a pharmacological process.
gical process.

Re-administration of caffeine reverses abstinence effects
After a period of abstinence during which the severity or incidence of a symptom or sign develops, the demonstration that re-administration of caffeine rapidly and dose dependently reverses the abstinence effects helps to confirm that the withdrawal effect reflects a pharmacological process.

Symptoms and signs of caffeine withdrawal

Symptoms of caffeine withdrawal

In this section, the withdrawal symptoms (i.e., categories of self-reported changes in mood or behavior) that were assessed in the studies listed in Tables 1, 2, and 3, and summarized in Table 4, are individually discussed and evaluated. For some symptoms, relevant case reports are also discussed if they contribute to the evaluation. As in Table 4, the symptoms are sequenced from those that were documented in the greatest number of studies to those that were documented in the fewest number.

Of relevance to the assessment of each symptom is information about the “hit-rate” (i.e., the ratio of the number of times a symptom was found to be significant relative to the number of times it was assessed). For this analysis, the number of studies in which each symptom was documented was taken from the 57 experimental studies described in Tables 1 and 2 (and summarized in columns 2–6 of Table 4). The number of studies in which each symptom was assessed was obtained from an evaluation of the same 57 experimental studies. In several studies (Comer et al. 1997; Schuh and Griffiths 1997; Garrett and Griffiths 1998; Jones et al. 2000) all of the symptoms assessed could not be determined from the published article, and this information was obtained from the authors. [Methodological note: in the rare instance in which a study used a compound symptom descriptor (e.g., alert/attentive/observant/able to concentrate) that could potentially apply to two symptom categories (e.g., alert/attentiveness and difficulty concentrating), the data were counted in the single category that it best represented (e.g., alert/attentiveness was counted as assessed and difficulty concentrating was coded as not assessed).]

In interpreting the hit-rate, it should be noted that most of the statistically significant effects reported are based on group mean data. Thus, a high hit-rate indicates that the abstinence condition is readily differentiated from the comparison condition and usually reflects an intermediate to high incidence of the symptom. A low hit-rate indicates that the abstinence condition is not readily differentiated from the comparison condition. However, it is important to recognize that a low hit-rate does not mean that a symptom is not valid because such a symptom may have a low incidence and thus be undetected in a group statistical analysis. Furthermore, a symptom with a low hit-rate may also be clinically important to the extent that it may have profound effects in a small percentage of the population (e.g., psychotic symptoms in the general population). Finally, a failure to detect a particular effect may reflect methodological shortcomings of a study (e.g., insufficient period of abstinence, small sample size, insensitive measures).

Validity criteria For purposes of this review, the criteria for concluding that a symptom is valid was the statistical demonstration of the symptom in six or more studies that include two or more double-blind studies that used methodologies in which the conclusion of caffeine-withdrawal effects was not confounded by the direct effects of caffeine (i.e., Table 4, columns 4–6). As a conservative approach, two studies that used the same group of subjects (i.e., studies 5 and 6 in Table 4) were considered to be a single study for purposes of judging validity.

Headache (descriptors: headache and headachy) Headache has been the most frequently assessed symptom (48 experimental studies and 6 survey studies). Headache was found in 37 of 48 (77%) of the experimental studies in which it was assessed. The median percentage of individuals reporting headache in 19 experimental studies was 47%, ranging from 9% to 100% across studies (Table 4). Of the 7 experimental studies that assessed headache severity, the median percentage of subjects reporting moderate to severe or maximum headache was 50%. In 7 survey studies, the median percentage of caffeine users reporting caffeine-withdrawal headache was 24%, ranging from 8% to 56%.

Headache as a caffeine-withdrawal symptom has been demonstrated in studies comparing acute caffeine abstinence with a preceding baseline condition, a caffeine administration condition, and a chronic caffeine abstinence condition (Table 4) and by comparing acute caffeine abstinence in caffeine consumers with non-consumers (Table 4). Studies have also shown that abstinence-induced headache is time-limited (Table 4) and is rapidly (usually within 30–60 min) and often completely reversed after re-administration of caffeine (Driesbach and Pfeiffer 1943; Goldstein et al. 1969; Roller 1981; Couturier et al. 1997; Tinley et al. 2003), with the magnitude of reversal being an increasing function of the re-administered caffeine dose (Goldstein et al. 1969). Studies have also shown that the severity and incidence of headache after abstinence were increasing functions of caffeine maintenance dose (Goldstein 1964; Silverman et al. 1992; Evans and Griffiths 1999) and duration of caffeine dosing (Evans and Griffiths 1999) before abstinence. Finally, when different caffeine doses were substituted for the usual maintenance dose, the severity and incidence of headache increased as the substituted dose decreased (Evans and Griffiths 1999). Caffeine-withdrawal headache has been characterized in these experimental studies as well as in case reports as being gradual in development (Driesbach and Pfeiffer 1943; Greden et al. 1980; Roller 1981; Griffiths et al. 1990), diffuse (Driesbach and Pfeiffer 1943; Greden 1974; Greden et al. 1980; Lader et al. 1996),
Experimental studies (75%). In 4 experimental studies, vigor, lively, stronger/more vigorous/more energy) energy, energetic, active, stimulated/active/energetic/excited, decreased energy/activeness (descriptors: decreased energy/activeness is demonstrated under a wide range of methodological conditions. It is concluded that tiredness/fatigue is a valid withdrawal symptom. Decreased energy/activeness has been demonstrated in 21 of 27 experimental studies (78%). Cross 5 experimental studies providing incidence data (Table 4), the median percentage of individuals showing decreased energy/activeness was 36%. Decreased energy/activeness has been demonstrated in studies comparing acute caffeine abstinence with a preceding baseline condition, a caffeine administration condition, and a chronic caffeine abstinence condition (Table 4), and by comparing acute caffeine abstinence in caffeine consumers with that in non-consumers (Table 4). Studies have have also shown that abstinence-induced decreased energy/activeness is time limited (Table 4) and is rapidly (i.e., within 30–60 min) and completely reversed after re-administration of caffeine (Goldstein et al. 1969). Studies have also shown that the severity of decreased energy/activeness is an increasing function of the caffeine maintenance dose before abstinence (Rogers et al. 1995; Evans and Griffiths 1999). In summary, decreased energy/activeness has been very frequently studied, has an intermediate incidence, and has been demonstrated under a wide range of different methodological conditions. It is concluded that decreased energy/activeness is a valid withdrawal symptom.

Tiredness/fatigue (descriptors: tiredness, tired, fatigue, lazy, sluggish, lazy/sluggish/slow-moving, lethargy/fatigue/tired/sleepy, sluggish/slowed down, worn-out, and lassitude) Tiredness/fatigue was demonstrated in 32 of 38 studies (84%). In 7 experimental studies providing incidence data (Table 4), the median percentage of individuals showing tiredness/fatigue was 27%. In 3 survey studies, the percentage of subjects reporting tiredness/fatigue ranged between 21% and 56%. Tiredness/fatigue has been demonstrated in studies comparing acute caffeine abstinence with a preceding baseline condition, a caffeine administration condition, and a chronic caffeine abstinence condition (Table 4), and by comparing acute caffeine abstinence in caffeine consumers with that in non-consumers (Table 4). Studies have also shown that abstinence-induced tiredness/fatigue is time limited (Table 4), completely reversed after re-administration of caffeine (Roller 1981), and an increasing function of the duration of caffeine maintenance before abstinence (Evans and Griffiths 1999). Furthermore, when different caffeine doses are substituted for the usual maintenance dose, the magnitude of tiredness/fatigue increases as the substituted dose decreases (Evans and Griffiths 1999). Severity of tiredness/fatigue also appears to increase at higher daily caffeine maintenance doses (Rogers et al. 1995).

In summary, tiredness/fatigue has been very frequently studied, has a low to moderate incidence, and has been demonstrated under a wide range of methodological conditions. It is concluded that tiredness/fatigue is a valid withdrawal symptom.

Decreased alertness/attentiveness (descriptors: decreased alertness, alert/attentive/observant, alert/attentive/able to concentrate, and activity/alertness) Decreased alertness/attentiveness was demonstrated in 22 of 31 experimental studies (71%). Two experimental studies reported incidence data of 27% and 50% (Table 4). Decreased alertness/attentiveness has been demonstrated in studies comparing acute caffeine abstinence with a preceding baseline condition, a caffeine administration condition, and a chronic caffeine abstinence condition (Table 4), and by comparing acute caffeine abstinence in caffeine consumers with that in non-consumers (Table 4). Studies have also shown that abstinence-induced decreased alertness/attentiveness is time limited (Table 4) and is rapidly (i.e., within 30–60 min) and completely reversed after re-administration of caffeine (Goldstein et al. 1969), with the magnitude of reversal being an increasing function of the re-administered caffeine dose (Goldstein et al. 1969). In addition, deficits in alertness/attentiveness are an increasing function of caffeine maintenance dose before abstinence (Rogers et al. 1995; Evans and Griffiths 1999). Finally, when different caffeine doses are substituted for the usual maintenance dose, the magnitude of alertness/attentiveness decreases as the substituted dose decreases (Evans and Griffiths 1999).

In summary, decreased alertness/attentiveness has been very frequently studied, may have an intermediate incidence, and has been demonstrated under a wide range of methodological conditions. It is concluded that decreased alertness/attentiveness is a valid withdrawal symptom.

Decreased energy/activeness was demonstrated in 24 of 32 experimental studies (75%). In 4 experimental studies providing incidence data (Table 4), the median percentage of individuals showing decreased energy/activeness was 36%. Decreased energy/activeness has been demonstrated in studies comparing acute caffeine abstinence with a preceding baseline condition, a caffeine administration condition, and a chronic caffeine abstinence condition (Table 4), and by comparing acute caffeine abstinence in caffeine consumers with that in non-consumers (Table 4). Studies have have also shown that abstinence-induced decreased energy/activeness is time limited (Table 4) and is rapidly (i.e., within 30–60 min) and completely reversed after re-administration of caffeine (Goldstein et al. 1969). Studies have also shown that the severity of decreased energy/activeness is an increasing function of the caffeine maintenance dose before abstinence (Rogers et al. 1995; Evans and Griffiths 1999).

In summary, decreased energy/activeness has been very frequently studied, has an intermediate incidence, and has been demonstrated under a wide range of methodological conditions. It is concluded that decreased energy/activeness is a valid withdrawal symptom.

Drowsiness/sleepiness (descriptors: drowsiness, sleepy, drowsy/sleepy/tired, sedated, feel half awake, and decreased wakefulness) Drowsiness/sleepiness was demonstrated in 21 of 27 experimental studies (78%). Across 5 experimental studies providing incidence data (Table 4),
the median percentage of individuals showing drowsiness/sleepiness was 45%. In 3 survey studies, the percentage of subjects reporting tiredness/fatigue ranged between 18% and 59%. Drowsiness/sleepiness has been demonstrated in studies comparing acute caffeine abstinence with a preceding baseline condition, a caffeine administration condition, and a chronic caffeine abstinence condition (Table 4), and by comparing acute caffeine abstinence in caffeine consumers with that in non-consumers (Table 4). Studies have also shown that abstinence-induced drowsiness/sleepiness is time limited (Table 4) and is rapidly (i.e., within 30–60 min) and completely reversed after re-administration of caffeine (Goldstein et al. 1969), with the magnitude of reversal being an increasing function of the re-administered caffeine dose (Goldstein et al. 1969). Severity is positively correlated with daily caffeine dose before abstinence (Silverman et al. 1992).

In summary, drowsiness/sleepiness has been very frequently studied, has an intermediate incidence, and has been demonstrated under a wide range of methodological conditions. It is concluded that drowsiness/sleepiness is a valid withdrawal symptom.

**Decreased contentedness/well-being (descriptors: decreased contentedness, content/satisfied, well-being, day positive, cheerful, happy, and increased miserable)**

Decreased contentedness/well-being was demonstrated in 17 of 28 experimental studies (61%). In 2 experimental studies, 18% and 64% of participants showed decreases in measures of contentedness/well-being (Table 4). Decreased contentedness/well-being has been demonstrated in studies comparing acute caffeine abstinence with a preceding baseline condition, a caffeine administration condition, and a chronic caffeine abstinence condition (Table 4), and by comparing acute caffeine abstinence in caffeine consumers with that in non-consumers (Table 4). Studies have also shown that abstinence-induced decreased contentedness/well-being is time limited (Table 4) and is rapidly (i.e., within 30–60 min) and completely reversed after re-administration of caffeine (Goldstein et al. 1969), with the magnitude of reversal being an increasing function of the re-administered caffeine dose (Goldstein et al. 1969). Severity also appears to increase with higher daily maintenance caffeine doses before abstinence (Rogers et al. 1995).

In summary, decreased contentedness/well-being has been very frequently studied, may have an intermediate incidence, and has been demonstrated under a wide range of methodological conditions. It is concluded that decreased contentedness/well-being is a valid withdrawal symptom.

**Decreased desire to socialize (descriptors: decreased desire to socialize, talkativeness, social disposition, and friendliness)**

Decreased desire to socialize was demonstrated in 15 of 28 experimental studies (54%). One experimental study reported incidence data of 79% for decreased social disposition and a second study reported 9% for decreased talkativeness (Table 4). Decreased desire to socialize has been demonstrated in studies comparing acute caffeine abstinence with a preceding baseline condition, a caffeine administration condition, and a chronic caffeine abstinence condition (Table 4). Studies have also shown that abstinence-induced decreased desire to socialize is time limited (Table 4) and that the magnitude is an increasing function of caffeine maintenance dose before abstinence (Evans and Griffiths 1999).

In summary, decreased desire to socialize has been very frequently studied with varied incidence. Although it has been demonstrated under several different methodological conditions, it does not presently fulfill the criteria for validity.

**Flu-like symptoms (descriptors: “flu-like symptoms” and “flu-like feelings”)**

An increase in this category was demonstrated in 9 of 17 (53%) experimental studies. One experimental study reported incidence data of 31% for flu-like symptoms (Table 4). Flu-like symptoms as a symptom category have been demonstrated in studies comparing acute caffeine abstinence with a preceding baseline condition, a caffeine administration condition, and a chronic caffeine abstinence condition (Table 4). Studies have also shown that abstinence-induced flu-like symptoms are time-limited (Table 4) and that the severity of flu-like symptoms is an increasing function of caffeine maintenance dose before abstinence (Lane and Phillips-Bute 1998; Evans and Griffiths 1999).

In summary, the flu-like symptoms category has been frequently studied, appears to have an intermediate incidence, and has been demonstrated under several methodological conditions. In addition to the foregoing studies, one study (Swedlow et al. 2000) showed caffeine-abstinence-induced increases in a composite scale that included flu-like symptoms (i.e., feel sick, queasy, dizzy, and perspiring). It seems plausible that endorsement of flu-like symptoms is related to a constellation of somatic symptoms that include nausea/vomiting, muscle pain/stiffness, and heavy feelings in arms and legs (these individual symptoms are discussed below). Although most of the experimental studies demonstrating statistical increases in flu-like symptoms have involved comparisons of caffeine abstinence to a caffeine administration condition, the category appears to reflect a genuine withdrawal effect because endorsement of flu-like symptoms is time limited (Griffiths et al. 1990) and it is implausible that such placebo versus caffeine differences represent a direct effect of caffeine in suppressing naturally occurring flu-like symptoms. Thus, even though the flu-like symptoms category fails to meet our a priori criteria for validity, the category appears to be a valid caffeine-withdrawal effect. Furthermore, the flu-like symptoms category may be clinically important because it may reflect significant distress.

**Depressed mood (descriptors: depression, dejection, sad/depressed, and elevated scores on the Beck Depression Inventory)**

Symptoms of depressed mood were demonstrated in 9 of 29 experimental studies (31%). In 4
experimental studies providing incidence data, the median percentage of individuals reporting depressed mood was 16% (range 11–36%; Table 4). In 2 survey studies, the percentage of subjects reporting depression was 4% and 9%. Depressed mood has been demonstrated in studies comparing acute caffeine abstinence with a preceding baseline condition, a caffeine administration condition, and a chronic caffeine abstinence condition (Table 4), and by comparing acute caffeine abstinence in caffeine consumers with that in non-consumers (Table 4). Studies also showed that abstinence-induced depressed mood is time limited (Table 4).

In summary, depressed mood has been very frequently studied, has a low to moderate incidence, and has been demonstrated under a wide range of methodological conditions. It is concluded that depressed mood is a valid withdrawal symptom.

**Difficulty concentrating (descriptors: difficulty concentrating, decreased concentration, and decreased ability to concentrate)** Difficulty concentrating was demonstrated in 8 of 12 experimental studies (67%). One experimental study reported incidence data of 79% and one survey study found that 11% of respondents reported difficulty concentrating (Table 4). Difficulty concentrating has been demonstrated in studies comparing acute caffeine abstinence with a preceding baseline condition, a caffeine administration condition, and a chronic caffeine abstinence condition (Table 4). Studies have also shown that abstinence-induced difficulty concentrating is time limited (Table 4), and the severity is positively correlated with daily caffeine dose before abstinence (Lane 1997).

In summary, difficulty concentrating has been moderately frequently studied, may have a high incidence, and has been demonstrated under a wide range of methodological conditions. It is concluded that difficulty concentrating is a valid withdrawal symptom.

**Irritability (descriptors: irritability, irritable/cross/grumpy, and irritable/frustrated/angry/cross)** Irritability was demonstrated in 8 of 23 experimental studies (35%). Two experimental studies reported incidence data of 29% and 9% (Table 4). In 2 survey studies, the percentage of subjects reporting irritability during caffeine abstinence was 21% and 20%. Irritability has been demonstrated in studies comparing acute caffeine abstinence with a preceding baseline condition, a caffeine administration condition, and a chronic caffeine abstinence condition (Table 4), and by comparing acute caffeine abstinence in caffeine consumers with that in non-consumers (Table 4). Studies have also shown that abstinence-induced irritability is time limited (Table 4) and is rapidly (i.e., within 60 min) and completely reversed after re-administration of caffeine (Goldstein et al. 1969), with the magnitude of reversal being an increasing function of the re-administered caffeine dose (Goldstein et al. 1969).

In summary, irritability has been very frequently studied, has a low to moderate incidence, and has been demonstrated under a wide range of methodological conditions. It is concluded that irritability is a valid withdrawal symptom.

**Unmotivated for work (descriptors: decreased motivation for work, urge to do tasks/work-related activities, and increased unmotivated)** Being unmotivated for work was demonstrated in 8 of 16 experimental studies (50%). One experimental study reported incidence data of 57% for decreased motivation for work and 64% for decreased urge to do work (Table 4). In 2 survey studies, the percentage of subjects reporting being unmotivated for work was 16% and 18%. Unmotivated for work has been demonstrated in studies comparing acute caffeine abstinence with a preceding baseline condition and with a caffeine administration condition (Table 4). Studies have also shown that abstinence-induced “unmotivated for work” is time limited (Table 4), and the severity is positively correlated with daily caffeine dose before abstinence (Lane 1997).

In summary, unmotivated for work has been frequently studied, has an intermediate incidence, and has been demonstrated under several methodological conditions. It has also been described anecdotally (Driesbach and Pfeiffer 1943). Although the data are suggestive, further research is needed to determine the validity of being unmotivated for work as a withdrawal symptom.

**Muzzy/foggy/not clearheaded (descriptors: muzzy/foggy/not clearheaded, muzziness, muddled, and decreased clearheaded)** Muzzy/foggy/not clearheaded was demonstrated in 7 of 18 experimental studies (39%). One experimental study reported incidence data of 71% (Table 4). Muzzy/foggy/not clearheaded has been demonstrated in studies comparing acute caffeine abstinence with a preceding baseline condition, a caffeine administration condition, and a chronic caffeine abstinence condition (Table 4), and by comparing acute caffeine abstinence in caffeine consumers with that in non-consumers (Table 4). Studies have also shown that abstinence-induced muzzy/foggy/not clearheaded is time limited (Table 4), and the severity is positively correlated with daily caffeine dose before abstinence (Lane 1997).

In summary, muzzy/foggy/not clearheaded has been frequently studied, may have a high incidence, and has been demonstrated under a wide range of methodological conditions. It is concluded that muzzy/foggy/not clearheaded is a valid withdrawal symptom.

**Yawning (no other descriptors)** Self-reported yawning was demonstrated in 7 of 12 experimental studies (58%). One experimental study reported incidence data of 43% and one survey study found that 21% of respondents reported yawning (Table 4). Yawning has been demonstrated in studies comparing acute caffeine abstinence with a preceding baseline condition, a caffeine administration condition, and a chronic caffeine abstinence condition (Table 4). Studies have also shown that abstinence-induced yawning is time limited (Table 4), and the severity is positively correlated with daily caffeine dose before abstinence (Silverman et al. 1992).
In summary, yawning has been moderately frequently studied, may have an intermediate incidence, and has been demonstrated under several methodological conditions. Although the data are suggestive, further research is needed to determine the validity of yawning as a withdrawal symptom.

**Decreased self-confidence (descriptors: decreased self-confidence and confident)** Decreased self-confidence was found in 7 of 17 experimental studies (41%). One experimental study reported incidence data of 64% (Table 4). Decreased self-confidence has been demonstrated in studies comparing acute caffeine abstinence with a preceding baseline condition, a caffeine administration condition, and a chronic caffeine abstinence condition (Table 4). Studies have also shown that abstinence-induced decreased self-confidence is time limited (Table 4).

In summary, decreased self-confidence has been frequently studied and may have an intermediate incidence. Although it has been demonstrated under a several different methodological conditions, it does not presently fulfill criteria for validity.

**Confusion–bewilderment [POMS] (no other descriptors)** Confusion/bewilderment was found in 6 of 21 experimental studies (29%). Confusion–bewilderment has been demonstrated in studies comparing acute caffeine abstinence with a preceding baseline condition, a caffeine administration condition, and a chronic caffeine abstinence condition (Table 4). Studies have also shown that abstinence-induced confusion–bewilderment is time limited (Table 4).

In summary, confusion–bewilderment has been very frequently studied and has been demonstrated under several methodological conditions. Although the data are suggestive, it does not presently fulfill criteria for validity.

**Total mood disturbance [POMS] (no other descriptors)** Total mood disturbance has been shown in 6 of 12 experimental studies (50%). Total mood disturbance has been demonstrated in studies comparing acute caffeine abstinence with a preceding baseline condition, a caffeine administration condition, and a chronic caffeine abstinence condition (Table 4). Studies have also shown that the severity of total mood disturbance is an increasing function of caffeine maintenance dose before abstinence (Evans and Griffiths 1999).

In summary, total mood disturbance has been moderately frequently studied and has been demonstrated under several methodological conditions. Although the data are suggestive, further research is needed to determine validity.

**Nausea/vomiting (descriptors: nausea/vomiting, nausea and sickness, sick/nauseated/vomiting, vomiting, queasy, and upset stomach)** Nausea/vomiting has been demonstrated in 6 of 24 experimental studies (25%). In 4 experimental studies providing incidence data (Table 4), the median percentage of individuals reporting nausea/vomiting was 21% (range 10–33%). In 2 survey studies, the percentage of subjects reporting nausea/vomiting during caffeine abstinence was 3% and 21%. Nausea/vomiting has been demonstrated in studies comparing acute caffeine abstinence with a caffeine administration condition (Table 4).

In summary, nausea/vomiting has been very frequently studied and has a low to moderate incidence. Although in experimental studies nausea/vomiting has only been demonstrated statistically by comparing caffeine abstinence with a caffeine administration condition, it appears to be a genuine withdrawal symptom because it is implausible that such placebo versus caffeine differences represent a direct effect of caffeine in suppressing naturally occurring nausea/vomiting. Instances of withdrawal-induced nausea/vomiting have been reported in case reports (Rainey 1985; Cacciapuoti et al. 1996), experimental studies (Griffiths et al. 1990; Silverman et al. 1992; Strain et al. 1994), and survey studies (Hughes et al. 1998; Oberstar et al. 2002). Thus, even though nausea/vomiting fails to meet our a priori criterion for validity based on experimental studies alone, nausea/vomiting appears to be a valid caffeine-withdrawal symptom. Furthermore, it may be a clinically important symptom because it is likely to reflect significant distress. It seems plausible that this symptom is related to a constellation of symptoms that include other somatic complaints and endorsement of flu-like symptoms.

**Muscle pain/stiffness (descriptors: muscle pain/stiffness, muscle joint ache, general muscle pains, and leg muscle pains)** Muscle pain/stiffness has been demonstrated in 4 of 15 experimental studies (27%). The incidence of muscle pain/stiffness was 43% in one study (Table 4). Muscle pain/stiffness has been demonstrated in studies comparing acute caffeine abstinence with a preceding baseline condition, a caffeine administration condition, and a chronic caffeine abstinence condition (Table 4). One study also showed that abstinence-induced muscle pain/stiffness is time limited (Table 4).

In summary, muscle pain/stiffness has been studied frequently, may have an intermediate incidence, and has been demonstrated under a range of methodological conditions. The symptom of muscle pain/stiffness has been described in case reports (Cobbs 1982; Stringer and Watson 1987) and is consistent with one report, which involved a musculoskeletal examination during caffeine abstinence (Reeves et al. 1997). Although additional studies are needed to fulfill our a priori validity criteria, the conclusion that muscle pain/stiffness represents a true withdrawal symptom unconfounded by the direct effects of caffeine seems reasonable because it is improbable that caffeine suppresses naturally occurring muscle pain/stiffness.
Anxiety/nervousness (descriptors: anxiety, anxious, nervous, tense, and elevated scores on the State-Trait Anxiety Inventory) Increased anxiety/nervousness has been demonstrated in 4 of 34 experimental studies (12%; Table 4). However, decreased anxiety/nervousness has been demonstrated in 2 of the 36 studies (Griffiths et al. 1990; Comer et al. 1997). One experimental study reported incidence data of 8% for increased anxiety (Table 4). In 3 survey studies, the percentage of subjects reporting increased anxiety during abstinence ranged between 10% and 29%. Anxiety/nervousness has been demonstrated in studies comparing acute caffeine abstinence with a preceding baseline condition and a caffeine administration condition (Table 4), and by comparing acute caffeine abstinence in caffeine consumers with that in nonconsumers (Table 4).

Despite the fact that marked anxiety is proposed as a caffeine-withdrawal symptom in DSM-IV-TR (American Psychiatric Association 2000), anxiety/nervousness does not fulfill the validity criteria. It is of note that 32 experimental studies using a variety of methodologies failed to show increased anxiety/nervousness, none of the positive studies used the most rigorous experimental design involving a chronic abstinence condition, and 2 experimental studies demonstrated significant decreases (Griffiths et al. 1990; Comer et al. 1997). However, it is also noteworthy that anxiety/nervousness has been described as a withdrawal symptom in case reports (Gibson 1981; Cobbs 1982; Rainey 1985; Adams et al. 1993) and that anxiety/nervousness is also endorsed as a withdrawal symptom at low to moderate rates in three survey studies (Table 4). Although further research is needed, it may be that the increased anxiety/nervousness associated with caffeine withdrawal in non-blind case reports and survey studies reflects increased anxiety in anticipation of experiencing unpleasant effects of caffeine abstinence.

Heavy feelings in arms and legs (no other descriptors) Heavy feelings in arms and legs were demonstrated in 4 of 9 experimental studies (44%). Heavy feelings in arms and legs have been demonstrated in studies comparing acute caffeine abstinence with a preceding baseline condition, a caffeine administration condition, and a chronic caffeine abstinence condition (Table 4). Although the data are suggestive, further research is needed to determine the validity of heavy feelings in arms and legs as a withdrawal symptom.

Increased nighttime sleep duration/quality (descriptors: self-report ratings of sleep quality, duration, and onset to sleep) Self-reported increased nighttime sleep duration/quality was demonstrated in 4 of 4 experimental studies. In the two studies that assessed sleep duration, the increase was about 30 min (Höfer and Bättig 1994b; James 1998). Increased nighttime sleep duration/quality has been demonstrated in studies comparing acute caffeine abstinence with a preceding baseline condition and a caffeine administration condition (Table 4).

Although increased nighttime sleep has been rarely studied during caffeine abstinence, the magnitude of increased sleep duration is notable. The limited methodologies in which it has been assessed do not permit differentiation between an effect of caffeine in decreasing sleep and a time limited caffeine-withdrawal effect. Further research is needed to determine whether this is a valid withdrawal symptom.

Analgesic use (no other descriptors) Self-reported analgesic use was demonstrated in 3 of 3 experimental studies. Two of these studies explicitly discouraged analgesic use (Silverman et al. 1992; Strain et al. 1994). Two experimental studies reported incidence data of 13% and 45% (Table 4). Analgesic use has been demonstrated in studies comparing acute caffeine abstinence with a preceding baseline condition and a caffeine administration condition (Table 4). Studies also showed that abstinence-induced analgesic use was time limited (Table 4).

Although analgesic use during caffeine abstinence has been rarely assessed, it is a potential indicator of the clinical significance of caffeine-withdrawal distress and is worthy of future study. Whether avoidance of caffeine withdrawal contributes to chronic use of caffeine-containing analgesics as suggested by some reports (Strain and Griffiths 1998; Bigal et al. 2002; but cf. Feinstein et al. 2000) also merits further study.

Craving/strong desire to use (no other descriptors) Craving/strong desire to use was demonstrated in 2 of 2 experimental studies. One experimental study reported incidence data of 43% and one survey study reported that 28% of respondents reported a strong desire to use (Table 4). Craving/strong desire to use has been demonstrated in studies comparing acute caffeine abstinence with a caffeine administration condition and has been shown to be time limited (Table 4).

Although craving is often reported anecdotally during caffeine abstinence in the natural environment (Rippen 1984; Gilbert 1986), it has been rarely assessed as a caffeine-withdrawal symptom. Further research is needed to determine its validity. Given its potential importance for understanding habitual caffeine consumption, a priority should be given to including measures of craving in future caffeine-withdrawal research.

Blurred vision (no other descriptors) Blurred vision was demonstrated in 2 of 11 experimental studies (18%). The incidence of blurred vision was 14% in one experimental study (Table 4). It has been demonstrated in studies comparing acute caffeine abstinence with a preceding baseline condition, a caffeine administration condition, and a chronic caffeine abstinence condition (Table 4). One study also showed that abstinence-induced blurred vision was time limited (Table 4).

In summary, blurred vision has been studied moderately frequently, may have a low incidence, and has been demonstrated under several methodological conditions. The symptom of blurred vision is also consistent with a
Limb tremor (no other descriptors) Lightheaded/dizzy was demonstrated in 3 of 18 experimental studies (17%). Lightheaded/dizzy has been demonstrated only in studies comparing acute caffeine abstinence with a caffeine administration condition.

Although lightheaded/dizzy has been frequently studied, it has only been demonstrated in three studies, one of which was not blind. It is concluded that there is little evidence supporting lightheaded/dizzy as a caffeine-withdrawal symptom.

Anger/hostility (descriptors: anger–hostility and angry) Anger/hostility was demonstrated in 2 of 20 experimental studies (10%). It has been demonstrated in studies comparing acute caffeine abstinence with a caffeine administration condition and a chronic caffeine abstinence condition (Table 4), and by comparing acute caffeine abstinence in caffeine consumers with that in non-consumers (Table 4). Studies have also shown that abstinence-induced anger/hostility is time limited (Table 4).

Although anger/hostility has been very frequently studied using various methodologies, it has only been demonstrated in two studies, one of which was not blind. It is concluded that there is little evidence to suggest that anger/hostility is a caffeine-withdrawal symptom.

Hot and cold spells (no other descriptors) This symptom was demonstrated in 2 of 8 experimental studies (25%). Hot and cold spells have been demonstrated only in studies comparing acute caffeine abstinence with a baseline or a caffeine administration condition. Further research is needed to determine the validity.

Rhinorrhea (runny nose) [(descriptors: rhinorrhea and runny nose)] Not shown in Table 4, rhinorrhea was demonstrated in one non-blind study (Roller 1981) of 12 experimental studies that used a variety of methodologies. Caffeine-withdrawal-induced rhinorrhea was also described anecdotally in two reports (Driesbach and Pfeiffer 1943; Greden et al. 1980). It is concluded that there is little evidence to suggest that rhinorrhea is a caffeine-withdrawal symptom.

Impaired behavioral and cognitive performance This category is comprised of tasks designed to assess various aspects of performance impairment. Of 23 experimental studies that assessed performance during caffeine abstinence, 11 (48%) reported significant impairment on one or more measures. More specifically, impairment of tapping speed occurred in 3 of 8 studies (Bruce et al. 1991; Silverman et al. 1992; Strain et al. 1994), impairment of visual vigilance occurred in 2 of 4 studies (Lane and Phillips-Bute 1998; Yeomans et al. 2002b), decreased reaction time occurred in 2 of 7 studies (Rizzo et al. 1988; Robelin and Rogers 1998), impaired performance on a digit symbol substitution task occurred in 2 of 11 studies (Liguori and Hughes 1997; Liguori et al. 1997b), impaired performance on a character recognition task occurred in 1 study (James 1998), and impairment in 5 of 7 measures in a complex cognitive problem-solving task occurred in 1 study (Streufert et al. 1995). Four studies assessing memory found no evidence for impairment during caffeine abstinence. With regard to incidence, one experimental study reported incidence data of 55% for decreased tapping speed (Strain et al. 1994). Methodologically, impaired performance has been demonstrated in studies comparing acute caffeine abstinence with a preceding baseline condition (Silverman et al. 1992), a caffeine administration condition (Silverman et al. 1992; Strain et al. 1994; Streufert et al. 1995; Liguori and Hughes 1997; Liguori et al. 1997b; James 1998; Lane and Phillips-Bute 1998; Robelin and Rogers 1998; Yeomans et al. 2002b), and a chronic caffeine abstinence condition (Bruce et al. 1991), and by comparing acute caffeine abstinence in caffeine consumers with that in non-consumers (Rizzo et
al. 1988). Studies have also shown that abstinence-induced impaired behavioral performance is time limited (Bruce et al. 1991).

In summary, impaired behavioral or cognitive performance has been very frequently studied, may have an intermediate incidence, and has been demonstrated under a range of methodological conditions. Furthermore, the types of impairments observed (i.e., psychomotor speed, vigilance, and cognitive performance) appear consistent with the profile of validated withdrawal symptoms such as tiredness/fatigue, decreased alertness, and difficulty concentrating. It should be noted, however, that this category is comprised of heterogeneous measures of performance impairment and, at present, there is not enough information to reach a conclusion about the validity of any specific performance measure. Future research should focus on measures that appear to be most sensitive to caffeine abstinence, including tapping, vigilance, reaction time, and complex cognitive problem solving.

**Increased cerebral blood flow**

Increased cerebral blood flow velocity has been demonstrated in 4 of 4 experimental studies (Mathew and Wilson 1985; Couturier et al. 1997; Jones et al. 2000; Field et al. 2003; cf. Tables 1, 2). Increased cerebral blood flow has been demonstrated in studies comparing acute caffeine abstinence with a preceding baseline condition (Mathew and Wilson 1985; Couturier et al. 1997) and a caffeine administration condition (Jones et al. 2000; Field et al. 2003). The magnitude of this effect is positively correlated with daily caffeine dose before abstinence (Mathew and Wilson 1985; Field et al. 2003).

The several studies indicating that caffeine abstinence is associated with increases in cerebral blood flow are of particular interest because the effect may be related to a vascular mechanism underlying the common withdrawal symptom of headache (cf. Jones et al. 2000). However, the methodologies of the studies conducted to date do not permit differentiation of the effects of caffeine per se on cerebral blood flow from the effects of caffeine withdrawal. Further research is needed to determine the validity of increased cerebral blood flow as a withdrawal sign.

**Changes in EEG**

Changes in quantitative electroencephalography (EEG) during caffeine abstinence were demonstrated in 3 of 3 experimental studies (Lader et al. 1996; Jones et al. 2000; Reeves et al. 2002; cf. Tables 1, 2). Effects included increases in EEG theta power (Jones et al. 2000; Reeves et al. 2002), which have been associated with drowsiness. However, across studies, findings have been inconsistent across different EEG measures. Changes in EEG have been demonstrated in studies comparing acute caffeine abstinence with a preceding baseline condition (Lader et al. 1996; Reeves et al. 2002) and a caffeine administration condition (Jones et al. 2000).

Although a few studies suggest that quantitative EEG measures might provide a physiological measure or correlate of caffeine withdrawal, the methodologies do not permit differentiation of the effects of caffeine per se on EEG from the effects of caffeine withdrawal. Further research is needed to validate changes in EEG as a withdrawal sign.

**Decreased blood pressure**

Of 11 experimental studies that assessed blood pressure, three demonstrated decreases in systolic blood pressure (Streufert et al. 1995; Lader et al. 1996; Phillips-Bute and Lane 1998) and one each showed decreases in diastolic (Phillips-Bute and Lane 1998) and arterial (Lane 1997) blood pressure. The small magnitude of this effect (about 6 mmHg for systolic blood pressure) suggests that, if this effect is time limited, it is not clinically important. Decreased blood pressure has been demonstrated in studies comparing acute caffeine abstinence with a preceding baseline condition (Lader et al. 1996) and a caffeine administration condition (Streufert et al. 1995; Lane 1997; Phillips-Bute and Lane 1998). One study showed the effect to be time limited (Lader et al. 1996).

The studies cited above, as well as several studies that explicitly focused on cardiovascular outcome measures (Robertson et al. 1981; Ammon et al. 1983), do not provide convincing evidence that caffeine abstinence acutely decreases blood pressure.

**Decreased motor activity**

Decreased motor activity assessed using electronic activity monitors was demonstrated in 2 of 2 experimental studies (Höfer and Bättig 1994a,b; cf. Table 2). Decreased motor activity has been demonstrated in studies comparing acute caffeine abstinence with a preceding baseline condition (Höfer and Bättig 1994a,b), and the effect was time limited (Höfer and Bättig 1994a).

Although decreased motor activity has been only rarely studied, it has face validity as a caffeine-withdrawal sign because it is plausibly could co-vary with the well-validated symptoms of increased tiredness/fatigue and decreased energy/activeness. Further research is needed to establish the validity.

**Skin conductance**

One experimental study assessed and found a significant effect (decrease) in skin conductance during caffeine abstinence relative to a preceding baseline condition (Lader et al. 1996; cf. Table 1). Further research is needed to establish its validity.

**Urinary epinephrine and norepinephrine**

Of two studies that assessed urinary epinephrine and norepinephrine (Robertson et al. 1981; Lane 1994; cf. Tables 1, 2), one showed decreased levels of epinephrine during caffeine abstinence relative to a caffeine condition (Lane 1994). The author interpreted this as an effect of caffeine rather than caffeine withdrawal. Further research is needed.

**Heart rate**

Of 9 experimental studies that assessed heart rate, only one reported a significant effect (increase) during caffeine abstinence (Höfer and Bättig 1994a). The effect was neither time limited nor reversed by re-administration of caffeine (Höfer and Bättig 1994a). It is
concluded that there is no meaningful evidence that caffeine abstinence affects heart rate.

**Hand tremor** Of 7 experimental studies that assessed objective measures of hand tremor, none showed a significant effect of caffeine abstinence. These observations are consistent with 10 studies that showed that caffeine abstinence did not affect subjective measures of limb tremor (reviewed in Tables 1, 2, and discussed above). It is concluded that there is little to suggest that caffeine abstinence affects hand tremor.

### Incidence of clinically significant distress or impairment in daily functioning

In individuals reporting caffeine-withdrawal symptoms, the severity can vary from mild to extreme. Clinically significant distress and/or impairment of normal daily activities (e.g., work absence or going to bed because of symptoms) upon caffeine abstinence have been reported in clinical evaluations and experimental studies dating back over 170 years (Kingdon 1883; Bridge 1893; Driesbach and Pfeiffer 1943; Goldstein and Kaizer 1969; Greden et al. 1980; Rainey 1985; Smith 1987; Griffiths et al. 1990; Silverman et al. 1992; Adams et al. 1993; Weil and Rosen 1993, p 187; Hampl et al. 1994; Strain et al. 1994; Cacciatore et al. 1996; Lader et al. 1996). Information about the proportion of regular caffeine consumers who are at risk for experiencing clinically significant distress and/or functional impairment during abrupt caffeine abstinence is available from both prospective experimental studies and retrospective survey studies.

Prospective experimental studies showing clinically significant distress or impairment

Six prospective experimental studies provide information on the incidence of clinically significant distress or functional impairment during caffeine abstinence. With regard to the experimental studies, in one double-blind study of 11 people who fulfilled criteria for DSM-IV substance dependence applied to caffeine use (median intake 357 mg/day), 73% experienced significant disruptions in normal daily activities during a period of experimentally induced caffeine abstinence, including leaving or missing work, making errors or costly mistakes at work, inability to care for children, and inability to complete school work (Strain et al. 1994). Five experimental studies have been conducted in normal subject populations. One study, conducted in 22 medical and graduate students who consumed 650–780 mg/day experimentally administered caffeine before abstinence, reported that headache “as extreme in severity as the subjects had ever experienced” occurred in 55% of 38 trials (Driesbach and Pfeiffer 1943). A double-blind study of 62 caffeine consumers (mean intake 235 mg/day), who had no knowledge that the study was about caffeine, found that during caffeine abstinence 52% of subjects reported moderate to severe headache, and 8–11% showed abnormally high scores on standardized depression and fatigue scales (Silverman et al. 1992). In an open-ended interview, several of these subjects also reported severe functional impairment. In another double-blind study, 45% of 40 caffeine consumers (mean intake 360 mg/day) reported a “diffuse, throbbing headache,” with 28% of those also reporting “nausea and sickness” (Lader et al. 1996). A third double-blind study evaluated the incidence of functional impairment during abrupt caffeine abstinence in a group of 18 subjects (mean intake 231 mg/day) who reported having had problems or symptoms when previously stopping caffeine (Dews et al. 1999). The study found that 39% of subjects spontaneously reported caffeine-withdrawal symptoms and 22% showed substantial decreases (≥1.5 points on a 4 point scale) in their ratings of daily functioning (e.g., at work and leisure activities), although none of the subjects reported symptoms judged to be “incapacitating” by the authors. Limitations of this study, which have been discussed elsewhere (Griffiths et al. 2003), include the relatively unstructured and unmonitored conditions under which data were obtained and the relatively low caffeine maintenance dose. Finally, one non-blind trial of caffeine abstinence reported 10% of 20 caffeine consumers reported nausea and vomiting (Couturier et al. 1997).

Overall, the incidence of clinically significant distress or functional impairment in prospective experimental studies with normal subjects has varied from about 10% to as high as 55% (median 13%). The estimate of 13% is lower than the 73% rate of functional impairment shown in subjects who met DSM-IV criteria for substance dependence applied to caffeine (Strain et al. 1994). The difference is not due to differences in caffeine intake (the usual maintenance dose in the dependent subjects was in the range of that of the studies in the normal subjects), suggesting that the caffeine-dependent subjects may represent a subpopulation vulnerable to severe withdrawal effects. It should be recognized that the 13% incidence rate for normal subjects could underestimate the rate of occurrence in the general population. Although unstudied, it seems reasonable to suppose that there may be a substantial subject selection bias in caffeine research in the general population, with individuals who experience clinically significant withdrawal being less likely to volunteer for a study in which caffeine abstinence might be a possibility.

Retrospective survey studies showing clinically significant distress or impairment

Four retrospective survey studies provide information on the percentage of respondents reporting clinically significant distress or functional impairment during caffeine abstinence. A survey study of 183 women showed that the percentage endorsing that they would experience classic caffeine-withdrawal symptoms (headache, lethargy, and
sleepiness) without their morning coffee increased as a function of the number of cups of coffee consumed (Goldstein and Kaizer 1969). Consistent with functional impairment, endorsement of being “unable to work effectively” also increased with cups consumed, with the effect reported by 9% of all coffee consumers and 16% of heavy consumers (>5 cups/day). In a population-based random digit dial telephone survey study, caffeine consumers were questioned about their experience with caffeine withdrawal (Hughes et al. 1998). Of 71 who reported having stopped or reduced caffeine for at least 24 h during the past year, 11% reported experiencing headache and other withdrawal symptoms that interfered with their performance. This figure was 24% among the subgroup who reported stopping caffeine in an attempt at permanent abstinence. A third study asked a wide variety of questions of people who telephoned to volunteer for a paid clinical research trial (Dews et al. 1999). Of 6,815 daily caffeine consumers, 2.6% reported that problems or symptoms during caffeine abstinence were severe enough to interfere with normal activity (e.g., inability to concentrate at work; lost time at work). The low rate of endorsement of functional impairment in this study may have been due to a failure to exclude individuals who had not actually abstained from caffeine, as well as possible under reporting of symptoms because of a desire to participate in a paid research trial. A final retrospective survey was conducted in 36 adolescent caffeine consumers who endorsed two or more DSM-IV substance dependence criteria applied to caffeine use (Oberstar et al. 2002). Twenty-one percent reported the caffeine-withdrawal symptom of “sick/nauseated/vomiting.”

Overall, the percentage of respondents reporting clinically significant distress or functional impairment in retrospective survey studies varied between 2.6% and 11% (median 9%) in general survey studies, and was 21% in caffeine-dependent adolescent subjects. The median estimate from the general survey studies is quite similar to the estimate from prospective experimental studies (9% and 13%, respectively). As with the experimental studies, there are several limitations inherent to survey studies that may result in underestimates. First, a portion of habitual caffeine consumers may be unaware of caffeine-withdrawal symptoms because they never have had a period of sustained abstinence. Furthermore, it has been demonstrated that as little as 25 mg/day of caffeine can prevent some withdrawal symptoms (Evans and Griffiths 1999). Thus, small amounts of caffeine that are unknowingly consumed on days believed to be “caffeine-free” days may lead to an underestimation of the incidence of clinically significant distress during complete abstinence. Finally, it is possible that caffeine-withdrawal symptoms such as headache, fatigue, nausea, and muscle aches could be misattributed to other causes or ailments (e.g., common cold).

**Expectancies and caffeine withdrawal**

It has been speculated that knowledge and expectation are the prime determinants of caffeine-withdrawal symptoms (Rubin and Smith 1999; Dews et al. 2002), leading to the conclusion that caffeine withdrawal is “controversial” (Dews et al. 1999; Rubin and Smith 1999). In drug research, the importance of expectancies has long been acknowledged (Marlatt and Rohsenow 1980; Fillmore 1994), and double-blind experimental methodologies were explicitly developed to help control for expectancy effects. However, what is known about withdrawal symptoms from abused drugs, including alcohol, opioids, sedatives, and cocaine, has been inferred from clinical observations rather than documented in experimental studies in which placebo was substituted for active drug under blind conditions (Martin 1977; Weddington et al. 1990). In contrast, the great majority (74%) of the 57 experimental studies of caffeine withdrawal outlined in Tables 1 and 2 were conducted under double-blind conditions, with an additional 11% conducted under single-blind conditions. Thus, caffeine-withdrawal research, in particular, seems to distinguish itself as having controlled for expectancy effects better than most other research on drug withdrawal.

One approach to assessing the impact of expectancies on caffeine withdrawal is to compare the incidence of caffeine-withdrawal headache that has been reported in double-blind and single-blind studies (studies 1–48 in Tables 1 and 2) to that reported in non-blind studies (studies 49–55 in Table 2). The median incidence of headache (derived from column 7 of Table 4) for the blind and non-blind studies that reported such data is 45% and 57%, respectively, suggesting a modest effect of expectancy.

However, it is important to recognize that the impact of expectancies is not entirely eliminated using double-blind methods. Even in double-blind studies, expectancies can play a role if subjects have knowledge of the purpose and conditions of the study. For instance, in extreme cases, subjects could be told that the purpose of a study is to investigate caffeine withdrawal and that they will be receiving either caffeine or placebo. This information could functionally unblind subjects if they could discriminate between conditions based on immediate pharmacological effects or on early symptoms of withdrawal (Dews et al. 1999; Rubin and Smith 1999).

To address this issue, the blind studies in Tables 1 and 2 were re-reviewed for information relevant to possible expectancy effects. This analysis revealed that many of the blind studies provided or probably provided sufficient information such that subjects could have been aware that the study involved caffeine or caffeine withdrawal. However, a substantial number of blind studies went to some lengths to keep subjects uninformed about purpose or experimental conditions. For example, several studies conducted at Johns Hopkins (Silverman et al. 1992; Schuh and Griffiths 1997; Garrett and Griffiths 1998; Jones et al. 2000) instructed subjects that they could receive a variety of compounds found in foods and beverages (e.g.,
chlorogenic acids, determines, caffeine, tannin, sugar, theophylline, or inactive placebo). To further divert attention away from caffeine, the dietary restrictions were written without reference to caffeine and included various foods and substances that do not contain caffeine (i.e., saccharin, aspartame [NutraSweet], oysters, mussels, almonds, coconuts, poppy seeds, and all beverages except milk, fruit juice, and water). Furthermore, as the research unit at Johns Hopkins conducts a large number of studies with a wide range of drugs other than caffeine, it is unlikely that conduct of these studies at that site would have created subject expectations that the study involved caffeine. Two studies from other laboratories also used instructions and methods that reduced the likelihood of expectancy effects (Comer et al. 1997; Dews et al. 1999). Finally, several studies ruled out expectancy effects by using debriefing procedures to explicitly determine what subjects understood or inferred about the experimental conditions (van Dusseldorp and Katan 1990; Rogers et al. 1995; James 1998; Robelin and Rogers 1998; Yeomans et al. 1998, 2002b; Tinley et al. 2003). From these studies in which expectancy effects are judged to be unlikely, the median incidence of headache (derived from column 7 of Table 4) for the studies that reported such data is 42%, which is similar to the incidence of headache for all blind studies (45%). Furthermore, the profile of other symptoms demonstrated in these studies in which expectancy effects were unlikely is similar to those demonstrated in the larger set of studies.

In conclusion, while it is undoubtedly true that, as with the assessment of other clinical phenomena, expectancies could play some role in caffeine withdrawal, the evidence that caffeine withdrawal is pharmacologically based is overwhelming, and analysis of the published data does not support the hypothesis that expectancies are a primary determinant of caffeine-withdrawal symptoms. It should also be recognized that, to the extent that expectancies may modestly enhance caffeine-withdrawal symptoms (as suggested from the comparison of blind and non-blind studies described above), blind research studies may actually underestimate the incidence and severity of withdrawal that occurs under more naturalistic clinically relevant conditions. Future research should evaluate this possibility using a methodology such as the balanced placebo design, which experimentally manipulates both caffeine abstinence and expectancy of abstinence (Marlatt et al. 1998; Tinley et al. 2003), which is consistent with the short half-life of caffeine (4–6 h). Some evidence suggests that symptoms may emerge later (>24 h) after abstinence from higher doses (e.g., 900 mg/day) of caffeine (Bruce et al. 1991; Evans and Griffiths 1992). In the few studies that provided detailed time-course information for individual subjects, onset of withdrawal symptoms have been reported as early as 6 h (Roller 1981) and as late as 43 h (Griffiths et al. 1990) after abstinence. Caffeine-withdrawal symptoms have been shown to reach peak intensity between 20 h and 51 h after abstinence (Griffiths et al. 1986, 1990; Evans and Griffiths 1992; Brauer et al. 1994; Höfer and Bättig 1994a,b; Lader et al. 1996). The duration of caffeine withdrawal has been shown to be 2–9 days (Griffiths et al. 1986, 1990; van Dusseldorp and Katan 1990; Höfer and Bättig 1994a), and the possibility of withdrawal headaches occurring up to 21 days has been suggested (Richardson et al. 1995).

### Parametric determinants of caffeine withdrawal

**Chronic caffeine maintenance dose**

There is good evidence that the incidence or severity of caffeine withdrawal increases with increases in the chronic daily caffeine maintenance dose. The best evidence for this relationship comes from a prospective study that experimentally manipulated caffeine maintenance dose (100, 300, and 600 mg/day) and showed monotonic increases in several withdrawal measures, with significantly greater headache and headache/poor mood demonstrated after abstinence from 600 mg than 100 mg/day (Evans and Griffiths 1999). This study and a previous study (Griffiths et al. 1990) also demonstrated that significant caffeine withdrawal occurred after abstinence from a dose as low as 100 mg/day.

The relationship between withdrawal incidence or severity and usual caffeine dose has also been demonstrated in studies of self-reported caffeine maintenance dose, including retrospective survey studies (Goldstein and Kaizer 1969), experimental studies (Goldstein 1964; Silverman et al. 1992; Rogers et al. 1995; Lader et al. 1996; Lane 1997; Lane and Phillips-Bute 1998), and studies of post-operative headache (Galletly et al. 1989; Fennelly et al. 1991). It should be noted, however, that the relationship between withdrawal and self-reported caffeine intake appears relatively weak because it has not been demonstrated in some studies (Verhoef and Millar 1990; Hughes et al. 1993; Höfer and Bättig 1994a), and significant correlations between withdrawal measures and caffeine intake are low and inconsistent across different withdrawal measures (Silverman et al. 1992; Lane 1997; Lane and Phillips-Bute 1998).

**Acute decreases in caffeine maintenance dose**

When lower caffeine doses are substituted for the usual maintenance dose, withdrawal severity increases as the substituted dose decreases. One study maintained indivi-
duals on 300 mg caffeine/day and then substituted a range of lower doses (Evans and Griffiths 1999). The study showed that a substantial reduction in caffeine dose (to ≤100 mg/day) was necessary to produce caffeine withdrawal and that even a dose of 25 mg/day was sufficient to avoid significant caffeine-withdrawal headache. This result suggests that a substantial percentage reduction in caffeine consumption is necessary to manifest the full caffeine-withdrawal syndrome.

Duration of caffeine maintenance

Three studies demonstrated that caffeine withdrawal can occur after a relatively short duration of caffeine maintenance (Driesbach and Pfeiffer 1943; Griffiths et al. 1986; Evans and Griffiths 1999). One of these studies (Evans and Griffiths 1999), which was conducted in caffeine consumers who were caffeine abstinent for 7 days before the period of caffeine maintenance, showed no withdrawal effects after a single day of exposure to caffeine (300 mg/day). However, significant withdrawal symptoms occurred after 3 consecutive days of caffeine, with somewhat greater severity demonstrated after 7 and 14 consecutive days of exposure. Another study (Driesbach and Pfeiffer 1943) showed that caffeine-withdrawal headache occurred in three individuals who normally totally abstained from caffeinated beverages, but who were given increasing doses of caffeine over 6 days or 7 days up to 650–780 mg/day.

Within-day frequency of dosing during caffeine maintenance

Most studies of caffeine withdrawal have involved caffeine abstinence after a maintenance period that involved multiple caffeine doses each day. The only study to vary the within-day frequency of caffeine dosing showed that the range and severity of caffeine-withdrawal symptoms was similar when caffeine maintenance involved 300 mg taken as single dose in the morning compared with 100 mg taken at three time points across the day (Evans and Griffiths 1999). This indicates that once-a-day dosing with caffeine is sufficient for producing withdrawal symptoms.

Re-administration of caffeine reverses abstinence effects

After a period of abstinence during which the severity or incidence of a symptom or sign develops, re-administration of caffeine rapidly (usually within 30–60 min) and often completely reverses withdrawal (Driesbach and Pfeiffer 1943; Goldstein et al. 1969; Roller 1981; Couturier et al. 1997; Tinley et al. 2003), with the magnitude of reversal being an increasing function of the re-administered caffeine dose (Goldstein et al. 1969).

Individual differences in caffeine withdrawal

There are differences within and across individuals with respect to the incidence of caffeine withdrawal. As discussed above and shown in Table 4, only about 50% of subjects in experimental studies report headache after any single occasion of caffeine abstinence, and the severity of headache can vary from mild to extreme. One study that examined six repeated blind abstinence trials in seven subjects maintained on 100 mg/day of caffeine documented differences within and across subjects: one subject never showed substantial headache, some subjects showed consistent headaches, while others reported headaches on some trials but not others (Griffiths et al. 1990). A second study that analyzed the effects of six repeated abstinence trials showed that at least 36% of subjects, who showed statistically significant elevations in headache, failed to report this effect consistently across repeated trials (Hughes et al. 1993). There is evidence that genetic factors may play a role in some differences among individuals. One study of 1,934 female twins found that there was significantly greater concordance of DSM-IV-defined caffeine withdrawal among monozygotic twins (41%) than dizygotic twins (18%), yielding an estimated broad heritability of 35% (Kendler and Prescott 1999).

Other than the role of chronic maintenance dose (discussed previously), very little is known about the determinants of individual differences in caffeine withdrawal. The results of one study suggested that individuals who eliminated caffeine slowly were less likely to experience sedation during withdrawal (Lader et al. 1996). Whether females, individuals with histories of previous drug dependence including cigarette smoking, or individuals with polymorphisms in the A1 and A2A adenosine receptor genes are at greater risk of caffeine withdrawal is worthy of future research (Strain et al. 1994; Dews et al. 1999; Alsene et al. 2003).

The role of caffeine withdrawal in the habitual consumption of caffeine

Research indicates that avoidance of abstinence-associated withdrawal symptoms plays a central role in the habitual consumption of caffeine. This relationship has been shown in retrospective questionnaire studies (Goldstein and Kaizer 1969) and in double-blind experimental studies that assessed direct behavioral measures of caffeine reinforcement or preference (Griffiths et al. 1986; Hughes et al. 1993; Liguori and Hughes 1997; Schuh and Griffiths 1997; Garrett and Griffiths 1998) and beverage flavor preferences (Rogers et al. 1995; Yeomans et al. 1998, 2000a, 2001, 2002a; Tinley et al. 2003).

One study of caffeine reinforcement, for example, showed that moderate caffeine consumers who reported caffeine-withdrawal symptoms (i.e., headache, drowsiness) after drinking decaffeinated coffee were more than twice as likely to choose caffeinated over decaffeinated
coffee in choice tests (Hughes et al. 1993). In studies that prospectively manipulated caffeine physical dependence, subjects chose caffeine more than twice as often when they were physically dependent than when they were not physically dependent (Griffiths et al. 1986; Garrett and Griffiths 1998).

Withdrawal also plays an important role in the development of preferences for flavors paired with caffeine. In these studies, caffeine consumers who abstained from caffeine overnight and were repeatedly exposed to a novel flavored drink paired with caffeine showed increased ratings of drink pleasantness or preference compared with caffeine consumers who received placebo-paired drinks (Rogers et al. 1995; Yeomans et al. 1998). It has been demonstrated that the development of such flavor preference requires that subjects be caffeine deprived at training and testing (Yeomans et al. 1998, 2000b, 2001, 2002a). Furthermore, the effects are not observed in caffeine non-consumers or in long-term abstinent consumers (Rogers et al. 1995; Tinley et al. 2003). It seems likely that, in the natural environment, withdrawal-dependent conditioned flavor preferences play an important role in development of strong consumer preferences for specific kinds of caffeine-containing beverages.

**DSM-IV-TR and ICD-10 diagnostic criteria for caffeine withdrawal**

The potential for caffeine withdrawal to cause clinically significant distress or impairment in functioning is reflected by the inclusion of caffeine withdrawal as an official diagnosis in ICD-10 (World Health Organization 1992a,b) and as a proposed research diagnosis in DSM-IV-TR (American Psychiatric Association 2000). Caffeine withdrawal was included in DSM-IV as a proposed diagnosis rather than an official diagnosis to encourage further research on the range and specificity of caffeine-withdrawal symptoms (Hughes 1994). The DSM-IV-TR proposed research criteria for withdrawal are: (A) prolonged daily use of caffeine; (B) abrupt cessation of caffeine use or reduction in the amount of caffeine used, closely followed by headache and one (or more) of the following symptoms: (1) marked fatigue or drowsiness, (2) marked anxiety or depression, (3) nausea or vomiting; (C) the symptoms in criterion B cause clinically significant distress or impairment in social, occupational, or other important areas of functioning; (D) the symptoms are not due to the direct physiological effects of a general medical condition (e.g., migraine, viral illness) and are not better accounted for by another mental disorder.

The official ICD-10 diagnosis for withdrawal from caffeine does not specify specific symptoms for making the diagnosis (World Health Organization 1992a,b). The ICD-10 diagnostic criteria for research includes the diagnosis of a “withdrawal state from other stimulants, including caffeine” (World Health Organization 1993). The criteria involve: (A) cessation or reduction of caffeine use after prolonged use; (B) dysphoric mood (for instance, sadness or anhedonia); and (C) two or more of the following: (1) lethargy and fatigue, (2) psychomotor retardation or agitation, (3) craving for stimulant drugs, (4) increased appetite, (5) insomnia or hypersomnia, (6) bizarre or unpleasant dreams.

**Proposed revision of DSM-IV-TR and ICD-10 diagnostic criteria for caffeine withdrawal**

The great majority of the research literature on caffeine withdrawal has been published since the DSM-IV Work Group (in 1994) and the World Health Organization (in 1993) formulated their respective criteria for proposed research diagnoses (cf. Tables 1, 2, 3). This section focuses on a proposed revision of the DSM, although many of the considerations would be applicable to a revision of ICD-10. The research summarized in this review further documents that caffeine withdrawal may be severe enough to warrant clinical attention, which was questioned in DSM-III-R (American Psychiatric Association 1987). Moreover, the research literature now provides a sound empirical basis for updating the criteria for caffeine withdrawal. Problems with the DSM-IV-TR criteria are that they (1) do not reflect the apparent independence of headache and non-headache withdrawal symptoms (cf. section on headache); (2) do not include symptoms that have now been well-validated; and (3) include a symptom (i.e., anxiety) for which there is little empirical support.

Based on the current evaluation of the research literature, we now propose that part B of the DSM research criteria be changed to indicate that a caffeine-withdrawal diagnosis requires: abrupt cessation of caffeine use or reduction in the amount of caffeine used, closely followed by three or more of the following: (1) headache, (2) fatigue or drowsiness, (3) dysphoric mood, depressed mood, or irritability, (4) difficulty concentrating, and (5) flu-like somatic symptoms, nausea, vomiting, or muscle pain/stiffness.

The proposed five clusters of symptoms are based on the 13 symptoms from this review that are the best candidates for describing the caffeine-withdrawal syndrome. The new criteria address the 1994 DSM-IV Work Group concerns that there were too few validated symptoms and there may be overlap between fatigue and drowsiness (Hughes 1994). The decision to propose the diagnosis based on three of the five clusters is a conservative strategy to prevent over-diagnosis of the disorder, which was also a concern of the DSM-IV Work Group (Hughes 1994). Although some of the proposed symptoms have high prevalence and other etiologies, this is also true of other withdrawal diagnoses recognized by DSM (e.g., cocaine withdrawal, nicotine withdrawal, opioid withdrawal).

It should be recognized that although the individual symptoms have been empirically validated, the symptom clusters are conceptually rather than empirically derived. It would be informative if future research with a suitably
large group of individuals assessed all of the symptoms validated in this review and used statistical procedures to empirically differentiate among clusters of symptoms (e.g., cluster analysis or multiple regression), as well as determine their ability to predict clinically significant distress.

Summary/conclusions

The present paper represents the most comprehensive review and analysis of the effects of caffeine abstinence in humans published to date. The purpose of this analysis was to empirically validate specific symptoms and signs, and to appraise important features of the caffeine-withdrawal syndrome.

Of 49 symptom categories and 9 sign categories identified from 57 experimental and 9 retrospective survey studies, the following 10 symptom categories fulfilled methodologically rigorous validity criteria: headache, tiredness/fatigue, decreased energy/activeness, decreased alertness/attentiveness, drowsiness/sleepiness, decreased contentedness/well-being, depressed mood, difficulty concentrating, irritability, and muzzy/foggy/not clearheaded. In addition, flu-like symptoms, nausea/vomiting, and muscle pain/stiffness were judged likely to represent valid symptom categories. The percentage of subjects reporting headache was 50% in experimental studies and 24% in retrospective survey studies. The percentage of subjects reporting clinically significant distress or functional impairment was 13% in prospective experimental studies and 9% in retrospective survey studies.

Data supporting the following 13 symptom and sign categories were judged as suggestive and in need of further research: decreased desire to socialize, unmotivated for work, decreased self-confidence, total mood disturbance (POMS), yawning, heavy feelings in arms and legs, increased nighttime sleep quality/duration, analgesic use, craving/strong desire to use, impaired behavioral and cognitive performance (objectively measured), decreased motor activity (objectively measured), increased cerebral blood flow, and EEG changes.

Onset of withdrawal symptoms typically occurs 12–24 h after abstinence, with peak intensity occurring at 20–51 h, and duration of withdrawal ranging between 2 days and 9 days. Re-administration of caffeine rapidly and often completely reverses withdrawal. The incidence or severity of symptoms increases with increases in the chronic daily maintenance dose. Symptoms may occur upon abstinence from chronic caffeine exposure at doses as low as 100 mg/day, and upon abstinence following only 3–7 days of caffeine exposure at higher doses. There is good evidence that avoidance of withdrawal symptoms plays a central role in the habitual consumption of caffeine by increasing the reinforcing effects of caffeine and preference for tastes paired with caffeine. Overall, the evidence that caffeine withdrawal is pharmacologically based is overwhelming, and analysis of the published data does not support the hypothesis that expectancies are a primary determinant of caffeine-withdrawal symptoms.

In addition to the previously discussed research priorities concerning the DSM-IV diagnosis of caffeine withdrawal, future studies should determine the validity of the symptom and sign categories identified above as in need of further research, and investigate how vulnerability to caffeine withdrawal is affected by gender, drug abuse histories, caffeine metabolism, genetics, behavioral conditioning, personality, and other factors. It would be valuable if future reports of research on caffeine withdrawal provided individual subject data in addition to the usual group data to provide further information about individual differences in severity of the withdrawal syndrome. Future research should provide more detailed information about the time course (i.e., onset, duration, and offset) of individual withdrawal symptoms. Furthermore, the impact of gradual reduction of caffeine on withdrawal symptoms should be characterized. Although tolerance and withdrawal are thought to be functionally related phenomena, no research has investigated the relationship between the extent of caffeine tolerance and magnitude of withdrawal. Finally given the high rate of caffeine use in children, caffeine withdrawal in children deserves much more systematic study (Goldstein and Wallace 1997; Bernstein et al. 1998).

In conclusion, although descriptions in the medical literature of the caffeine-withdrawal syndrome date back more than 170 years, a solid empirical parametric analysis of the phenomenon has only begun to emerge in recent years. Arguably, the caffeine-withdrawal syndrome has been more rigorously and completely characterized than withdrawal from any other drug and can now serve as a model system for evaluating drug withdrawal phenomena. Despite this progress, we can anticipate that future research with caffeine will provide further valuable insights into the world’s most widely consumed mood-altering drug, as well as insights into drug withdrawal effects more generally.

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## Appendix A—Studies that did not statistically document symptom or sign

<table>
<thead>
<tr>
<th>Symptom or sign</th>
<th>Studies(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alertness/attentiveness</td>
<td>10, 14, 16, 22, 23, 28, 38, 46, 55</td>
</tr>
<tr>
<td>Anger/hostility</td>
<td>4, 6, 9, 10, 15, 18, 22, 25, 26, 27, 28, 33, 34, 35, 36, 37, 46, 55</td>
</tr>
<tr>
<td>Anxiety/nervousness</td>
<td>4, 5, 6, 10, 14, 15, 16, 17, 18, 19, 20, 21, 23, 24, 25, 26, 27, 28, 29, 33, 34, 35, 36, 37, 41, 44, 45, 46, 55, 56</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>3, 13, 38, 43, 44, 45, 54</td>
</tr>
<tr>
<td>Blurred vision</td>
<td>5, 19, 21, 25, 26, 37, 46, 55, 56</td>
</tr>
<tr>
<td>Calm/relaxed</td>
<td>16, 17, 18, 21, 27, 31, 39</td>
</tr>
<tr>
<td>Chills</td>
<td>21</td>
</tr>
<tr>
<td>Circular lights task (^b)</td>
<td>4</td>
</tr>
<tr>
<td>Confusion—bewilderment</td>
<td>4, 5, 10, 15, 17, 18, 21, 25, 28, 33, 35, 36, 37, 46, 55, 56</td>
</tr>
<tr>
<td>Constipation</td>
<td>5, 6</td>
</tr>
<tr>
<td>Contentedness/well-being</td>
<td>4, 10, 14, 15, 20, 27, 28, 29, 37, 39, 46</td>
</tr>
<tr>
<td>Depressedness</td>
<td>2, 4, 10, 18, 19, 20, 21, 22, 23, 24, 25, 26, 29, 33, 34, 35, 36, 37, 46, 55</td>
</tr>
<tr>
<td>Desire to socialize</td>
<td>15, 18, 19, 21, 23, 24, 25, 26, 27, 28, 29, 35, 36</td>
</tr>
<tr>
<td>Diaphoresis</td>
<td>5, 6, 9, 14, 15, 19, 20, 21, 22, 23, 24, 25, 26, 37, 46, 55, 56</td>
</tr>
<tr>
<td>Diarrhea (^b)</td>
<td>5, 6, 14, 15, 22, 23, 24</td>
</tr>
<tr>
<td>Difficulty sleeping/insomnia</td>
<td>9, 14, 15, 19, 21, 22, 23, 24, 26, 46, 55</td>
</tr>
<tr>
<td>Difficulty concentrating</td>
<td>21, 25, 28, 46</td>
</tr>
<tr>
<td>Digit symbol substitution task</td>
<td>9, 14, 15, 20, 21, 22, 28, 37, 55</td>
</tr>
<tr>
<td>Divided attention (^b)</td>
<td>21</td>
</tr>
<tr>
<td>Drowsiness/sleepiness</td>
<td>5, 10, 18, 22, 25, 37</td>
</tr>
<tr>
<td>Energy/activation</td>
<td>10, 14, 16, 17, 25, 36, 42, 46</td>
</tr>
<tr>
<td>Flu-like symptoms</td>
<td>19, 21, 26, 28, 35, 37, 46, 56</td>
</tr>
<tr>
<td>Frequent urination (^b)</td>
<td>9, 14, 15, 19, 22, 23, 24, 25, 26, 37, 46, 55, 56</td>
</tr>
<tr>
<td>Grammatical/logical reasoning</td>
<td>28, 55</td>
</tr>
<tr>
<td>Hand tremor (objectively measured) (^b)</td>
<td>14, 15, 17, 20, 22, 23, 24</td>
</tr>
<tr>
<td>Headache</td>
<td>14, 19, 22, 24, 26, 28, 29, 31, 37, 40, 41</td>
</tr>
<tr>
<td>Heart pounding/palpitations</td>
<td>9, 14, 15, 19, 20, 21, 22, 23, 24, 25, 26, 37, 46, 55, 56</td>
</tr>
<tr>
<td>Heart rate</td>
<td>3, 13, 19, 38, 43, 45, 54, 55</td>
</tr>
<tr>
<td>Heavy feelings in arms and legs</td>
<td>19, 21, 25, 26, 56</td>
</tr>
<tr>
<td>Hot and cold spells</td>
<td>19, 25, 26, 37, 46, 55</td>
</tr>
<tr>
<td>Hunger/appetite (^b)</td>
<td>17, 18, 20, 21, 22, 23, 24, 29, 31, 42</td>
</tr>
<tr>
<td>Irregular heartbeat</td>
<td>14, 15, 22, 23, 24</td>
</tr>
<tr>
<td>Irritability</td>
<td>4, 10, 14, 15, 16, 20, 21, 23, 24, 25, 28, 29, 37, 46, 55</td>
</tr>
<tr>
<td>Jittery/shaky (^b)</td>
<td>5, 6, 9, 28, 31, 37, 40, 41, 42, 46, 56</td>
</tr>
<tr>
<td>Lightheaded/dizzy</td>
<td>5, 9, 14, 15, 19, 20, 21, 22, 23, 24, 25, 26, 37, 46, 56</td>
</tr>
<tr>
<td>Limb tremor (^b)</td>
<td>5, 6, 9, 19, 25, 26, 37, 46, 55, 56</td>
</tr>
<tr>
<td>Loss of sex drive (^b)</td>
<td>9, 19, 25, 26, 37, 46, 55, 56</td>
</tr>
<tr>
<td>Memory/recall (^b)</td>
<td>9, 21, 28, 37</td>
</tr>
<tr>
<td>Muscle cramps (^b)</td>
<td>9, 19, 25, 26, 37, 46, 55, 56</td>
</tr>
<tr>
<td>Muscle pain/stiffness</td>
<td>9, 19, 20, 21, 25, 26, 37, 44, 46, 55, 56</td>
</tr>
<tr>
<td>Muscle twitches</td>
<td>14, 15, 22, 23, 24</td>
</tr>
<tr>
<td>Muzzy/foggy/not clear-headed</td>
<td>5, 8, 19, 25, 26, 28, 29, 31, 37, 42, 46</td>
</tr>
<tr>
<td>Nausea/vomiting/upset stomach</td>
<td>2, 4, 5, 6, 9, 10, 14, 15, 19, 20, 21, 25, 26, 37, 45, 46, 55, 56</td>
</tr>
<tr>
<td>Numbness or tingling in extremities</td>
<td>21</td>
</tr>
</tbody>
</table>

\(^a\)Numbers in table refer to the entry number in Tables 1 and 2.

### References


Cobb’s LW (1982) Lethargy, anxiety, and impotence in a diabetic. Hosp Pract (Off Ed) 17:67 (see also pages 70 and 73)


Gilbert RJ (1986) Caffeine, the most popular stimulant. Chelsea House, New York


Höfer I, Bättig K (1994b) Psychophysiological effects of switching to caffeine tablets or decaffeinated coffee under field conditions. Pharmacopsychocologia 7:169–177


Caffeine Use Disorder: A comprehensive review and research agenda

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Abstract
Caffeine is the most commonly used drug in the world. Although consumption of low to moderate doses of caffeine is generally safe, an increasing number of clinical studies are showing that some caffeine users become dependent on the drug and are unable to reduce consumption despite knowledge of recurrent health problems associated with continued use. Thus, the World Health Organization and some health care professionals recognize caffeine dependence as a clinical disorder. In this comprehensive literature review, we summarize published research on the biological evidence for caffeine dependence; we provide a systematic review of the prevalence of caffeine dependence and rates of endorsement of clinically meaningful indicators of distress and functional impairment among habitual caffeine users; we discuss the diagnostic criteria for Caffeine Use Disorder—a condition for further study included in the Diagnostic and Statistical Manual of Mental Disorders (5th ed.); and we outline a research agenda to help guide future clinical, epidemiological, and genetic investigations of caffeine dependence. Numerous controlled laboratory investigations reviewed in this paper show that caffeine produces behavioral and physiological effects similar to other drugs of dependence. Moreover, several recent clinical studies indicate that caffeine dependence is a clinically meaningful disorder that affects a nontrivial proportion of caffeine users. Nevertheless, more research is needed to determine the reliability, validity, and prevalence of this clinically important health problem.

KEYWORDS: caffeine, caffeine dependence, caffeine use disorder, addiction, energy drink, withdrawal

Introduction
Caffeine is the most widely used drug in the world.(1) In the US, more than 90% of adults use it regularly, and, among them, average consumption is over 200 mg of caffeine per day(2)—more caffeine than is contained in two 6-ounce cups of coffee or five 12-ounce cans of soft drinks.(3, 4) Although consumption of low to moderate doses of caffeine is generally safe, consumption of higher doses by vulnerable individuals can lead to increased risk for negative health consequences, including cardiovascular problems and perinatal complications.(5, 6) Moreover, a number of recent studies show that some caffeine users become addicted to or dependent on caffeine.(7-23) Many of these individuals are unable to reduce consumption despite knowledge of recurrent health problems associated with continued caffeine use.(17, 20, 21)
In the present review, we first summarize the published literature on the biological evidence for caffeine dependence. We then discuss the diagnostic criteria for Caffeine Use Disorder, a condition for further study recently published in the Diagnostic and Statistical Manual of Mental Disorders (5th ed.; DSM-5). (24) We also provide a systematic review of the clinical evidence for Caffeine Use Disorder and rates of endorsement of the DSM-5 diagnostic criteria for this research diagnosis. Finally, because this diagnosis is intended to stimulate further study of Caffeine Use Disorder, we conclude the paper with a discussion of future research directions.

**Nomenclature**

For the purposes of this review, the terms used to describe an individual’s inability to control caffeine use despite negative physical or psychological consequences associated with continued use (i.e., caffeine dependence, Caffeine Dependence Syndrome, Caffeine Use Disorder, and caffeine addiction) will be used interchangeably, with preference given to the term, “caffeine dependence” because this term has been used most frequently in the published literature to date. Terms that refer to specific mental or behavioral diagnoses (e.g., Caffeine Dependence Syndrome, Caffeine Use Disorder, and Substance Use Disorder) as defined by health care organizations (i.e., World Health Organization and American Psychiatric Association) will be capitalized.

**Overview of Biological Evidence for Caffeine Dependence**

*Neuropharmacology*

Caffeine acts as an antagonist at adenosine receptors, thereby, blocking endogenous adenosine. (25, 26) Functionally, caffeine produces a range of effects opposite those of adenosine, including the behavioral stimulant effects associated with the drug. (27) Importantly, caffeine has been shown to stimulate dopaminergic activity by removing the negative modulatory effects of adenosine at dopamine receptors. (28) Studies suggest that dopamine release in the nucleus accumbens shell may be a specific neuropharmacological mechanism underlying the addictive potential of caffeine. (29-32) Notably, dopamine release in this brain region is also caused by other drugs of dependence, including amphetamines and cocaine. (33, 34) In addition to the direct effects of caffeine on adenosine receptors, a recent study has shown that paraxanthine, the primary metabolite of caffeine in humans, produces increased locomotor activity, as well as increases in extracellular levels of dopamine through a phosphodiesterase (PDE) inhibitory mechanism. (35)

Upregulation of the adenosine system following chronic caffeine administration appears to be a neurochemical mechanism underlying caffeine withdrawal syndrome. (36) This mechanism results in increased functional sensitivity to adenosine during caffeine abstinence, and it likely plays an important role in the behavioral and physiological effects produced by caffeine withdrawal.

*Behavioral pharmacology*

**Subjective effects.** Low to moderate doses of caffeine have been shown to increase self-reported liking of the drug (37) as well as other positive subjective effects, (38, 39) including increased well-being, energy, alertness, and sociability—drug effects that are qualitatively similar to some of the positive subjective effects produced by other stimulants (e.g., amphetamine and cocaine). Although the positive subjective effects of caffeine occur among nonhabitual users and those on caffeine-free diets, these effects are enhanced by physical dependence, likely due to suppression of withdrawal symptoms. (4)

**Reinforcement.** Reinforcement is an essential behavioral mechanism that influences rates of operant behavior, including drug use. A drug is said to function as a reinforcer when drug administration increases the future likelihood of drug use (e.g., increased drug self-administration or increased choice of drug over placebo). Low to moderate doses of caffeine have been shown to function as reinforcers in both human
and nonhuman animal subjects.(36, 40, 41) Although there is variability across subjects, human studies show that many individuals reliably choose caffeine over placebo. Moreover, research has shown that caffeine is more likely to function as a reinforcer among individuals with a history of heavy caffeine use,(27) and avoidance of caffeine withdrawal has been shown to play a central role in the reinforcing effects of caffeine in habitual users.(42-47) As might be expected, caffeine reinforcement has been shown to covary with the positive subjective effects of the drug. Individuals who choose caffeine tend to report positive subjective effects following drug administration, while those who do not choose caffeine tend to report more negative subjective effects.(38, 43)

**Conditioned taste preference.** When a reinforcer is repeatedly paired with a neutral stimulus, this stimulus can also acquire reinforcing properties by virtue of respondent (i.e., Pavlovian) conditioning. Thus, in studies using a conditioned flavor preference paradigm, caffeine can engender a preference for a novel flavored beverage when the drug is repeatedly paired with that flavor.(48-51) For example, ratings of how much individuals like a novel flavored beverage significantly increase when the beverage is paired with caffeine, while ratings for the beverage decrease when it is paired with a placebo.(52) Suppression of withdrawal symptoms plays a primary role in the development of caffeine flavor preferences,(51, 53, 54) and it seems likely that these conditioned taste preferences play an important role in the development of strong consumer preferences for specific types and brands of caffeinated beverages.(4)

**Withdrawal.** Caffeine withdrawal refers to a time-limited syndrome that develops after cessation of chronic (e.g., daily) caffeine administration. Caffeine withdrawal has been shown to occur in a range of nonhuman animal species,(40) and a clearly defined caffeine withdrawal syndrome has also been well documented in humans.(17, 55) Common symptoms include headache, fatigue, difficulty concentrating, and dysphoric mood.(55, 56) Low doses of caffeine have been shown to suppress these symptoms.(57) Tolerance to caffeine occurs when the physiological, behavioral, and/or subjective effects of caffeine decrease following repeated exposure to the drug, such that the same dose of caffeine no longer produces equivalent effects, or a higher dose of caffeine is needed to produce similar effects. Caffeine tolerance has been demonstrated among several nonhuman animal species (e.g., mice, rats, and monkeys), using a range of behavioral measures (e.g., locomotor activity, seizure, and drug discrimination).(27) Tolerance has also been reliably demonstrated in humans. For example, a number of studies have shown tolerance to the subjective effects of caffeine,(38) as well as to the drug’s sleep disruptive effects,(58) and several other physiological effects, including diuresis, oxygen consumption, and blood pressure.(59) Although complete tolerance does not occur at low doses, tolerance to some of the effects of caffeine can occur following chronic administration of very high doses of the drug (i.e., 750-1200 mg/day).(4)

**Genetics**

As with other drug dependencies, caffeine dependence appears to be influenced, in part, by genotype. Studies comparing human monozygotic and dizygotic twins have shown heritabilities of caffeine use, tolerance, and withdrawal ranging from 35% to 77%.(60-63) The magnitude of heritability for caffeine dependence markers is similar to those for nicotine and alcohol.(64, 65) Genetic polymorphisms in the adenosine A2A receptor gene (ADORA2A), are associated with caffeine consumption;(66) sensitivity to the effects of caffeine following sleep deprivation; and the effects of caffeine on anxiety,(67-69) sleep,(70, 71) blood pressure,(72) and psychomotor vigilance.(73) In addition, variability in the cytochrome P450 1A2 (CYP1A2) gene, which codes for the primary enzyme responsible for caffeine metabolism, is associated with variability in caffeine consumption.(74-76) Moreover, individuals who carry the variant of the CYP1A2 gene that slows caffeine metabolism have been shown to be at increased risk for hypertension and myocardial infarction associated with coffee use.(66, 77) Recent genome-wide meta-analyses have found associations between caffeine use and variants of the CYP12A gene and aryl hydrocarbon receptor gene (AHR), which regulates CYP1A2.(78-80)

**Current Status of Caffeine Dependence Diagnosis**
World Health Organization
The World Health Organization developed The International Statistical Classification of Diseases and Related Health Problems (10th Revision; ICD-10), the most recent international medical diagnostic system (http://apps.who.int/classifications/icd10/browse/2008/en#, accessed January 2013). The ICD-10 recognizes the diagnosis of Caffeine Dependence Syndrome. This disorder is defined as a cluster of behavioral, cognitive, and physiological phenomena that develop after repeated substance use and that typically include a strong desire to take the drug, difficulties in controlling use, persisting in use despite harmful consequences, a higher priority given to drug use than to other activities and obligations, increased tolerance, and sometimes a physical withdrawal state.

American Psychiatric Association
The American Psychiatric Association recently published the DSM-5, the latest edition of the Diagnostic and Statistical Manual of Mental Disorders. The nomenclature and diagnostic criteria of substance-related and addictive disorders differs between this edition of the manual and the DSM-IV.(83) Specifically, the disorders of Substance Abuse and Substance Dependence found in the DSM-IV have been combined and are now referred to as Substance Use Disorder in the DSM-5. Although neither the DSM-IV nor the DSM-5 officially recognizes these disorders applied to caffeine, the DSM-5 recognizes Caffeine Use Disorder as a condition for further study. Table 1 shows the diagnostic criteria for Caffeine Use Disorder. These criteria are similar, but not identical, to those for Caffeine Dependence Syndrome in the ICD-10. The Caffeine Use Disorder criteria also overlap considerably with the older DSM-IV criteria for Substance Abuse and Substance Dependence. A footnote in Table 1 notes many of the similarities and differences between the DSM-5 Caffeine Use Disorder diagnosis and the DSMIV diagnoses for Substance Abuse and Substance Dependence. The DSM-5 diagnostic schema for Substance Use Disorder includes 11 criteria. Endorsement of any two of these criteria will fulfill the diagnostic requirement. However, to ensure identification of only those cases with sufficient clinical importance to warrant labeling of a mental disorder, the DSM-5 diagnostic schema for the study of Caffeine Use Disorder requires that all three of the most clinically meaningful indicators of distress or impairment associated with caffeine dependence be fulfilled: 1) persistent desire or unsuccessful efforts to cut down or control caffeine use, 2) continued caffeine use despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by caffeine, and 3) characteristic caffeine withdrawal syndrome or caffeine use to relieve or avoid withdrawal symptoms. In addition to these three primary diagnostic criteria for Caffeine Use Disorder, six other criteria are also assessed (see Table 1).

Systematic Review of Clinical Evidence for Caffeine Dependence
Summary of Literature Search
On 17 July 2013, we conducted searches on three research databases (PubMed, Embase, and The Cochrane Library) using the following four search terms: “caffeine dependence,” “caffeine use disorder,” “caffeine addiction,” and “caffeinism.” In addition to literature searches conducted via electronic databases, the authors’ personal journal article collections were also searched, as well as the reference sections of review papers and studies that met inclusion criteria. After eliminating duplicates, 122 results were obtained. Studies selected for inclusion were peer-reviewed, published in English, used experimental or observational designs, and reported prevalence of caffeine dependence or rates of endorsement of caffeine dependence diagnostic criteria. The caffeine-related substance use disorders that were evaluated and the tools that were used to assess them varied across studies. For example, one study used only the generic DSM-IV diagnostic criteria for Substance Abuse applied to caffeine to assess “caffeine abuse,”(84) another study used the DSM-III-R diagnostic criteria for both Substance Abuse and Substance Dependence to determine the presence or absence of a “caffeine disorder,”(85) and several
studies assessed caffeine dependence using various substance dependence questionnaires [e.g., Leeds Dependence Questionnaire (LDQ)(23), Shorter PROMIS Questionnaire (SPQ)(19), or other surveys (7, 11)]. To be included in the present review, studies were required to evaluate DSM-IV Substance Dependence criteria as applied to caffeine, including, at minimum, the three most clinically meaningful indicators of distress or impairment listed in the DSM-5 Caffeine Use Disorder diagnostic schema [i.e., 1) persistent desire or unsuccessful efforts to cut down or control caffeine use, 2) continued caffeine use despite knowledge of having a persistent or 10 recurrent physical or psychological problem that is likely to have been caused or exacerbated by caffeine, and 3) characteristic caffeine withdrawal syndrome or caffeine use to relieve or avoid withdrawal symptoms]. Two studies assessed caffeine dependence using structured interviews that were informed by the DSM-IV Substance Dependence criteria applied to caffeine; however, one of these studies did not assess criterion 1,(15) and the other study reported rates of endorsement for criteria 1 and 3 in a manner that precluded interpretation of the results within the context of a DSM-IV Substance Dependence diagnosis or a DSM-5 Caffeine Use Disorder diagnosis.(12) In addition, although two studies met all inclusion criteria,(14, 86) data from both of these studies were collected from subjects who participated in two other studies that met our inclusion criteria and, therefore, overlapped with data reported in these studies.(87, 88) Thus, these studies will not be discussed in detail below. A total of 9 studies are included in the following systematic review.

Prevalence of Caffeine Dependence Diagnosis
Nine studies documented and characterized caffeine dependence in the general population and among other populations.(8-10, 13, 16, 17, 20-22) In addition to reporting the prevalence of caffeine dependence, eight of these studies also reported rates of endorsement of caffeine dependence diagnostic criteria.(8, 10, 13, 16, 17, 20-22) For these eight studies, the rates of endorsement of the DSM-5 diagnostic criteria for Caffeine Use Disorder and the prevalence of fulfilling the research diagnosis (i.e., endorsement of all three primary criteria) are presented in Table 2. Because Burgalassi et al.(9) did not report rates of endorsement of each diagnostic criterion, data from this study is not included in Table 2.

General Population. Hughes and colleagues(13) characterized caffeine dependence in the general population using a random-digit-dial telephone survey of Vermont residents (see first study in Table 2). This study found that 30% of 162 current caffeine users fulfilled the DSM-IV diagnosis for Substance Dependence as applied to caffeine by indicating that three or more of the seven criteria were met during the past year. Hughes et al.(13) also reported the percentage of participants who endorsed at least three of the four DSM-IV criteria that the authors considered to be the most clinically relevant to caffeine dependence (i.e., desire to cut down, use despite harm, withdrawal, and tolerance). Nine percent of their sample endorsed at least three of these four criteria. Three of these criteria (i.e., desire to cut down, use despite harm, and withdrawal) are now the primary diagnostic criteria for Caffeine Use Disorder in the DSM-5. Thus, as shown in Table 2, the prevalence of Caffeine Use Disorder among the general population (i.e., those endorsing all three primary diagnostic criteria) must be less than or equal to 9%—a much more conservative estimate than the 30% who fulfilled the DSM-IV diagnostic criteria for Substance Dependence as applied to caffeine. Two other studies also provided information about the prevalence of caffeine dependence in the general population. Ciapparelli et al.(10) interviewed 104 healthy control subjects from the general population in Pisa, Italy (see second study in Table 2). Participants who endorsed three of the seven DSM-IV Substance Dependence criteria as applied to caffeine were considered caffeine dependent. Six percent of the control subjects received the caffeine dependence diagnosis. The prevalence of Caffeine Use Disorder among participants in this study could not be determined from the data provided. However, Ciapparelli et al. reported the rates of endorsement of each diagnostic criterion. These data are shown in Table 2. Among the 104 participants in this study who were from the general population, rates of endorsement of many diagnostic criteria were considerably lower than the rates observed among those individuals who participated in the general population study that was conducted in the US.(13) These differences may be
due, in part, to cultural factors. They may also be due to differences in sampling or other study procedures. For example, Hughes et al. only included participants who consumed at least one caffeinated beverage per week, whereas Ciapparelli et al. included participants who were not regular caffeine consumers. In addition, Ciapparelli et al. excluded individuals with a personal or family history of substance abuse or dependence, whereas Hughes et al. used no such exclusion criterion. Burgalassi et al. (9) interviewed 15 control subjects in Pisa, Italy. Participants who endorsed at least three of six DSM-IV Substance Dependence criteria as applied to caffeine were considered caffeine dependent. The researchers noted that a seventh criterion, great deal of time spent with the drug, was not included during the caffeine dependence assessment because it was not considered applicable to caffeine use, due to the widespread availability of the drug. They found that only 2% of these participants from the general population fulfilled the caffeine dependence diagnosis.

Special Populations. Eight studies investigated caffeine dependence among other populations (data from seven of these studies are shown in Table 2). Population samples in these studies consisted of patients with eating disorders, (9) patients diagnosed with various other psychiatric disorders, (10) individuals with a recent history of licit or illicit drug use, (21) individuals who self-identified as physically or psychologically dependent on caffeine, (20, 89) adolescents and young adults who fulfilled two or more caffeine dependence diagnostic criteria, (8, 16) and pregnant women who received a lifetime (rather than previous 12-month) diagnosis of caffeine dependence. (22) The prevalence of Caffeine Use Disorder and the rates of endorsement of each diagnostic criterion were typically higher among participants in these studies relative to rates among participants in the general population. (10, 13)

Ciapparelli et al. (10) interviewed 369 in-and outpatient psychiatric patients who were diagnosed with various mental disorders, including mood disorders, anxiety disorders, schizophrenia spectrum disorders, and eating disorders. Participants who endorsed three of the seven DSM-IV Substance Dependence criteria as applied to caffeine were considered caffeine dependent. Among these patients, 17% were diagnosed as caffeine dependent—significantly more than the 6% of control subjects who were diagnosed with the disorder. The percentage of participants who met DSM-5 criteria for Caffeine Use Disorder could not be determined from the data provided in this study. Burgalassi et al. (9) interviewed 58 female patients with eating disorders (i.e., Anorexia Nervosa, Bulimia Nervosa, and Binge Eating Disorder). Sixteen percent of these patients met the DSM-IV criteria for Substance Dependence applied to caffeine. The percentage of participants who met DSM-5 criteria for Caffeine Use Disorder cannot be determined from the data reported in this study, and, as mentioned above, rates of endorsement of each diagnostic criterion were not reported in this study.

Striley et al. (21) interviewed a sample of 167 high school students, college students, pain clinic patients, and drug treatment patients with a recent history of caffeine use (previous 7 days) and other licit or illicit drug use (previous 12 months). Although 35% of their sample met the DSM-IV criteria for Substance Dependence as applied to caffeine by fulfilling three or more of the seven generic Substance Dependence criteria, only 20% of participants fulfilled the three primary diagnostic criteria required for a DSM-5 diagnosis of Caffeine Use Disorder (Table 2). Juliano and colleagues (17) interviewed 94 participants seeking treatment for caffeine dependence who self-identified as physically or psychologically dependent on caffeine, or who had tried unsuccessfully to quit using the drug. Given these inclusion criteria, it is not surprising that 93% of this sample met the DSM-IV criteria for Substance Dependence applied to caffeine, and 79% met the DSM-5 criteria for Caffeine Use Disorder (Table 2).

Svikis et al. (22) interviewed 44 caffeine-using pregnant women seeking prenatal care from a private obstetrical practice in a suburban community. Fifty-seven percent of these women endorsed at least three of the seven generic DSM-IV criteria for a lifetime diagnosis of Substance Dependence applied to caffeine. This relatively high prevalence of caffeine dependence likely resulted from lifetime, rather than past year assessment of the diagnostic criteria. An estimate of the prevalence of the DSM-5 diagnosis of
Caffeine Use Disorder could not be determined from the data reported in this study. A study conducted by Jones and Lejuez(16) included only college students who fulfilled the DSM-IV diagnosis for Substance Dependence as applied to caffeine (i.e., 100% of these participants were diagnosed with caffeine dependence). Given these inclusion criteria, it is not surprising that the study showed high rates of endorsement of each diagnostic criterion relative to the rates of endorsement observed in the general population (Table 2). An estimate of the prevalence of the DSM-5 diagnosis of Caffeine Use Disorder could not be determined from the data reported in this study.

Strain et al.(20) interviewed 27 caffeine users who self-identified as physically or psychologically dependent on caffeine. As might be expected, the majority of these participants (59%; n=16) fulfilled at least three of the following four criteria: desire to cut down, use despite harm, tolerance, and withdrawal. Because only three of these four criteria are now the primary DSM-5 diagnostic criteria for Caffeine Use Disorder (i.e., desire to cut down, use despite harm, and withdrawal), it is likely that less than 59% of this sample endorsed all three criteria and would have received a Caffeine Use Disorder diagnosis; especially, considering that a relatively large percentage of the sample endorsed tolerance, which is not one of the three primary criteria for Caffeine Use Disorder. Table 2 displays the rates of endorsement for each diagnostic criterion among only those 16 participants who fulfilled at least three of the four diagnostic criteria assessed by Strain and colleagues.

Bernstein and colleagues(8) characterized caffeine dependence in a sample of 36 daily caffeine consumers between 13 and 17 years of age who endorsed two or more of four DSM-IV diagnostic criteria for Substance Dependence as applied to caffeine (i.e., the same four criteria evaluated by Strain et al.(20); desire to cut down, use despite harm, tolerance, and withdrawal). They found that 22% of their sample endorsed at least three of these criteria. Thus, fewer than 22% of this sample likely endorsed all three primary criteria for the DSM-5 diagnosis of Caffeine Use Disorder (Table 2).

Clinically Meaningful Indicators of Distress or Impairment Associated with Caffeine Dependence

The DSM-5 indicates that the diagnostic schema for Caffeine Use Disorder was explicitly developed to be more restrictive than that for other Substance Use Disorders to prevent overdosage due to the high rate of non-problematic caffeine use in the general population. The DSM-5 further indicates that a key goal in including the proposed diagnostic criteria for Caffeine Use Disorder is to stimulate research that will determine the reliability, validity, and prevalence of Caffeine Use Disorder based on the new diagnostic schema, with particular attention to the association of the diagnosis with functional impairment as part of validity testing. In a recent survey of addiction professionals about the possible inclusion of a Caffeine Use Disorder diagnosis in the DSM-5, a minority of participants were concerned that the severity and clinical importance of the disorder had not been demonstrated.(90) This section summarizes data and observations that bear on the question about whether caffeine dependence is associated with clinically meaningful distress or impairment. The section is organized into three subsections based on the three primary DSM-5 diagnostic criteria for Caffeine Use Disorder (see Table 1) that summarize information from the clinical studies included in this review (see Table 2).

A persistent desire or unsuccessful efforts to cut down or control caffeine use. Perhaps the most distinguishing feature of any Substance Use Disorder is a persistent desire or unsuccessful efforts to cut down or control use. In a general population survey conducted in the State of Vermont, this criterion was the most frequently endorsed diagnostic criterion for caffeine dependence, with 56% of caffeine users reporting a desire or unsuccessful efforts to cut down(13) (Table 2).

In Italy, Ciapparelli et al. found that 10% of 104 participants from the general population endorsed this criterion.(10) This rate of endorsement was significantly lower than the rate observed among psychiatric patients in the same study—20% of these patients endorsed a
desire or unsuccessful efforts to cut down.

Striley and colleagues(21) found that 23% of a group of licit and illicit drug users endorsed a desire or unsuccessful efforts to cut down.

Juliano and colleagues(17) interviewed 94 participants seeking treatment for caffeine dependence who self-identified as being physically or psychologically dependent on caffeine. Participants in this study consumed 548 mg of caffeine per day (i.e., roughly twice the national average for regular caffeine users). As might be expected, a large proportion of these treatment seekers (89%) endorsed a desire or unsuccessful efforts to cut down (Table 2). Mean rating of desire to modify caffeine use on a 0 to 10 scale was 7.79 (SD = 2.18). Many of these caffeine users did not believe that they could control their caffeine use without assistance. Sixty-five percent of participants reported that they had previously tried to stop using caffeine, and 80% reported that they had tried to cut down. In addition, 59% of 258 initial responders expressed interest in attending face-to-face counseling to receive assistance in their quit attempts. Table 3 presents three previously unpublished case histories of participants who were sufficiently distressed by their dependence on caffeine to enroll in this study, undergo diagnostic interview, maintain a detailed record of caffeine consumption, and visit a clinic several times over a 6-month period.

Among the remaining studies summarized in Table 2, Svikis et al.(22) found that 45% of pregnant women seeking prenatal care endorsed a desire or unsuccessful efforts to cut down at some point during their lifetime. Jones and Lejuez(16) found that, among college students who fulfilled the DSM-IV diagnosis for Substance Dependence applied to caffeine, 60% endorsed this criterion. Among adults who fulfilled at least three of four DSM-IV diagnostic criteria for Substance Dependence applied to caffeine (i.e., desire to cut down, use despite harm, withdrawal, and tolerance), Strain et al.(20) found that 81% endorsed desire or unsuccessful efforts to cut down. Finally, Bernstein et al.(8) reported that, among adolescents who fulfilled two or more of the DSM-IV diagnostic criteria for Substance Dependence applied to caffeine, 39% endorsed this criterion (Table 2).

Continued caffeine use despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by caffeine. The diagnostic criterion that may be of most concern to health care professionals is continued caffeine use despite harm. Caffeine consumption has been associated with a number of negative health consequences, including anxiety, insomnia, hypertension, myocardial infarction, bladder instability, gastroesophageal reflux, spontaneous abortion, and reduced fetal growth.(89)

In a general population survey in the US, 14% of caffeine users endorsed use despite harm(13) (Table 2). Many participants from this study (13%) reported that a physician or counselor had advised them to stop or reduce caffeine consumption within the last year. Medical and psychological problems that participants attributed to caffeine, included heart, stomach, and urinary problems, and complaints of anxiety, depression, insomnia, irritability, and difficulty thinking. In addition, two-thirds of those surveyed endorsed at least one symptom associated with Caffeine Intoxication, a clinical disorder recognized by the ICD-10 (i.e., Caffeine Acute Intoxication) and the DSM-5 (see Table 4). For example, 39% of participants from this general population study endorsed insomnia, 30% endorsed nervousness, 24% endorsed heart pounding, 18% endorsed stomachache, and 10% endorsed muscle twitching. Seven percent of participants reported that these symptoms interfered with their performance at work, home, or school.

Ciapparelli et al.(10) found that only 7% of participants from the general population in
Italy endorsed *use despite harm*. The rate of endorsement of this criterion was significantly higher among psychiatric patients participating in the study—24% of these patients endorsed *use despite harm*.

Six studies among other populations show considerable variability in the rates of endorsement of *use despite harm*. In a sample of adolescents who fulfilled two or more of the *DSM-IV* diagnostic criteria for Substance Dependence as applied to caffeine, Bernstein *et al.* (8) reported that 17% of these daily caffeine users endorsed this criterion (Table 2). Jones and Lejuez (16) characterized caffeine dependence in college students who fulfilled the *DSM-IV* diagnosis for Substance Dependence as applied to caffeine and found that 57% of these caffeine users endorsed *use despite harm* (Table 2). Striley and colleagues (21) found that 44% of licit and illicit drug users endorsed *use despite harm* (Table 2). Ten percent of participants in this study reported having talked to a physician about problems associated with consuming caffeinated beverages, and 11% continued to use caffeine after learning that a health problem could be exacerbated by continued consumption. Physical and psychological problems attributed to caffeine included: trouble falling or staying asleep (36%); feeling very anxious (19%); stomach problems (16%); fast or irregular heartbeat or chest pain (11%); and feeling irritable or angry (10%). In a study of pregnant women who had been advised to quit caffeine use during pregnancy, Svikis *et al.* (22) reported that 43% of these caffeine users endorsed *use despite harm* (Table 2). Twenty-one percent indicated that they had previously been told by a health care professional that they should reduce or quit caffeine because of medical conditions, including fibrocystic breast disease, headaches, pregnancy, insomnia, and stomach problems.

In addition to diagnostic assessment, Svikis *et al.* conducted a prospective study demonstrating that some of these pregnant women were unable to quit using caffeine. Participants were given written and verbal instructions by a physician to quit caffeine during their pregnancy in order to avoid adverse birth consequences. Of those who did not fulfill diagnostic criteria for caffeine dependence, none continued to consume caffeine in amounts greater than those thought to be safe during pregnancy (i.e., >300 mg/day). In contrast, 28% of women who fulfilled the caffeine dependence diagnosis continued to consume caffeine at unsafe levels. Reasons participants provided for failing to eliminate or cut back caffeine use included: cravings, headache, nervousness; migraines; need to stay awake; severe withdrawal; and inability to concentrate at work. Examples of functional impairment reported by women who attempted to reduce caffeine consumption included: “less active at work;” “flu symptoms prevented going to work;” and “it is difficult to concentrate.”

Not surprisingly, the rates of endorsement for *use despite harm* were highest in studies of individuals who self-identified as psychologically or physically dependent on caffeine. In two such studies, the prevalence was 94% and 87%, respectively, in Strain *et al.* (20) and Juliano *et al.* (89) (Table 2). In the study described by Strain *et al.* , 44% of participants reported that physical conditions such as pregnancy, palpitations, gastrointestinal problems, and acne rosacea had led physicians to recommend reduction or cessation of caffeine. All participants reported that they failed to comply with these recommendations. In the study by Juliano *et al.*, 59% of participants reported health problems or concerns as a reason for seeking treatment for caffeine dependence. Forty-three percent reported being advised by a health care professional to modify caffeine use for reasons including cardiovascular problems, fibrocystic breast disease, pregnancy, anxiety, headaches, urinary problems, gastric problems, hypoglycemia, and sleep difficulties.

*Withdrawal.* It is well documented that habitual caffeine users can experience a well-defined
withdrawal syndrome upon acute abstinence from caffeine, and many caffeine dependent individuals report continuing to use caffeine to avoid experiencing withdrawal symptoms. Both the ICD-10 and the DSM-5 recognize a clinical diagnosis of Caffeine Withdrawal. Although Caffeine Withdrawal is an independent diagnosis, it can also be a feature of Caffeine Dependence Syndrome (ICD-10) or Caffeine Use Disorder (DSM-5). Table 5 shows the DSM-5 diagnostic criteria for Caffeine Withdrawal.

In the general population study conducted in the US by Hughes and colleagues, the rate of endorsement of withdrawal was 18% (Table 2), with most participants (17%) endorsing the sub-criterion of using caffeine to relieve or avoid withdrawal symptoms (see Table 1). Ciapparelli et al. showed that rates of withdrawal were considerably lower among control subjects (6%) and psychiatric patients (2%) in Italy.

As shown in Table 2, all six studies in other populations show considerably higher rates of endorsement of withdrawal: 26%, 96%, 77%, 73%, 94%, and 78% respectively in Striley et al., Juliano et al., Svikis et al., Jones and Lejuez, Strain et al., and Bernstein et al. (Table 2). In the two studies that provided information about the rate of endorsement of the sub-criterion, use of caffeine to relieve or avoid withdrawal symptoms, 24% of participants in Striley et al. and 92% of participants in Juliano et al. endorsed this sub-criterion.

Severity of Withdrawal. Based on a comprehensive review of the literature, the incidence of clinically significant distress or functional impairment in prospective experimental studies of caffeine withdrawal in normal subjects varied from about 10% to 55%, with a median of 13%. In the general population, withdrawal symptoms plus interference with performance was reported by 7% of caffeine users who cut down or stopped caffeine use temporarily and 24% of those who tried to stop permanently. Moreover, 33% of caffeine users reported that they needed the drug to function.

Among licit an illicit drug users with a recent history of caffeine consumption, 26% reported that they needed caffeine to function. Thirteen percent of participants from this study reported that caffeine withdrawal symptoms interfered with functioning, and 13% endorsed difficulty concentrating after 12 hours without caffeine.

The incidence of functional impairment is greater among those with a caffeine dependence diagnosis. Strain et al. conducted a prospective experimental assessment of the rate of functional impairment during caffeine withdrawal in 11 caffeine dependent individuals. This double-blind study showed that 73% of participants reported significant disruptions in normal daily activities during caffeine abstinence, including leaving or missing work, making errors or costly mistakes at work, inability to care for children, and inability to complete school work.

In a survey about the effects of withdrawal in a group of caffeine treatment seekers, Juliano et al. showed that 43% reported functional impairment due to withdrawal. Headache was the most frequently endorsed withdrawal symptom in this study (89%) and vomiting was the least frequently endorsed symptom (approximately 5%). Examples of functional impairment reported by these participants included inability to work, sleeping at work, missing activities on vacation, and inability to attend church. Other studies have also reported severe withdrawal-induced functional impairment as well as nausea and sickness in as many as 13% of normal subjects and 33% of caffeine dependent adolescents.

Assessing the Severity of Caffeine Dependence
The DSM-5 lists nine diagnostic criteria for Caffeine Use Disorder. In addition to the
three primary criteria, which are required for the diagnosis, six other criteria are also assessed (see Table 1). Among these additional criteria, Hughes et al.(13) found that 28% of the general population endorsed use more than intended, 15% endorsed use results in role dysfunction, 2% endorsed use despite interpersonal problems, 50% endorsed use more than intended, 15% endorsed use results in role dysfunction, and 19% endorsed craving (Table 2). The relatively low rate of endorsement for use despite interpersonal problems is not surprising given the wide availability and social acceptance of caffeine use. Likely, for similar reasons, the DSM-IV Substance Dependence criterion “Important social, occupational, or recreational activities are given up or reduced because of substance use” was also endorsed at only very low rates in many of the studies discussed in this review: <1% in Hughes et al.; 1% in Ciapparelli et al.(10); 1% in Striley et al.(21); 0% in Svikis et al.(22); 2005; 8% in Juliano et al.(89); and 20% in Jones and Lejuez(16) (data not shown). Nevertheless, evaluating additional diagnostic criteria such as these can contribute to an assessment of the severity of caffeine dependence.

For Substance Use Disorders, the DSM-5 indicates that the number of diagnostic criteria endorsed can be used as a measure of severity (i.e., endorsement of two or three diagnostic criteria = mild; four or five = moderate; and six or more = severe). Although Hughes et al.(13) evaluated all nine proposed DSM-5 criteria for Caffeine Use Disorder, they did not report the proportion of participants diagnosed with Caffeine Use Disorder who also endorsed one or more of the six additional criteria. Therefore, it is not possible to estimate the severity of Caffeine Use Disorder in participants from the general population. However, data from two studies provide more information about Caffeine Use Disorder severity in other populations. Striley et al.(21) evaluated four of the six other Caffeine Use Disorder diagnostic criteria.

After that study was published, Striley and Cottler conducted subsequent analyses of these data (Catherine Striley and Linda Cottler, personal communication, February 7, 2013). These analyses showed that, among licit and illicit drug users who fulfilled the three primary diagnostic criteria for Caffeine Use Disorder, 9%, 30%, 33% and 24%, respectively, endorsed 1, 2, 3, and 4 additional criteria, with 58% endorsing use more than intended, 85% endorsing use more than intended, 85% endorsing use results in role dysfunction, and 82% endorsing craving. These data were also expressed in accordance with the DSM-5 recommendations for assessing Substance Use Disorder severity. Even though Striley et al.(21) evaluated only seven of the nine Caffeine Use Disorder diagnostic criteria, their data indicate that the severity of the disorder among participants who met the three primary criteria was mild in 3% (i.e., endorsed no criteria in addition to the three primary diagnostic criteria), moderate in 39% (i.e., endorsed one or two criteria in addition to the three primary criteria), and severe in 57% (i.e., endorsed three or more additional criteria).

Among treatment-seeking caffeine users, Juliano et al.(89) evaluated the same seven criteria that were assessed by Striley and colleagues.(21) The authors of the present review analyzed data from this study which showed that, among participants diagnosed with Caffeine Use Disorder, 12%, 24%, 31%, and 28%, respectively, endorsed 1, 2, 3, and 4 of the additional criteria, with 42% endorsing use more than intended, 62% endorsing use results in role dysfunction, and 74% endorsing use results in role dysfunction, and 89% endorsing craving. The severity of Caffeine Use Disorder among participants who met the three primary criteria was mild in 4% (i.e., endorsed no additional criteria), moderate in 36% (i.e., endorsed one or two additional criteria), and severe in 59% (i.e., endorsed three or more additional criteria).

Future Research Directions
Although a clinical diagnosis of Caffeine Use Disorder is not recognized by the DSM-5, a research diagnosis may still benefit patients by providing clinicians with the diagnostic tools
necessary to recognize an otherwise unspecified caffeine-related disorder. However, the most important function of the Caffeine Use Disorder research diagnosis is to stimulate further study of the disorder. Therefore, this section provides a discussion of important areas for future research.

**Caffeine consumption and prevalence of Caffeine Use Disorder**

The most recent data comprising the largest database on caffeine intake among US consumers was collected over 15 years ago. Thus, there is a critical need for epidemiological studies to examine caffeine consumption among US children and adults. In addition, studies should compare caffeine consumption in the US to consumption in other countries, as some research suggests that rates of consumption vary between nations. There is also a critical need for studies to determine the prevalence of Caffeine Use Disorder and rates of endorsement of each diagnostic criterion in the US and in other countries. As discussed in this review, one general population study has been conducted in the US, and it included only 162 regular caffeine users from the State of Vermont. Two studies conducted in Italy also included individuals from the general population. Notably, the prevalence of caffeine dependence was lower among participants from the general population of Italy relative to the prevalence of the disorder among participants from the general population of the US. It is not clear whether the observed difference in prevalence was due to cultural factors, different sampling procedures, or other factors. Thus, rigorous general population surveys are still needed in the US and in other countries.

The prevalence of Caffeine Use Disorder and rates of endorsement of each diagnostic criterion should also be determined among special populations, including individuals seeking treatment for symptoms related to Caffeine Withdrawal and Caffeine Intoxication (e.g., headache, insomnia, and anxiety). Results from the studies reviewed in this paper suggest that some individuals may endorse certain diagnostic criteria more or less frequently than others. For example, endorsement of use despite harm varied from 14% in the general population to 44% in licit and illicit drug users and 87% in caffeine users seeking treatment for caffeine dependence (Table 2). A diagnosis of Caffeine Use Disorder or rates of endorsement of each diagnostic criterion may also vary as a function of other variables, such as gender, age, ethnicity, education, or cultural background. Thus, future studies should examine the prevalence of Caffeine Use Disorder and rates of endorsement of each diagnostic criterion in both the general population and among special populations to determine whether certain populations are more susceptible to developing Caffeine Use Disorder than others.

**Reliability and validity of diagnostic criteria**

Studies investigating the reliability and validity of the DSM-5 Caffeine Use Disorder research diagnosis are also needed. Factor analyses of the diagnostic criteria have not been conducted, and more test-retest evaluations are still needed. The only test-retest evaluation of caffeine dependence diagnostic criteria that has been conducted to date found high reliability for a DSM-IV diagnosis of Substance Dependence as applied to caffeine. However, no internal reliability studies have been conducted using the DSM-5 criteria for Caffeine Use Disorder. There is also a need for studies to investigate the external validity of the Caffeine Use Disorder research diagnosis. Prospective studies of caffeine reinforcement can be used to validate the diagnosis. For example, a small study of caffeine self-administration showed that subjects who had met criteria for caffeine dependence tended to be more likely to demonstrate reliable reinforcement (75%) than those who were not dependent (20%). Perhaps the most important indicator of the external validity of the Caffeine Use Disorder
diagnosis is its ability to predict whether an individual can quit using caffeine. To date, the only prospective study to use this clinically meaningful outcome measure showed that pregnant women with a lifetime DSM-IV Substance Dependence diagnosis applied to caffeine were more likely to consume caffeine at unsafe levels throughout pregnancy than those without the diagnosis. More studies focusing on pregnant women and other vulnerable populations are still needed. Understanding whether individuals with a diagnosis of Caffeine Use Disorder are less likely than others to quit using the drug is particularly important in populations with comorbid medical conditions that are exacerbated by caffeine consumption.

In addition to investigating the reliability and validity of the Caffeine Use Disorder diagnostic criteria, studies should compare the DSM-5 diagnostic schema for Caffeine Use Disorder to other diagnostic schemas (e.g., the generic DSM-5 Substance Use Disorder criteria) to determine if the proposed Caffeine Use Disorder diagnostic schema provides a more reliable and valid assessment of caffeine dependence than other diagnostic algorithms. The research reviewed in this paper suggests that the use of the Caffeine Use Disorder diagnostic schema produces relatively conservative estimates of the prevalence of caffeine dependence, but more research is needed to determine whether these estimates are more reliable or valid than those produced by other diagnostic schemas.

**Objective measures of caffeine consumption**

In many clinical investigations of caffeine dependence, caffeine consumption has been assessed via retrospective surveys in which participants are asked to report how many caffeinated beverages they consume on a daily basis. However, because caffeine concentration varies considerably within and across foods and beverages (e.g., 54 mg to 210 mg in a 6 oz cup of brewed coffee), researchers should use methods to more accurately measure caffeine consumption (e.g., through the use of detailed food diaries or timeline follow-back approaches). Thus, future research should include studies designed to develop and evaluate methods to assess caffeine consumption frequently and accurately.

**Functional impairment and severity**

The DSM-5 states that one key goal for the proposed research diagnosis of Caffeine Use Disorder is to stimulate research investigating the association of the diagnosis with clinically significant distress and functional impairment. Although some studies have already shown functional impairment during withdrawal, additional studies using a range of volunteer-rated, observer-rated, behavioral, and cognitive measures of impairment during withdrawal will be important.

Whether distress and functional impairment occur at times other than during caffeine abstinence should also be investigated. Thus, more prospective empirical investigations of the relationship between Caffeine Use Disorder and functional impairment during chronic caffeine administration are needed. Future research should also include surveys administered in medical settings to patients and health care providers (e.g., primary care physicians) to assess the prevalence, severity, and functional impairment of Caffeine Use Disorder among patients who present with symptoms of the disorder.

As discussed above, the DSM-5 indicates that the severity of Substance Use Disorders should be estimated based on the number of criteria scored (two or three criteria = mild, four or five = moderate, and six or more = severe). To date, the only information on the severity of Caffeine Use Disorder is presented in this article based on re-analyses of data from two
previously published studies that collected data on only seven of the nine DSM-5 diagnostic criteria. (17, 21) Thus, it will be important for future researchers to attempt to validate the proposed DSM-5 severity scoring approach by scoring all criteria and by comparing DSM-5 severity scores with other measures of addiction severity.

Genetics
Further research investigating the role of genetic determinants in the development of Caffeine Use Disorder is needed. Although heritabilities for some markers of Caffeine Use Disorder have been demonstrated, (60-63) no studies have examined the heritability of Caffeine Use Disorder per se nor the heritability of the primary diagnostic criteria of desire to cut down or use despite harm. Also, given that genetic polymorphisms in the ADORA2A gene have been shown to predict the subjective effects of caffeine, (67, 68) future research should investigate the role of specific polymorphisms in Caffeine Use Disorder.

Comorbidity with non-drug psychiatric disorders
Because drug dependence diagnoses are frequently associated with non-drug psychiatric disorders, (93) more studies are needed to examine the relationship between Caffeine Use Disorder and non-drug psychiatric disorders. Ciapparelli et al. (10) showed that psychiatric patients were more likely than healthy controls to fulfill DSM-II Substance Dependence diagnostic criteria as applied to caffeine. Other research has shown an association between various comorbid conditions and excessive caffeine consumption. For example, excessive caffeine consumption is common among psychiatric patients, particularly those diagnosed with schizophrenia. (94-98) In addition, some features of caffeine dependence, including tolerance and withdrawal, have been associated with major depression, adult antisocial disorder, panic disorder, and generalized anxiety disorder. (64, 99) Moreover, excessive caffeine consumption is believed to exacerbate chronic psychiatric conditions, including anxiety. (100-104) Thus, future research should investigate whether a diagnosis of Caffeine Use Disorder predicts or exacerbates other non-drug psychiatric disorders.

Relationship between Caffeine Use Disorder and other Substance Use Disorders
Although comorbidity among Substance Use Disorders is common, only a few studies have examined the relationship between caffeine dependence and other drug dependencies. Among a group of pregnant women, Svikis et al. (22) found that those who received a lifetime diagnosis of caffeine dependence were nearly nine times more likely than those without the diagnosis to report a history of cigarette smoking (44% vs. 5%). Further, women with both caffeine dependence and a family history of alcoholism were six times more likely to have a lifetime diagnosis of alcohol abuse or dependence. Among licit and illicit drug users, Striley et al. (21) found a non-significant trend (p<0.10) in the proportion of caffeine dependent participants who were also alcohol dependent (i.e., 42% of caffeine dependent alcohol users were also alcohol dependent and 28% of caffeine dependent alcohol users were not alcohol dependent; n=142). They also found a non-significant trend (p<0.10) in the proportion of caffeine dependent participants who were nicotine dependent (i.e., 37% of caffeine dependent nicotine users were also nicotine dependent and 19% of caffeine dependent nicotine users were not nicotine dependent; n=120). Among individuals from the general population, Hughes and colleagues (105) found a weak correlation between the severity of caffeine dependence and alcohol dependence, but they found no correlation between caffeine dependence and nicotine dependence. In addition, some twin studies examining caffeine use, alcohol use, and cigarette smoking found that a common genetic factor (i.e., polysubstance use) underlies the use of these three substances, with 28 to 41% of the heritable effects of caffeine use (or heavy use) shared with alcohol and smoking. (63, 106) However, other twin studies suggest that caffeine and nicotine use and dependence are substantially influenced by genetic factors unique to these drugs. (64, 65)
Thus, much more clinical, epidemiological, and genetic research is needed to determine the relationship between Caffeine Use Disorder and other Substance Use Disorders. In addition to further investigations of comorbidity, studies that directly compare Caffeine Use Disorder with other Substance Use Disorders are also needed. To our knowledge, only two such studies have been conducted to date. In a group of 148 licit and illicit drug users who used caffeine, nicotine, and alcohol, Striley et al. (21) compared the proportion of participants who endorsed the seven DSM-IV diagnostic criteria for Substance Dependence as applied to caffeine to the proportion who endorsed the criteria as applied to alcohol and nicotine. The proportion endorsing each criterion for caffeine was generally lower than the proportion endorsing each criterion for nicotine and for alcohol, consistent with the finding that the prevalence of caffeine dependence (about 28%) was lower than that for nicotine (80%) and alcohol (50%) in this group of drug users. Miyata and colleagues (107) compared individuals who met the DSM-IV Substance Dependence diagnosis for either caffeine or nicotine before they experienced a controlled 7-day abstinence from their respective drug of dependence. The results showed no differences between nicotine and caffeine in craving, or social functioning.

Although the incidence of irritability was higher with nicotine than caffeine, there were no differences in irritability on a well-being questionnaire. The authors concluded that, overall, there were no meaningful differences between nicotine and caffeine in the difficulties of abstaining. Additional qualitative and quantitative comparisons of Caffeine Use Disorder with other Substance Use Disorders are needed.

Future research is also needed to determine whether the development of Caffeine Use Disorder is predictive of the development of subsequent licit or illicit Substance Use Disorders. If this is the case, such research could have important implications for the early identification and treatment of subpopulations vulnerable to the development of more harmful substance abuse.

**Caffeine Use Disorder among energy drink consumers**

Future research should investigate the prevalence and severity of Caffeine Use Disorder among consumers of caffeinated energy drinks. Although energy drinks often contain other compounds, including taurine, glucuronolactone, and B-vitamins, research suggests that the effects of these additives contribute little beyond the effects of caffeine on physical and cognitive performance. (108, 109) However, more research is needed to evaluate the independent subjective and behavioral effects of these ingredients and the effects of the additives when combined with caffeine.

The primary psychoactive ingredient in energy drinks is caffeine. The amount of caffeine contained in energy drinks can range from 50 mg to 505 mg. (110) Therefore, these beverages allow consumers to drink large quantities of caffeine in a relatively small number of servings. Research suggests that this level of consumption can produce Caffeine Intoxication (89) (see Table 4), resulting in serious adverse health consequences and, in rare cases, death. (111) Due to recent increases in the popularity of caffeinated energy drinks, there is growing concern among health professionals about the short- and long-term negative health consequences associated with energy drink consumption, especially among children, adolescents, and young adults. (112-114) Indeed, a 2013 report from the Drug Abuse Warning Network (DAWN) showed that emergency room visits involving energy drinks doubled in the US from 2007 to 2011. (115)

Some research has shown that energy drink consumption is associated with increased
marijuana use, alcohol consumption, and alcohol-related risky behavior, including riding with an intoxicated driver, taking advantage of another sexually, and being physically hurt or injured.(116-118) Moreover, studies have found that energy drink consumption is associated with an increased risk for alcohol dependence(119) and predicts subsequent nonmedical prescription drug use.(120) Thus, researchers should explore whether a diagnosis of Caffeine Use Disorder among adolescents and adults who consume energy drinks is associated with increased energy drink consumption, subsequent licit and illicit drug dependencies, or negative health consequences.

Course and treatment
No studies have investigated the course and history of Caffeine Use Disorder. However, like other Substance Use Disorders, Caffeine Use Disorder appears to be a chronic relapsing condition. For example, studies have shown that caffeine dependent participants report frequent relapses due to failed efforts to quit caffeine.(17, 20) The research discussed in the present review shows that some individuals desire to quit using caffeine but are unable to do so. Moreover, some caffeine users are sufficiently distressed by their caffeine use to seek assistance to quit or reduce caffeine consumption. For example, Juliano and colleagues(17) reported that 47% of 258 individuals seeking treatment for caffeine use were interested in one-on-one counseling, 12% were interested in group counseling, 25% were interested in a self-help booklet, and 4% were interested in phone-based assistance. Future research should determine the extent of the demand for Caffeine Use Disorder treatment.

Unfortunately, few treatments for Caffeine Use Disorder are currently available. To date, only a handful of studies have examined individuals receiving treatment for problems related to caffeine use.(121-125) More research on this population is needed to determine what methods work best in promoting caffeine reduction and cessation, ideally, so that brief, inexpensive interventions can be developed to assist caffeine users with their quit attempts.

Summary and Conclusion
The literature reviewed in this paper shows that caffeine produces behavioral and physiological effects similar to those produced by other drugs of dependence. Indeed, an abundance of evidence from controlled laboratory studies with human and nonhuman animal subjects demonstrates the biological plausibility of caffeine dependence. Further, several recent clinical studies show that a nontrivial proportion of caffeine users develops clinically meaningful features of caffeine dependence, including a persistent desire or unsuccessful efforts to cut down or control caffeine use, continued use despite harm, and a characteristic withdrawal syndrome.

Although the World Health Organization already recognizes a diagnosis of Caffeine Dependence Syndrome in the ICD-10, the American Psychiatric Association has indicated that more research is needed to determine the clinical significance of Caffeine Use Disorder before the diagnosis may be recognized in the DSM as a clinical disorder. Indeed, there is a critical need for more clinical, epidemiological, and genetic research on caffeine dependence. To date, no national population-based study has been conducted to investigate the prevalence and severity of caffeine dependence in the general population, and most studies that have characterized caffeine dependence in the general population and among special populations relied on relatively small sample sizes. Nevertheless, several recent reports have shown that caffeine dependence can result in clinically significant distress and functional impairment, and many individuals are sufficiently distressed by their caffeine dependence to seek treatment. Due
to this new evidence, Caffeine Use Disorder is now recognized by the *DSM-5* as a condition in need of further study.

The inclusion of Caffeine Use Disorder in the *DSM-5* should help stimulate more research on caffeine dependence. More studies are needed to determine the prevalence of Caffeine Use Disorder and the severity of functional impairment associated with the disorder. In addition, research is needed to evaluate the reliability and validity of the Caffeine Use Disorder diagnostic schema and the relationship between Caffeine Use Disorder and other behavioral and mental disorders. Most importantly, however, more research is needed to determine which methods work best to treat individuals who are already distressed by this clinically important health problem.

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**Author Disclosure Statement**

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**References**

measurement framework. Psychol Addict Behav. 2009 Sep;23(3):500-11.
36
37
31. Quarta D, Ferré S, Solinas M, You ZB, Hockemeyer J, Popoli P, et al. Opposite modulatory roles for adenosine A1 and A2A receptors on glutamate and dopamine release in the shell of...
40
70. Byrne EM, Johnson J, McRae AF, Nyholt DR, Medland SE, Gehrman PR, et al. A genomewide association study of caffeine-related sleep disturbance: Confirmation of a role for a
common variant in the adenosine receptor. Sleep. 2012 Jul 1;35(7):967-75.
42
98. Strassnig M, Brar JS, Ganguli R. Increased caffeine and nicotine consumption in community-dwelling patients with schizophrenia. Schizophr Res. 2006 Sep;86(1-3):269-75.
114. Torpy JM LE. Energy drinks. JAMA. 2013 January 16;309(3):297-.

For TABLES and FIGURES, please send a request to jmulligan@nas.edu
Caffeine’s effects go beyond a cup of coffee
By Charles O’Brien

Caffeine is part of many people’s daily lives – in the coffee and soda they drink, the candy and cereal they eat, the pain relievers and weight-loss pills they take. It’s found so often in common products that people may not realize caffeine is indeed a drug. And although most individuals consume it with no ill effects, there is substantial evidence that too much caffeine, or abrupt changes in its use, can have very impairing consequences for sensitive people. These include headaches, muscle twitching, and vomiting. Extremely high amounts of caffeine can even be fatal.

The new Diagnostic and Statistical Manual of Mental Disorders (DSM-5) details both caffeine intoxication and caffeine withdrawal to help clinicians identify patients who are experiencing serious effects of caffeine use. Although the most severe symptoms are rare, especially considering the widespread use of caffeine, they can hinder a person’s ability to work, socialize, or manage family responsibilities.

Caffeine intoxication describes someone who is experiencing clinically significant distress or impairment in occupational, social, or other important areas of functioning. This disorder was included in DSM-IV and remains largely unchanged in the new manual. It is characterized by symptoms such as muscle twitching, flushed face, insomnia, and psychomotor agitation, which typically appear when a person is consuming 1 gram or more of caffeine daily. To put that amount in perspective, 10 cups of coffee contain about a gram of caffeine. Adults who regularly consume caffeine ingest about 280 milligrams a day on average.

Caffeine withdrawal is a new diagnosis in DSM-5, moved from the previous manual’s appendix section based on growing research about the condition and its frequency. Caffeine withdrawal is marked by drowsiness, headaches, difficulty concentrating, and flu-like symptoms of nausea, vomiting, muscle pain, and stiffness. Its primary trigger is an abrupt cessation, or substantial reduction, in caffeine use after prolonged daily use. Symptoms normally begin within 24 hours and can peak after one to two days without caffeine. They also cause clinically significant distress or impairment.

Symptoms of caffeine withdrawal are more often experienced when a person attempts to permanently avoid all caffeine. One study found that more than 70 percent of individuals may experience at least one symptom (i.e., headaches), and 24 percent may experience headaches plus one or more other symptoms and functional impairment. To help decrease the severity of caffeine withdrawal, a person should gradually reduce caffeine consumption over a period of days or weeks rather than stop abruptly.

In addition to these two formal disorders, DSM-5 includes another caffeine-related diagnosis in Section III of the manual. This section notes conditions that require more research before being considered as formal disorders. While caffeine use has been well-studied, more data are needed to determine whether the condition is clinically significant. By including caffeine use disorder in Section III, we aim to encourage the further study needed.
DSM is designed to help clinicians distinguish the individuals who need professional help from those who don’t. In the case of the drug caffeine, most people do not need clinical treatment. That’s why the criteria for caffeine intoxication and caffeine withdrawal require that symptoms cause clinically significant distress or impairment to signal that they go beyond most people’s everyday experiences and drastically upend lives. It’s my hope that both diagnoses will ensure clinicians provide the best care possible for affected patients and increase awareness of the use of caffeine as a drug.
DAY 2, SESSION 1: OTHER COMPOUNDS IMPACTING CAFFEINE EFFECTS


SCIENTIFIC OPINION

The use of taurine and D-glucurono-γ-lactone as constituents of the so-called “energy” drinks

Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food

(Question No EFSA-Q-2007-113)

Adopted on 15 January 2009

PANEL MEMBERS

SUMMARY

Following a request from the Commission, the Scientific Panel on Food Additives and Nutrient Sources added to Food (ANS) has been asked to evaluate the safety-in-use of taurine and D-glucurono-γ-lactone as constituents of the so-called “energy” drinks.

In the present opinion the Panel evaluates the safety of taurine and D-glucurono-γ-lactone as individual ingredients of so-called “energy” drinks based on the new studies provided by the petitioner.

The Panel does not evaluate the safety of “energy” drinks as such.

Taurine and D-glucurono-γ-lactone are constituents of the so-called “energy” drinks, but they also occur at much lower levels as natural ingredients in food, and they are also normal human metabolites. Previous Scientific Committee on Food (SCF) Opinions have summarized safety studies of taurine and D-glucurono-γ-lactone, but the safety-in-use of these


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two ingredients at the levels of exposure expected from their use in “energy” drinks could not be established at that time.

In the absence of new chronic and acute exposure data, the exposures used in this opinion are based on the data reported by the SCF in 2003, i.e. a daily mean chronic consumption of 0.5 cans per person and a high chronic exposure at the 95th percentile of 1.4 cans per regular consumer. The Panel notes that actual exposure data on “energy” drink consumption, especially for adolescents and young adults, may need to be collected. Based on the assumption that a can contains 250 mL and 4000 mg/L taurine and 2400 mg/L D-glucurono-\(\gamma\)-lactone, the SCF calculated that these values result in a mean daily exposure to 500 mg taurine (8.3 mg taurine/kg bw/day for a 60 kg person) and 300 mg D-glucurono-\(\gamma\)-lactone (5.0 mg D-glucurono-\(\gamma\)-lactone/kg bw/day for a 60 kg person). The 95th percentile exposure of regular users would amount to 1400 mg taurine/day (23.3 mg/kg bw/day for a 60 kg person) and 840 mg D-glucurono-\(\gamma\)-lactone /day (14 mg/kg bw/day for a 60 kg person).

If it is assumed that this amount of chronic consumption is relevant to occasional exposure of children of 25 kg body weight, then their exposure (on a body weight basis) would be about 2.5 times higher than that for adults (60-65 kg body weight). It must be emphasized that these estimates relate to chronic exposures by average and high consuming adults, and would not reflect the occasional and sporadic exposure that might occur in children. If the frequency of exposure for children would be 2.5 times lower than for adults, then the average chronic exposure for children, on a body weight basis, would be the same as for adults. If children were to consume the adult intake of 0.5 and 1.4 cans per person only once per week, then the average chronic exposure to “energy” drinks and their constituents for children, on a body weight basis, would be one third of that for adults.

These estimates for the daily exposure to taurine and D-glucurono-\(\gamma\)-lactone from “energy” drinks are higher than the estimated mean daily exposure to taurine from omnivore diets which was estimated to be at most 400 mg/day, and that of D-glucurono-\(\gamma\)-lactone from naturally occurring sources in the diet estimated to amount to 1-2 mg/day.

The SCF Opinion of 2003 used 3 cans/day as a reasonable high (acute) consumption, this amount being higher than the 90th percentile recorded in the Austrian survey (2.6 cans/day) and being the average reported in the Irish survey for the highest number of cans consumed in a single session. The SCF also indicated that it was aware that amounts of up to 8-12 cans/day were reported by a few extreme consumers in both surveys, which would result in an intake of 4800-7200 mg D-glucurono-\(\gamma\)-lactone and 8000-12000 mg taurine per day, equivalent to 80-120 mg D-glucurono-\(\gamma\)-lactone/kg bw/day and 133-200 mg taurine/kg bw/day for a 60 kg person.

The SCF Opinion of 2003 and the recent BfR Opinion mention a number of anecdotal and case reports of acute, adverse effects, including fatalities, in individuals consuming “energy” drinks, containing caffeine, taurine and D-glucurono-\(\gamma\)-lactone. In these cases “energy” drinks had either been consumed in very high amounts (1420 mL), in combination with physical exercise, or more frequently together with alcohol. The Panel considers that it is possible that the effects reported in recent publications by Iyadurai and Chung, Nagajothi et al. and Terlizzi et al. could be due to the well known side effects of high caffeine intake, while the assumption of a causal relationship with taurine intake is lacking scientific evidence.

**Taurine**

Upon oral exposure taurine is readily bioavailable in the systemic circulation. The Panel concludes that new ADME data support the contention that oral exposure to taurine was not
increasing taurine levels in the brain, because in rat studies, brain taurine levels did not increase after dosage.

The SCF already concluded in 1999 that toxicological studies did not reveal any indication for a genotoxic, teratogenic or carcinogenic potential of taurine.

It can be concluded that the NOAEL derived from a new 13-week oral neurotoxicity study in male and female rats including functional observational battery and locomotor activity tests, confirmed the NOAEL established in the prior 13-week study, described already by the SCF in 2003, of 1000 mg taurine/kg bw/day, and provided evidence for a NOAEL of 1500 mg taurine/kg bw/day for behavioural effects. The results of this study were sufficient to address the concerns raised previously, notably the observation of increased activity and possible decrements in motor skills on the rotarod.

The NOAEL of at least 1000 mg/kg bw/day for pathological changes is respectively 120-fold higher than the estimated mean and 43-fold higher than the estimated 95th percentile exposure to taurine from “energy” drinks only, when calculated for a 60 kg person. Given that taurine is a natural body constituent, the Panel concludes that these margins of safety are sufficiently large to conclude that exposure to taurine at the levels mentioned above is not of safety concern.

**D-glucurono-γ-lactone**

The SCF already concluded that the available data indicate that D-glucurono-γ-lactone administered orally to humans is rapidly absorbed, metabolised and excreted as glucaric acid, xylitol and L-xylulose. Animals, such as rodents, which can synthesise vitamin C endogenously do so from glucuronic acid and such animals can also convert exogenously administered D-glucurono-γ-lactone into vitamin C. However, primates, including man, and guinea pigs do not possess this metabolic pathway. The SCF concluded that for this reason, the rodent may be an inappropriate model for man in the study of the effects of D-glucurono-γ-lactone. The Panel concludes that data in the literature indicate that synthesis of vitamin C is only a minor pathway of D-glucurono-γ-lactone metabolism in the rat and of limited relevance to the safety assessment of exogenous D-glucurono-γ-lactone.

In 2003 the SCF evaluated a 13-week oral toxicity rat study with D-glucurono-γ-lactone and concluded that the cause of the kidney lesions remained unclear.

The petitioner has now provided data from a new 13-week oral (gavage versus drinking water) toxicity study of D-glucurono-γ-lactone in rats, with specific focus on the kidneys. This study used the same rat strain as the previous study reported in the SCF Opinion of 2003. Extensive urinalysis and histopathological examinations demonstrated no treatment-related effects. Based on the results of this study, the NOAEL for daily oral administration of D-glucurono-γ-lactone in rats was 1000 mg/kg bw/day, the highest dose tested.

Toxicological studies on the genotoxic, teratogenic or carcinogenic potential of D-glucurono-γ-lactone were not available. However, D-glucurono-γ-lactone is a normal human metabolite formed from glucose and there are no structural alerts for mutagenicity or carcinogenicity. At physiological pH it is in equilibrium with glucoronic acid, its immediate precursor. D-glucurono-γ-lactone and its hydrolysis product glucuronic acid are endogenous metabolites in humans and other mammals, they occur naturally in several dietary sources and are readily metabolized to innocuous products and excreted. Furthermore, in the high dose 13-week rat studies there was no evidence of any effect on the gonads which might indicate the need for reproductive toxicity studies.
The NOAEL for D-glucurono-γ-lactone of 1000 mg/kg bw/day is 200-fold higher than the estimated mean and 71-fold higher than the estimated 95th percentile exposure to D-glucurono-γ-lactone from “energy” drinks only, when calculated for a 60 kg person.

Given the fact that D-glucurono-γ-lactone is a natural body constituent the Panel concludes that these margins of safety are sufficiently large to conclude that exposure to D-glucurono-γ-lactone at the levels mentioned above is not of safety concern.

Overall, the Panel concludes that the exposure to taurine and D-glucurono-γ-lactone at the levels currently used in “energy” drinks and mentioned in the present opinion is not of safety concern.

The ANS Panel agrees with the considerations of the SCF Opinion from 2003 on the fact that it is unlikely that D-glucurono-γ-lactone would have any interaction with caffeine, taurine, alcohol or the effects of exercise. The Panel also concludes, based on the new data available, that additive interactions between taurine and caffeine on diuretic effects are unlikely. Other interactions between taurine and caffeine were not investigated.

**Key words:**

The use of taurine and D-glucurono-γ-lactone as constituents of the so-called “energy” drinks

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BACKGROUND AS PROVIDED BY THE COMMISSION

On 21 January 1999 the Scientific Committee on Food (SCF) expressed an opinion on caffeine, taurine and D-glucurono-γ-lactone as constituents of the so-called “energy” drinks. For taurine and D-glucurono-γ-lactone, the SCF was unable to conclude that the safety-in-use of taurine and D-glucurono-γ-lactone in the concentration ranges reported for these constituents in “energy” drinks had been adequately established. It indicated that further studies would be required to establish upper safe levels for daily intake of taurine and D-glucurono-γ-lactone.

In 2002, following the submission of new information by a manufacturer of “energy” drinks and the publication of reports and statements on the issue by the Australian New Zealand Food Safety Authority and the UK Food Standard Agency, the SCF was asked by the Commission to indicate if the conclusions of its opinion of 1999 needed to be revised. On 5 March 2003, the SCF expressed an opinion on additional information on “energy” drinks.

Concerning taurine, the SCF indicated that the new 13-week study in rats provided further useful information and that it showed no significant changes in pathological measures, but it did show the occurrence of significant behavioural effects (increased activity and self-chewing), and possibly impaired motor performance, which could be mediated via a pharmacological action on the central nervous system. In view of this, the SCF was of the opinion that focused neurological studies were needed. The SCF concluded that these effects should be taken into account in human risk assessment, noting that behavioural effects were observed at the lowest dose tested of 300 mg/kg bw/day. This effect level is 36-fold above the estimated human intake of taurine (8.3 mg/kg bw/day for a 60 kg adult) at the mean chronic daily intake for “energy” drinks, and 6-fold above the more relevant estimate for acute intake (50 mg/kg bw/day for a 60 kg adult). The absence of a NOAEL for these effects precludes the setting of an upper safe level for daily intake of taurine. The SCF’s reservations were expressed in the context of an estimated acute intake of taurine up to 3000 mg/day from consumption of “energy” drinks, compared with the highest estimated intake of taurine from naturally occurring sources in the diet of 400 mg/day.

Concerning D-glucurono-γ-lactone, the new 13-week study provided useful information indicating that in rats there were no adverse effects except on the kidney. The NOAEL for these effects was 300 mg/kg bw/day, which is around 20-fold above the estimate of high chronic intake of D-glucurono-γ-lactone of 14 mg/kg bw/day for a 60 kg adult. The hamster study and the new 13-week rat study both provided information showing no effects on body weight gain in growing animals. However, the 1999 Opinion also pointed out that rodents may not be an appropriate model for man since they can metabolise exogenous glucuronolactone to vitamin C whereas primates including man do not possess this metabolic pathway. The SCF therefore reiterated its earlier conclusion (SCF, 1999) that there is lack of evidence to support the safety of D-glucurono-γ-lactone present in beverages at concentrations that may result in intakes several-fold higher than that usually obtained from the rest of the diet. Due to the lack of relevant data, it was not possible to set an upper safe level for daily intake of D-glucurono-γ-lactone. The SCF’s reservations were expressed in the context of an estimated high chronic intake of D-glucurono-γ-lactone of 840 mg/day and an acute intake of up to 1800 mg/day from consumption of “energy” drinks, compared with the estimated intake of D-glucurono-γ-lactone from naturally occurring sources in the diet of 1-2 mg/day.
Following these opinions, and taking into account the remarks made by the SCF, a manufacturer of “energy” drinks has submitted new data on the safety-in-use of taurine and D-glucurono-\(\gamma\)-lactone as constituents of the so-called “energy” drinks.

**TERMS OF REFERENCE AS PROVIDED BY THE COMMISSION**

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to:

- review the data submitted on the safety-in-use of taurine and D-glucurono-\(\gamma\)-lactone as constituents of the so-called “energy” drinks
- consider, if appropriate, to provide a scientific opinion on the safety-in-use of taurine and D-glucurono-\(\gamma\)-lactone as constituents of the so-called “energy” drinks.

**ACKNOWLEDGEMENTS**

The use of taurine and D-glucurono-\(\gamma\)-lactone as constituents of the so-called “energy” drinks

ASSESSMENT

In the present opinion the Panel evaluates the safety of taurine and D-glucurono-\(\gamma\)-lactone as individual ingredients of the so-called “energy” drinks based on the new toxicological studies provided by the petitioner. The Panel does not evaluate the safety of “energy” drinks as such.

1. Technical data

1.1. Chemistry

Taurine

Taurine (CAS No. 107-35-7) occurs naturally in food, especially in seafood and meat, and it is a normal metabolite in humans. It is a metabolic product of sulphur-containing amino acids, and it is mainly biosynthesised from cysteine in the liver (SCF, 1999). Its molecular weight is 125.15 g/mol, the molecular formula is \(\text{C}_2\text{H}_7\text{NO}_3\text{S}\) and the structural formula is as shown:

\[
\text{Taurine}
\]

\[
\text{O} \quad \text{S} \quad \text{NH}_2 \\
\text{HO} \quad \text{SO} \quad \text{O}
\]

D-glucurono-\(\gamma\)-lactone

D-glucurono-\(\gamma\)-lactone (CAS No. 32449-92-6) is a normal human metabolite formed from glucose. At physiological pH it is in equilibrium with glucuronic acid, its immediate precursor (see figure below). Glucuronic acid occurs in plants, mainly in gums, but as it is in polymeric combination with other carbohydrates it is not readily available. Glucuronic acid is also an important constituent of fibrous and connective tissues in all animals (SCF, 1999). The molecular weight of D-glucurono-\(\gamma\)-lactone is 176.12 g/mol, the molecular formula is \(\text{C}_6\text{H}_8\text{O}_6\) and the structural formula is as shown:

\[
\text{D-glucurono-\(\gamma\)-lactone}
\]

\[
\text{O} \quad \text{O} \\
\text{O} \quad \text{O} \\
\text{O} \quad \text{OH} \\
\text{HOOC} \quad \text{OH} \quad \text{OH} \\
\text{HO} \quad \text{OH} \quad \text{OH}
\]

\[
\text{D-glucuronic acid}
\]

\[
\text{HO} \quad \text{OH} \quad \text{OH} \\
\text{HOOC} \quad \text{OH} \quad \text{OH}
\]
1.2. Manufacturing Process

The petitioner provided adequate information on the production process of taurine, retrieved from supplier information describing the general principle of the process. Taurine can be made from monoethanolamine and sulphuric acid as the starting materials or from ethylene oxide and sodium hydrogen sulphate as the starting materials. The crude production is followed by purification steps.

The petitioner provided adequate information on the production process of D-glucurono-\(\gamma\)-lactone, retrieved from supplier information describing the general principle of the process. D-glucurono-\(\gamma\)-lactone is made from nitric acid and starch as the starting materials. The crude production is followed by purification steps.

1.3. Specifications

The petitioner indicated that taurine used in “energy” drinks complies with US pharmacopoeia specifications (US Pharmacopeia, 2005). Purity is not less than 98.5 %.

The petitioner provided the following specifications for D-glucurono-\(\gamma\)-lactone and indicated that the methods for determining these specifications meet the requirements of several pharmacopoeias or regulations describing testing methods. D-glucurono-\(\gamma\)-lactone is a white crystalline powder and its purity is not less than 98.5 %. Impurities identified by HPLC include glucuronic acid <0.19%, other identified constituents each at <0.05 % and in total <0.15% and unknown constituents each at <0.05 % in total <0.15 %. Melting range is 170-176 °C, its appearance in solution is clear and colourless, the acidity of a 10% solution in water is 3.7-4.1, the loss on drying is <0.2%, heavy metals are present at <0.001% (10 mg/kg), arsenic at <2 mg/kg, iron at <2 mg/kg, copper at <2 mg/kg, chloride at <100 mg/kg, ammonia at <200 mg/kg and sulphate at <100 mg/kg. Specifications for microbial purity were also provided by the petitioner.

1.4. Methods of analysis in foods

The petitioner indicated that taurine can be determined in “energy” drinks after derivatization with dabsylchloride by HLPC with UV detection, and that D-glucurono-\(\gamma\)-lactone can be quantified in “energy” drinks by HPLC without derivatization.

1.5. Reaction and fate in foods, stability

The petitioner indicates that samples of “energy” drinks were stored at 20 °C for 24 months and tested at defined time-points for the levels of taurine and D-glucurono-\(\gamma\)-lactone. Based on the results obtained it could be concluded that taurine and D-glucurono-\(\gamma\)-lactone in the “energy” drink were stable during these 24 months of storage at 20 °C.

1.6. Case of need and use levels

According to the petitioner taurine and D-glucurono-\(\gamma\)-lactone are to be used as constituents of so-called “energy” drinks together with caffeine.
A submission from the Austrian National Food Authority (1996) included a list of the content of 32 “energy” drinks taken from a published review of drinks on the Austrian market. Some “energy” drinks did not contain taurine. In those drinks in which taurine was present and its concentration declared, one contained 300 mg/L, one 2000 mg/L and 11 contained 4000 mg/L. For the exposure estimates done in 2003 the SCF assumed that the “energy” drinks contained maximum levels of 4000 mg/L taurine and 2400 mg/L of D-glucurono-γ-lactone (SCF, 2003).

1.7. Exposure

No new data on exposure were available to the Panel.

In 2003 the SCF established a mean chronic consumption of 0.5 cans per person per day (250 mL per can) (SCF, 2003). High chronic exposure was estimated by the SCF to be 1.4 cans per person per day. This figure was based on the 95th percentile exposure of regular users, a group which represents 12% of the total population.

Based on the assumption that a can contains 250 mL and 4000 mg/L taurine and 2400 mg/L D-glucurono-γ-lactone the SCF calculated that these values result in a mean daily exposure to 500 mg taurine (8.3 mg taurine/kg bw/day for a 60 kg person) and 300 mg D-glucurono-γ-lactone (5.0 mg D-glucurono-γ-lactone/kg bw/day for a 60 kg person). The 95th percentile exposure to regular users would amount to 1400 mg taurine/day (23.3 mg/kg bw/day for a 60 kg person) and 840 mg D-glucurono-γ-lactone/day (14 mg/kg bw/day for a 60 kg person).

The mean daily exposure to taurine from omnivore diets was determined to be around 58 mg (range from 9 to 372 mg) and to be low or negligible from a strict vegetarian diet (Rana and Sanders, 1986). In another study, taurine exposure was estimated to be generally less than 200 mg/day, even in individuals eating a high meat diet (Laidlaw et al., 1990). And in another study, taurine consumption was estimated to vary between 40 and 400 mg/day (Hayes and Trautwein, 1994).

The SCF Opinion mentioned an estimated exposure to D-glucurono-γ-lactone from naturally occurring sources in the diet of 1-2 mg/day (SCF, 2003). The SCF also concluded that “Human metabolic considerations indicate the body is likely to handle small quantities of glucuronolactone without any problems. However, the exposure to glucuronolactone resulting from consumption of “energy” drinks is possibly as much as two orders of magnitude greater than that from the rest of the diet.” The mean exposure to D-glucurono-γ-lactone would be 300 mg/day, and at the 95th percentile exposure would be 840 mg/day.

The chronic exposures by average and high adult consumers have been estimated by the SCF (2003) to be 0.5 and 1.4 cans per person per day. The petitioner indicates that if it was assumed that this level of chronic consumption is relevant to occasional exposure of children of 25 kg body weight (about 6 to 7 years of age), then their exposure (on a body weight basis) would be about 2.5 times higher than that of adults (60-65 kg body weight). The petitioner emphasizes that these estimates relate to chronic exposures by average and high consuming adults, and would not reflect the occasional and sporadic exposure that might occur in children. If the frequency of exposure of children would be 2.5 times lower than in adults, then the average chronic exposure of children, on a body weight basis, would be the same as for adults. If children were to consume the adult intake of 0.5 and 1.4 cans per person only once per week, then the average chronic exposure of children, on a body weight basis, would be one third of that in adults.
The SCF Opinion (2003) used 3 cans/day as a reasonable high (acute) consumption, this amount being higher than the 90th percentile recorded in the Austrian survey (2.6 cans/day) and being the average reported in the Irish survey for the most number of cans consumed in a single session. The SCF also indicated that it was aware that amounts up to 8-12 cans/day, were reported by a few extreme consumers in both surveys, which would result in an intake of 4800-7200 mg D-glucurono-γ-lactone and 8000-12000 mg of taurine per day, equivalent to 80 - 120 mg D-glucurono-γ-lactone/kg bw/day and 133-200 mg taurine/kg bw/day.

1.8. Existing authorisations and evaluations

In 1999, the SCF adopted an opinion on so-called “energy” drinks, which evaluated the safety of caffeine, taurine and D-glucurono-γ-lactone as constituents of “energy” drinks (SCF, 1999). The SCF (1999) concluded that toxicological studies did not reveal any indication for a genotoxic, teratogenic or carcinogenic potential of taurine.

At that time, the SCF was unable to conclude that the safety-in-use of taurine and glucuronolactone in the concentration ranges reported for "energy" drinks had been adequately established. The SCF commented that “there is insufficient information on which to set an upper safe level for daily intake of these constituents.”

In 2001, the Agence Française de Sécurité Sanitaire des Aliments (AFSSA), reviewed a 13-week mouse oral toxicity study on “energy” drinks, concluding that authorisation of the use of various substances in “energy” drinks was not acceptable since harmlessness at the concentrations recommended by the petitioner had not been demonstrated (AFSSA, 2001). The AFSSA reiterated the same conclusions in three subsequent opinions (AFSSA, 2003, 2006a, 2006b).

In 2003 the SCF was asked to review additional information submitted on “energy” drinks and indicate if the conclusions in its opinion of 21 January 1999 needed to be modified. The SCF was also asked by the petitioner to take into account published reports and statements from the Australian New Zealand Food Authority (ANZFA, 2000) and the UK Food Standards Agency (FSA, 2001; 2002).

The SCF (2003) concluded the following on taurine:

“The new 13-week study in rats provided further useful information in that it showed no significant changes in pathological measures, but it did show the occurrence of significant behavioural effects (increased activity and self-chewing), and possibly impaired motor performance, which could be mediated via a pharmacological action on the central nervous system. In view of this, the Committee is of the opinion that focused neurological studies are now needed and that the effects reported in a 13-week study should be taken into account in human risk assessment, noting that behavioural effects were observed at the lowest dose tested of 300 mg/kg bw/day. This effect level is 36-fold above the estimated human intake of taurine (8.3 mg/kg bw/d for a 60 kg adult) at the mean chronic daily intake for “energy” drinks, and 6-fold above the more relevant estimate for acute intake (50 mg/kg bw/d for a 60 kg adult). The absence of a NOAEL for these effects precludes the setting of an upper safe level for daily intake of taurine. The Committee’s reservations are expressed in the context of an estimated acute intake of taurine of up to 3000 mg/day from consumption of “energy” drinks, compared with the highest estimated intake of taurine from naturally occurring sources in the diet of 400 mg/day.”
The SCF (2003) concluded the following on glucurono-\(\gamma\)-lactone:

“The new 13-week study provided useful information indicating that in rats, there were no adverse effects except on the kidney. The NOAEL for these effects was 300 mg/kg bw/d, which is around 20-fold above the estimate of high chronic intake of glucuronolactone of 14 mg/kg bw/d for a 60 kg adult. The hamster study and the new 13-week rat study both provided information showing no effects on body weight gain in growing animals. However, the 1999 Opinion also pointed out that rodents may not be an appropriate model for man since they can metabolise exogenous glucuronolactone to vitamin C whereas primates, including man, do not possess this metabolic pathway.

The Committee therefore reiterates its earlier conclusion (SCF, 1999) that there is a lack of scientific evidence to support the safety of glucuronolactone present in beverages at concentrations that may result in intakes several-fold higher than that usually obtained from the rest of the diet. Due to the lack of relevant data it is not possible to set an upper safe level for daily intake of glucuronolactone. The Committee’s reservations are expressed in the context of an estimated high chronic intake of glucuronolactone of 840 mg/day and an acute intake of up to 1800 mg/day from consumption of “energy” drinks, compared with the estimated intake of glucuronolactone from naturally occurring sources in the diet of 1-2 mg/day.”

In 2005, a statement was expressed by the EFSA Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on studies designed to investigate the safety-in-use of taurine and D-glucurono-\(\gamma\)-lactone in "energy" drinks (EFSA, 2005).

2. Biological and toxicological data

Previous SCF Opinions have summarized safety studies of taurine and D-glucurono-\(\gamma\)-lactone (SCF, 1999; SCF, 2003) and a previous EFSA Statement from the AFC Panel commented on studies designed to further investigate the safety-in-use of taurine and D-glucurono-\(\gamma\)-lactone in “energy” drinks (EFSA, 2005).

The present opinion only describes in detail those studies that were submitted after the publication of the SCF Opinion in 2003 and the EFSA Statement in 2005.

2.1. Absorption, distribution, metabolism and excretion

Taurine

The SCF Opinion already concluded that new toxicokinetic data submitted at that time on taurine in rats showing ready bioavailability and peak plasma levels one hour after oral administration are in accordance with findings from the limited published data for humans (SCF, 2003). Human studies showed significant increases in plasma taurine 90 minutes after consumption of a taurine-rich meal with levels declining to background within 180-270 minutes (Trautwein and Hayes, 1995). The SCF indicates that these results also corroborate those from an unpublished human study using radiolabelled taurine, which showed peak serum levels at 1-2 hours after oral administration, declining by 7 hours (SCF, 2003). Other
human data suggest that taurine is absorbed orally via an active transport mechanism in the gut wall (Ahlman et al., 1993; 1995a, b).

Results from a new study on absorption, tissue distribution, metabolism and elimination of taurine given orally to rats were provided by the petitioner. In this study (Sved et al., 2007) three biodisposition studies with taurine were performed in male and female adult rats at dosages of 30 and 300 mg/kg bw. A single dose of $^{14}$C taurine was rapidly absorbed, distributed to tissues and excreted unchanged in the urine. Elimination of radioactivity from intracellular pools was slow. Pre-treatment of animals for 14 days with unlabelled taurine did not significantly affect the fate of $^{14}$C taurine. Daily administration of unlabelled taurine for 14 days did not result in an increase in total taurine in the brain. It was concluded that the data indicated that exogenous taurine rapidly equilibrates with endogenous body pools and that any excess is rapidly eliminated by the kidneys.

Based on these data which revealed that brain taurine levels did not increase after dosing, the petitioner concluded that the possibility that taurine may exhibit acute, central pharmacological effects mediated by an action on the central nervous system was scientifically improbable.

**D-glucurono-γ-lactone**

The SCF (1999) already concluded that the available data indicate that when D-glucurono-γ-lactone is administered orally to humans it is rapidly absorbed, metabolised and excreted as glucaric acid, xylitol and L-xylulose.

The SCF Opinion of 2003 stated that the toxicokinetic data on D-glucurono-γ-lactone in rats, showing bioavailability and lack of accumulation, with peak plasma levels 1-2 hours after oral administration, were in accordance with findings from the limited published data on humans (SCF, 2003).

Animals, such as rodents, which can synthesise vitamin C endogenously do so from glucuronic acid and such animals can also convert exogenously administered D-glucurono-γ-lactone into vitamin C (SCF, 1999). However, primates, including man, and guinea pigs do not possess this metabolic pathway. The SCF concluded that for this reason, the rodent may be an inappropriate model for man in the study of the effects of D-glucurono-γ-lactone.

In 2003, the SCF reiterated the 1999 Opinion that rodents may not be an appropriate model for humans since they can metabolise exogenous D-glucurono-γ-lactone to vitamin C whereas guinea pigs and primates, including humans, do not possess this metabolic pathway. However, the petitioner indicates that synthesis of vitamin C is only a minor pathway of D-glucurono-γ-lactone metabolism in the rat and argues that, although of nutritional importance, it is of limited relevance to the safety assessment of exogenous D-glucurono-γ-lactone. This is supported by more recent literature data demonstrating that D-glucurono-γ-lactone is predominantly metabolised in rats via the pentose pathway and that the flux through the pathway that results in synthesis of ascorbic acid from D-glucurono-γ-lactone is relatively small (Kondo et al., 2006; Linster and Van Schaftingen, 2007).
2.2. Toxicological data

2.2.1. Acute oral toxicity

In an acute toxicity study of taurine in Wistar rats, a 50% taurine suspension in 1.0% carboxymethylcellulose (CMC) solution was administered in a volume of 10 mL/kg bw. No dead animals were observed during the observation period of 14 days, and the authors indicate that this suggests that the LD$_{50}$ value of taurine is higher than 7000 mg/kg bw upon oral administration (Kihara et al., 1991).

2.2.2. Short-term and sub-chronic toxicity

**Taurine**

In 2003 the SCF evaluated a newly submitted 13-week rat study with taurine at dose levels of 0, 300, 600 and 1000 mg/kg bw/day which showed no significant changes in pathological measures, but did show the occurrence of significant behavioural effects (increased activity and self-injury such as self-chewing), and possibly impaired motor performance, which could have been mediated via a pharmacological action on the central nervous system. In view of this, the SCF was of the opinion that focused neurological studies were needed. The absence of a NOAEL for these effects precluded the setting of an upper safe level for daily exposure to taurine.

The petitioner argued that there had been bias in the original study observations and the EFSA Working Group (EFSA, 2005) agreed that the observations reported in this study on certain behavioural patterns of the animals had not been well described in the original submission and could be discounted since there was no evidence of self-injury. However, the EFSA Working Group also concluded that, even combined with the expert analyses provided, this information was insufficient in itself to address all the concerns raised previously, notably the observation on increased activity and possible decrements in motor skills on the rotarod.

Therefore, the petitioner has now provided data from a specifically-designed, new 13-week oral (gavage and drinking water) neurotoxicity study of taurine in male and female rats which was performed according to FDA and OECD principles of Good Laboratory Practice. The objective of this study was to evaluate any potential neurotoxic effects of taurine when administered to rats for 13 weeks either by gavage or by way of drinking water, and to address the reliability of observations noted in the previous 13-week taurine toxicity study. Beginning in the second week of the acclimatization period, all animals (180 males and 180 females) were tested twice in the functional observational battery (FOB) and locomotor activity paradigms. After initial evaluations, outliers in locomotor activity were eliminated from the study. The remaining animals were randomized based on their performance on the rotarod test. Finally, the mean and standard deviation of the locomotor activity results were analyzed to ensure that group means and variances were approximately equal before initiation of dosing, thereby minimizing subsequent skewing of these data. Potential functional deficits were assessed using a FOB and a measure of spontaneous locomotor activity. This study was conducted in a “blinded” manner, in which the actual dose level for each group (gavage and drinking water) were unknown to the personnel conducting the study, in order to remove human bias from all aspects of the study.
Taurine in the vehicle, deionized water, was administered orally by gavage once daily for 13 weeks to 2 groups of 20 male and 20 female Cr1:CD(SD) rats at dose levels of 600 and 1000 mg/kg bw/day. In addition taurine was administered ad libitum in drinking water for 13 weeks to 2 groups of 20 male and 20 female Cr1:CD(SD) rats at target dose levels of 1000 and 1500 mg/kg bw/day (actual mean taurine intake levels obtained with drinking water were 1095 and 1117 mg/kg bw/day for the males and females respectively in the low dose group and 1647 and 1656 mg/kg bw/day for the males and females respectively in the high dose group). Concurrent control groups received the vehicle by gavage and drinking water respectively, on comparable regimes. Clinical examinations were performed daily and detailed physical examinations were performed weekly. These examinations were conducted “blinded” with respect to treatment. Individual body weights and water consumption were recorded twice weekly and food consumption was recorded weekly. Functional observational battery and locomotor activity data were recorded for all animals prior to the initiation of dose administration and during study weeks 0, 6 and 12. Complete necropsies were conducted on all animals, and selected tissues and organs were collected at the scheduled necropsy.

The results indicated that there were no test-article-related deaths, clinical findings or macroscopic findings. No test-article-related effects were observed on body weights or food consumption. Some differences were observed in water consumption when rats were supplied taurine ad libitum in the drinking water. Increases in water consumption in the 1000 and 1500 mg/kg bw/day group males were noted only for study days 0 to 3 and/or 3 to 7 (both in g/animal/day and g/kg bw/day). The petitioner indicates that these differences were considered test-article-related, but not considered adverse effects and that they occurred temporarily and were considered to reflect adaptation to the osmotic property of the test article.

There were no test-article-related effects on FOB parameters (home cage, handling, open field, sensory, neuromuscular and physiological observations). Locomotor activity counts (total and ambulatory) and patterns were unaffected by test article administration.

Based on these results the petitioner concluded that the oral administration of taurine at dose levels of 600 and 1000 mg/kg bw/day was well tolerated by male and female rats and did not result in any behavioural changes. The Panel concluded that this study confirmed the NOAEL derived from the earlier study which included histopathology (1000 mg/kg bw/day – the highest dose tested). In addition, it provided evidence of a NOAEL of 1500 mg/kg bw/day (actual level approximately 1650 mg/kg bw/day) for behavioural effects.

D-glucurono-γ-lactone

For D-glucurono-γ-lactone the SCF concluded that the 13-week study in Cr1:CD(SD) rats showed that there were no significant, treatment-related effects, “apart from vacuolisation and inflammatory changes localised to the papilla of the kidney in females at 600 and 1000 mg/kg bw/day, with a NOAEL of 300 mg/kg bw/day” (SCF, 2003).

The study reported cytoplasmic vacuolation in 6/20 and 4/20 males in the control and 1000 mg/kg bw/day groups respectively and in 11/20, 9/20, 11/20 and 11/20 females in the control, 300, 600 and 1000 mg/kg bw/day groups respectively. The incidence was not increased by treatment. The lesions were described as mild (grade 2) rather than minimal (grade 1) in 1/20 and 0/20 males in the control and 1000 mg/kg bw/day groups and in 1/20, 1/20, 5/20 and 8/20 females in the control, 300, 600 and 1000 mg/kg bw/day groups respectively. Therefore the data indicated that there was a slight dose-related increase in severity in the treated females in comparison to the treated males. However the petitioner noted that for all rats used in the
The use of taurine and D-glucuronono-γ-lactone as constituents of the so-called “energy” drinks

study, a range of other effects in the kidneys were reported, such as inflammatory changes, nephropathy, pyelitis, indicative of renal problems.

The petitioner argued that the occurrence of the renal observations were incidental, related to background lesions usually occurring in this rat strain. The SCF concluded that the cause of the kidney lesions remained unclear. The petitioner also indicated that the occurrence of the lesions only in females may be related to the higher acidity and osmolality of urine in the female rat and went on to comment that the osmolality of human urine is considered less than of the Sprague-Dawley rat. However, the SCF stated that in their view the mechanistic cause of the kidney lesions remains unclear (SCF, 2003).

In a statement (EFSA, 2005) the Working Group on Additives of the AFC Panel noted that the guinea pig might be a better model for human, in terms of its metabolism of D-glucuronono-γ-lactone. But they expressed considerable reservations about a study on D-glucuronono-γ-lactone in guinea pigs as proposed by the petitioner. The Working Group particularly noted that the proposed study in guinea pigs with D-glucuronono-γ-lactone in drinking water could be difficult to perform (e.g. mortality) and interpret (e.g. lack of extensive background data on this species), and that in view of these potential difficulties, consideration should be given to whether this study could be justified on animal welfare grounds.

The Working Group suggested that a more productive strategy might be to undertake mechanistic studies to support the hypothesis put forward by the petitioner that the rat kidney effects were not relevant for human risk assessment.

The petitioner has now provided data from a new 13-week oral (gavage versus drinking water) toxicity study of D-glucuronono-γ-lactone in the Crl:CD(SD) rat strain, with specific focus on the kidneys. This study used the same rat strain as the previous study reported in the SCF Opinion of 2003 and was performed according to FDA and OECD principles of Good Laboratory Practice. In this new study, D-glucuronono-γ-lactone was administered orally by gavage once daily for 13 consecutive weeks to 4 groups of Crl:CD(SD) rats at dose levels of 0, 300, 600 and 1000 mg/kg bw/day. In addition D-glucuronono-γ-lactone was administered ad libitum in drinking water for 13 weeks to another 4 groups of Crl:CD(SD) rats at target dose levels of 0, 300, 600 and 1000 mg/kg bw/day. Each group consisted of 20 males and 20 females. Actual mean D-glucuronono-γ-lactone intake levels obtained in the drinking water groups were 311 and 322 mg/kg bw/day for the males and females respectively in the low dose group, 598 and 635 mg/kg bw/day for the males and females respectively in the mid dose group and 980 and 1066 mg/kg bw/day for the males and females respectively in the high dose group. Concurrent control groups of 20 males and 20 females received the vehicle by gavage and drinking water respectively on comparable regimens.

Clinical examinations were performed daily, and detailed physical examinations were performed weekly. Individual body weights and water consumption were recorded twice weekly. Serum chemistry evaluations were performed on all animals prior to the initiation of dose administration (study week 2), during study weeks 4 and 8, and at the scheduled necropsy (study week 13) and at time-points during 0 to 6 hours and 6 to 24 hours after dose administration from the gavage groups during study weeks 4, 8 and 13. Urine samples were collected from the drinking water groups on the same schedule (same time of day). Complete necropsies were conducted on all animals, and selected organs were weighed at the scheduled necropsy. Selected tissues were examined microscopically from all animals. Results revealed no test article-related deaths. There were no effects on clinical observations, food or water consumption, body weights, clinical pathology parameters, organ weights or clinical chemistry parameters representing renal function. Extensive urinalysis demonstrated no
The use of taurine and D-glucurono-γ-lactone as constituents of the so-called “energy” drinks

treatment related effects, and no differences between gavage and drinking water groups. There were no test article-related macroscopic or microscopic findings.

Histopathological examinations revealed focal inflammation in the kidneys in a few male and female animals, scattered among the groups, including controls. The petitioner indicates that inflammation was observed in only a small number of animals at each dose level, that it was unilateral and not treatment-related and that these background lesions are typical for this strain of rats.

There were no compound-related observations of vacuolization of the cells lining the collecting tubules. The petitioner also indicated that a greater number of rats in this new study had healthy kidneys in comparison to the first study. There were no differences between the gavage and drinking water groups. There was no significant incidence of cytoplasmic vacuolization in any groups. The petitioner also indicated that in light of the difference between the two studies the slides have been carefully reassessed, and that cytoplasmic vacuolization has been confirmed not to be present. The petitioner also stated that the pathologist who undertook the histopathological examination has indicated that the effect in the previous study was most likely a preparation artifact which was exacerbated by the generally poor health status of the kidneys in the rats at that time. Vacuolisation of renal collecting tubules may arise as an artifact using normal fixation techniques.

The petitioner concluded that based on the results of this and the previous study, the NOAEL for daily oral administration of D-glucurono-γ-lactone to rats was 1000 mg/kg bw/day, the highest dose tested in both studies. The Panel agrees with this NOAEL derived from the recent 13-week rat study which was performed under GLP.

2.2.3. Reproductive and developmental toxicity

The SCF already concluded in 1999 that toxicological studies did not reveal any indication for a teratogenic potential of taurine (SCF, 1999).

Studies on reproductive and developmental toxicity for D-glucurono-γ-lactone were not available. However the petitioner indicates that this substance and its hydrolysis product glucuronic acid are endogenous metabolites in humans and other mammals, that they occur naturally in several dietary sources and are readily metabolized to innocuous products and excreted. Furthermore there were no effects on the gonads in the 13-week rat studies. Therefore the Panel concluded that there was no need for reproductive toxicity studies.

There are no new studies available.

2.2.4. Mutagenicity

The SCF already concluded that toxicological studies did not reveal any indication for a genotoxic potential of taurine (SCF, 1999).

In a study on the antimutagenic activity of lactones in Escherichia coli, D-glucurono-γ-lactone was reported to be not mutagenic to E. coli strains WP2 and WPs (Kuroda et al., 1986).

There are no new studies available.
2.2.5. Carcinogenicity and long-term studies

The SCF already concluded that toxicological studies did not reveal any indication for a carcinogenic potential of taurine (SCF, 1999). But the SCF also indicated that there is no adequate chronic toxicity/carcinogenicity study for taurine.

Long term studies on D-glucurono-γ-lactone were not available. However this substance and its hydrolysis product glucuronic acid are endogenous metabolites in humans and other mammals, they occur naturally in several dietary sources and are readily metabolized to innocuous products and excreted. Furthermore, there was no evidence of any putative preneoplastic or hyperplastic lesions in the 13-week rat studies, which might indicate the need for a long term carcinogenicity study.

2.2.6. Human data

Available human data from the use of taurine in human medicine do not give any indication of safety concerns (Franconi et al., 1995; Takahashi and Nakane, 1978; Fukuyama and Ochiai, 1982; Airaksinen et al., 1980; Mantovani and DeVibo, 1979; Marchesi et al., 1975; Mutani et al., 1975; Azuma et al., 1983a; 1983b; 1985; 1992; 1994; Fujita et al., 1987; Yamori et al., 1996; Kroll and Lund, 1966; Yamamoto et al., 1994; Gentile et al., 1994; Matsuyama et al., 1983; Podd et al., 1990; Kimura et al., 1992; Obinata et al., 1996; Durelli et al., 1982; 1983; Nyland et al., 1989; Kopple et al., 1990; Thompson 1988; Darling et al., 1985; Carrasco et al., 1990; Belli et al., 1987; Colombo et al., 1988; Smith et al., 1991; De Curtis et al., 1992; Skopnik et al., 1991; Colombo et al., 1990).

In these separate studies taurine has been administered, mostly by oral ingestion on a daily basis for periods up to one year, and with daily doses generally in the 3-6 g range, to a large number of patients (adults, children and even infants) suffering from a wide variety of serious diseases. Taurine has also been administered parenterally at a daily dose of 0.64 g for 20 months or by intravenous administration at daily doses of 12 g for 15 days and 18 g for 60 days. Although the principal aim of these clinical studies was not to evaluate potential adverse effects of chronic administration of taurine it is apparent that these doses produced no adverse health effects. Such information has revealed that oral daily ingestion of taurine doses in the 3-6 g range for periods up to one year, did not produce adverse health effects.

The SCF Opinion of 2003 refers to a number of anecdotal reports of acute, adverse effects in young persons consuming “energy” drinks, containing caffeine, taurine and D-glucurono-γ-lactone, usually together with alcohol and/or ‘social drugs’, such as ecstasy and amphetamines. The adverse effects reported included tremors, seizures, drowsiness, muscle weakness, dizziness, nervousness, tachycardia, palpitations, nausea, vomiting, headache, bronchospasm, hyperventilation and also myocardial infarction and sudden unexplained death possibly resulting from cardiac dysrhythmia (SCF, 2003). The SCF already concluded the following: “The co-consumption of alcohol and/or drugs noted in most of these cases makes interpretation of effects due to the “energy” drinks particularly difficult. Thus there is no confirmation of any causal relationship between the reported effects and the consumption of “energy” drinks. Under these circumstances, the reports can only be noted”.

New human data on the assessment of “energy” drinks have been compiled in a recent BfR Opinion (BfR, 2008) reporting recent Swedish and American studies (Lehtihet et al., 2006; Wiklund et al., 2004; Steinke et al., 2007; American Heart Association; 2007; Iyadurai and Chung; 2007). In a Swedish publication possible adverse reactions of “energy” drinks
The use of taurine and D-glucurono-γ-lactone as constituents of the so-called “energy” drinks

including three cases of death are discussed, focussing on a potential contributing role of taurine associated with its known effects e.g. in osmoregulation and on the cardiovascular system. The three fatalities occurred after “energy” drinks had been consumed in combination with alcohol, whereby the forensic examinations including autopsy yielded negative results concerning medicaments and drugs, values between 0.59 and 0.87 parts per thousand of ethanol in blood samples, but no clear causes of death. In a further case, severe adverse effects arose after consumption of an “energy” drink in combination with physical efforts: A 31-year-old regularly trained man consumed 750 mL of an “energy” drink while taking part in a 3,000 m competition. He developed a poor general condition with a rhabdomyolysis and acute kidney failure with tubular necrosis diagnosed one week after the competition (Lehtihet et al., 2006).

Two new cases of “energy” drink-related tachycardias, in one individual associated with orthostatic intolerance, were reported by other authors (Nagajothi et al., 2008; Terlizzi et al., 2008). In the Terlizzi study, consumption was reported to amount to 4 to 5 cans of “energy drinks” a day.

In addition, cases of four patients who suffered generalised cerebral seizures after consuming a high dose of “energy” drinks, without there being any reports of parallel alcohol consumption were reported (Iyadurai and Chung, 2007).

Overall, the results also raised the issue of combination effects and possible interactions between, amongst others, taurine and alcohol, between taurine and caffeine and between taurine and D-glucurono-γ-lactone.

The SCF Opinion already evaluated the possibility of interactions between taurine, caffeine and D-glucurono-γ-lactone and considered it unlikely that D-glucurono-γ-lactone would have any interaction with caffeine and taurine. The SCF concluded (2003) “that consideration of the potential for interactions between caffeine and taurine has not ruled out the possibility of stimulatory effects from both substances at the levels of the central nervous system”.

The SCF also noted that “since caffeine and taurine act via different mechanisms, any diuretic effects could be additive” and that “Both taurine (Gentile et al., 1994) and alcohol centrally inhibit the release of the antidiuretic hormone, vasopressin and the Committee considered that they could act additively to increase water and sodium loss from the body in the short-term”.

New data have recently been published (Riesenhuber et al., 2006) describing results from a study investigating the possible additive diuretic effects of caffeine and taurine in a cross-over design in which 12 healthy male volunteers each received four different test drinks (750 mL of “energy” drink containing 240 mg caffeine and 3 g taurine, the three other test drinks that lacked caffeine, taurine or both). Effects on urinary output, urinary osmolarity and natriuresis were compared by mixed model analyses. Urinary output and natriuresis increased significantly with caffeine alone and in the caffeine-taurine group. This study demonstrated that the diuretic potential and natriuretic effects of the tested “energy” drinks were largely mediated by caffeine and that there were no additive interactions between taurine and caffeine. The petitioner concluded that this study does not support the possibility of interactions between taurine and caffeine.

To investigate possible cardiovascular effects of the combined exposure to caffeine and taurine with “energy” drinks an orientational study was conducted in healthy volunteers with low blood pressure (8 women, 7 men, average age of 26 years) in a state of physical rest (Steinke et al., 2007; American Heart Association, 2007). The test persons had abstained from caffeine for 48 h before the start of the study and throughout the study period. After an initial examination during which blood pressure and heart rate were measured and an ECG was...
carried out, each participant consumed 500 mL of “energy” drink containing a total of 80 mg caffeine and 1000 mg taurine. The examinations were repeated at intervals of up to 4 hours. On each of the following five days the participants again drank 500 mL and on the seventh day the procedure of the first day was repeated. Four hours after consumption of the beverage, systolic blood pressure had increased by 7.9 % (day one) or 9.6 % (day seven) and heart rate had been raised by 7.8 % (day one) or 11 % (day seven). Over the duration of the study this means an increase of blood pressure by 10 mm Hg and of heart rates of 5 to 7 beats per minute. No habituation could be determined following several days exposure since the effects were slightly enhanced on the seventh day. Until the submission of further findings the researchers recommended that patients with high blood pressure or cardiac diseases and corresponding medication should refrain from consuming “energy” drinks because of a possible health risk.

3. Discussion

In the absence of new chronic and acute exposure data, the exposure assessment is based on the data reported by SCF (2003), i.e. a daily mean chronic consumption of 0.5 cans per person and a high chronic exposure at the 95th percentile of 1.4 cans per regular consumer. The Panel notes that actual exposure data on “energy” drink consumption, especially for adolescents and young adults, may need to be collected.

These estimates for the daily exposure to taurine (mean 500 mg/day; 95th percentile 1400 mg/day) or D-glucurono-γ-lactone (mean 300 mg/day; 95th percentile 840 mg/day) from “energy” drinks are higher than the estimated mean daily exposure to taurine from omnivore diets which was estimated to be at most 400 mg/day and that of D-glucurono-γ-lactone from naturally occurring sources in the diet estimated to amount to 1-2 mg/day (SCF, 2003).

The Panel concludes that assuming that children were to consume within the adult intake range of 0.5 and 1.4 cans per person only once per week, then the average chronic exposure of children to “energy” drinks and their constituents, on a body weight basis, would be one third of that in adults.

The SCF Opinion (2003) used 3 cans/day as a reasonable high (acute) consumption, this amount being higher than the 90th percentile recorded in the Austrian survey (2.6 cans/day) and being the average reported in the Irish survey for the most number of cans consumed in a single session. The SCF also indicated that it was aware that amounts of up to 8-12 cans/day were reported by a few extreme consumers in both surveys.

**Taurine**

Results from a new study on absorption, tissue distribution, metabolism and elimination of taurine given orally to rats were provided by the petitioner (Sved *et al.*, 2007). The Panel concludes that these new ADME data support the contention that oral exposure to taurine was not increasing taurine levels in the brain.

In 2003 the SCF evaluated a 13-week rat oral toxicity study of taurine and concluded that focused neurological studies were needed and that the absence of a NOAEL for these effects precluded the setting of an upper safe level for daily exposure to taurine.
The Panel evaluated a new 13-week oral rat toxicity and neurotoxicity study in male and female rats which included FOB and locomotor activity tests. The new study confirmed the NOAEL of 1000 mg/kg bw/day for pathological changes established in the earlier 13-week study described already by the SCF in 2003 and provided evidence for a NOAEL of 1500 mg/kg bw/day for behavioural effects. The results of this study were sufficient to address the concerns raised previously, notably the observation of increased activity and possible decrements in motor skills on the rotarod.

The NOAEL of at least 1000 mg/kg bw/day for pathological changes is 120-fold higher than the estimated mean and 43-fold higher than the estimated 95th percentile exposure to taurine from “energy” drinks only, when calculated for a 60 kg person.

Given that taurine is a natural body constituent, the Panel concludes that these margins of safety are sufficiently large to conclude that exposure to taurine at the levels mentioned above is not of safety concern.

**D-glucurono-\(\gamma\)-lactone**

In 2003 the SCF evaluated a 13-week rat oral toxicity study with D-glucurono-\(\gamma\)-lactone and concluded that the cause of the kidney lesions remained unclear.

The petitioner has now provided data from a new 13-week oral (gavage versus drinking water) toxicity study of D-glucurono-\(\gamma\)-lactone in rats, with specific focus on the kidneys. This study used the same rat strain as the previous study reported in the SCF Opinion of 2003. Extensive urinalysis and histopathological examinations demonstrated no treatment-related effects. Based on the results of this study, the NOAEL for daily oral administration of D-glucurono-\(\gamma\)-lactone was 1000 mg/kg bw/day, the highest dose tested.

Toxicological studies on genotoxic, teratogenic or carcinogenic potential of D-glucurono-\(\gamma\)-lactone were not available. However, D-glucurono-\(\gamma\)-lactone is a normal human metabolite formed from glucose and there are no structural alerts for mutagenicity or carcinogenicity. At physiological pH it is in equilibrium with glucuronic acid, its immediate precursor. D-glucurono-\(\gamma\)-lactone and its hydrolysis product glucuronic acid are endogenous metabolites in humans and other mammals, they occur naturally in several dietary sources and are readily metabolized to innocuous products and excreted. Furthermore there was no evidence of any effect on the gonads in the high dose 13-week studies which might indicate the need for reproductive toxicity studies.

The NOAEL for D-glucurono-\(\gamma\)-lactone of 1000 mg/kg bw/day is 200-fold higher than the estimated mean and 71-fold higher than the estimated 95th percentile exposure to D-glucurono-\(\gamma\)-lactone from “energy” drinks only, when calculated for a 60 kg person.

Given the fact that D-glucurono-\(\gamma\)-lactone is a natural body constituent the Panel concludes that these margins of safety are sufficiently large to conclude that exposure to D-glucurono-\(\gamma\)-lactone at the levels mentioned above is not of safety concern.

**Combined exposure**

The SCF Opinion of 2003 and the recent BfR Opinion (BfR, 2008) mention a number of anecdotal and case reports of acute, adverse effects, including fatalities, in individuals consuming “energy” drinks, containing caffeine, taurine and D-glucurono-\(\gamma\)-lactone. In these cases, “energy” drinks had either been consumed in very high amounts (1420 mL), in combination with physical exercise or more frequently together with alcohol. The SCF
Opinion of 2003 also takes into account that drugs, such as ecstasy and amphetamines may have been involved. The effects mentioned included tremors, seizures, drowsiness, muscle weakness, dizziness, nervousness, tachycardia, palpitations, nausea, vomiting, headache, bronchospasm, hyperventilation and also myocardial infarction and sudden unexplained death possibly resulting from cardiac dysrhythmia (SCF, 2003; BfR, 2008; Lehtihet et al., 2006; Iyadurai and Chung; 2007, Nagajothi et al., 2008; Terlizzi et al., 2008). The SCF concluded the following: “The co-consumption of alcohol and/or drugs noted in most of these cases makes interpretation particularly difficult. Thus there is no confirmation of any causal relationship between the reported effects and the consumption of “energy” drinks. Under these circumstances, the reports can only be noted”. With regard to some actual reports (e.g. Iyadurai and Chung; 2007, Nagajothi et al., 2008; Terlizzi et al., 2008) the Panel considers that it is possible that the effects could be due to the well known side effects of high caffeine intake, while the assumption of a causal relationship with taurine intake is lacking scientific evidence.

These results also raised the issue of combination effects and possible interactions between, amongst others, taurine and alcohol, between taurine and caffeine and between taurine and D-glucurono-γ-lactone.

The SCF Opinion (SCF, 2003) already evaluated the possibility of interactions between taurine, caffeine and D-glucurono-γ-lactone and considered it unlikely that D-glucurono-γ-lactone would have any interaction with caffeine and taurine. The SCF concluded “that consideration of the potential for interactions between caffeine and taurine has not ruled out the possibility of stimulatory effects from both substances at the level of the central nervous system”.

Results from a new study provided by the petitioner (Sved et al., 2007) revealed that brain taurine levels did not increase after dosing. The Panel concludes that these new ADME data support the contention that oral exposure to taurine was not increasing taurine levels in the brain and that this largely rules out the possibility of stimulatory effects from taurine at the level of the central nervous system.

The SCF (2003) also noted that “since caffeine and taurine act via different mechanisms, any diuretic effects could be additive” and that “Both taurine (Gentile et al., 1994) and alcohol centrally inhibit the release of the antidiuretic hormone, vasopressin and the Committee considered that they could act additively to increase water and sodium loss from the body in the short-term”.

New data have recently been published (Riesenhuber et al., 2006) describing results demonstrating that the diuretic potential and natriuretic effects of the tested “energy” drinks were largely mediated by caffeine and that there were no additive interactions between taurine and caffeine. The Panel concludes that the diuretic potential and natriuretic effects of “energy” drinks may be largely mediated by caffeine and not by taurine.

In a recent study, possible cardiovascular effects of the combined exposure to caffeine and taurine with “energy” drinks were investigated (Steinke et al., 2007; American Heart Association, 2007). Four hours after consumption of 500 mL of “energy” drink containing a total of 80 mg caffeine and 1000 mg taurine, systolic blood pressure had increased by 7.9 % (day one) or 9.6 % (day seven) and heart rate had been raised by 7.8 % (day one) or 11 % (day seven). Over the duration of the study this means an increase of blood pressure by 10 mm Hg and of heart rates of 5 to 7 beats per minute. Until the submission of further findings the researchers recommended that patients with high blood pressure or cardiac diseases and corresponding medication should refrain from consuming “energy” drinks because of a
The use of taurine and D-glucurono-γ-lactone as constituents of the so-called “energy” drinks

possible health risk. The Panel notes that the studies were not designed to show whether the effects were due to caffeine or taurine.

Overall, the ANS Panel concludes that the diuretic potential and natriuretic effects of the tested “energy” drinks are largely mediated by caffeine. Other interactions between taurine and caffeine were not investigated.

CONCLUSIONS AND RECOMMENDATIONS

In the present opinion the Panel evaluates the safety of taurine and D-glucurono-γ-lactone as individual ingredients of the so-called “energy” drinks based on the new studies provided by the petitioner. The Panel does not evaluate the safety of “energy” drinks as such.

In the absence of new chronic and acute exposure data, the exposure is based on the data reported by the SCF in 2003. The Panel concluded that actual exposure data on “energy” drink consumption, especially for adolescents and young adults, may need to be collected.

The Panel concludes that the exposure to taurine and D-glucurono-γ-lactone at the levels presently used in “energy” drinks and mentioned above is not of safety concern.

The ANS Panel agrees with the considerations of the SCF Opinion from 2003 that it is unlikely that glucurono-γ-lactone would have any interaction with caffeine, taurine, alcohol or the effects of exercise. The Panel also concludes, based on the new data available, that additive interactions between taurine and caffeine on diuretic effects are unlikely. Other interactions between taurine and caffeine were not investigated.

DOCUMENTATION PROVIDED TO EFSA

1. Kroes R. and Renwick, A.G. Summary report regarding the safety in Use of Taurine and D-glucuronolactone as constituents of “energy” drinks.


5. Striker, G. Expert report of Professor Gary Striker, in concurrence with professor Robert Kroes, concerning the pathological kidney lesions found in toxicity studies.

REFERENCES

The use of taurine and D-glucuronolactone as constituents of the so-called “energy” drinks


The use of taurine and D-glucurono-γ-lactone as constituents of the so-called “energy” drinks

Commission compiled from information submitted by Member States. CS/PLEN/ENDRINKS/1 and Addendum. 2 December 1996 (cited in the SCF opinion, 1999).


The use of taurine and D-glucurono-γ-lactone as constituents of the so-called “energy” drinks


The use of taurine and D-glucurono-\(\gamma\)-lactone as constituents of the so-called “energy” drinks


Trautwein EA and Hayes KC, 1995. Plasma and whole blood taurine concentrations respond differently to taurine supplementation (humans) and depletion (cats). Z. Ernährungswiss 34, 137-142.


The use of taurine and D-glucurono-γ-lactone as constituents of the so-called “energy” drinks


### GLOSSARY / ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ADME</td>
<td>Absorption, Distribution, Metabolism, Excretion</td>
</tr>
<tr>
<td>AFSSA</td>
<td>Agence Française de Sécurité Sanitaire des Aliments</td>
</tr>
<tr>
<td>ANZFA</td>
<td>Australia New Zealand Food Authority</td>
</tr>
<tr>
<td>BfR</td>
<td>Federal Institute for Risk Assessment</td>
</tr>
<tr>
<td>CMC</td>
<td>Carboxymethylcellulose</td>
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<tr>
<td>EFSA</td>
<td>European Food Safety Authority</td>
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<tr>
<td>FSA</td>
<td>Food Standards Agency</td>
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<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>NOAEL</td>
<td>No Observable Adverse Effect Level</td>
</tr>
<tr>
<td>SCF</td>
<td>Scientific Committee on Food</td>
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Energy Beverages: Content and Safety

JOHN P. HIGGINS, MD, MPHIL; TROY D. TUTTLE, MS; AND CHRISTOPHER L. HIGGINS, BHMS (ExSc)

Exercise is making a resurgence in many countries, given its benefits for fitness as well as prevention of obesity. This trend has spawned many supplements that purport to aid performance, muscle growth, and recovery. Initially, sports drinks were developed to provide electrolyte and carbohydrate replacement. Subsequently, energy beverages (EBs) containing stimulants and additives have appeared in most gyms and grocery stores and are being used increasingly by “weekend warriors” and those seeking an edge in an endurance event. Long-term exposure to the various components of EBs may result in significant alterations in the cardiovascular system, and the safety of EBs has not been fully established. For this review, we searched the MEDLINE and EMBASE databases from 1976 through May 2010, using the following keywords: energy beverage, energy drink, power drink, exercise, caffeine, red bull, bitter orange, glucose, ginseng, guarana, and taurine. Evidence regarding the effects of EBs is summarized, and practical recommendations are made to help in answering the patient who asks, “Is it safe for me to drink an energy beverage when I exercise?”


EB = energy beverage; ECG = electrocardiogram; SD = sport drink

Red Bull was introduced in Austria in 1987 and in the United States in 1997. Since then, the energy beverage (EB) market has grown exponentially.1 Hundreds of different brands are now marketed, with caffeine content ranging from a modest 50 mg to an alarming 505 mg per can or bottle.2 In the United States, Red Bull enjoyed a 65% share of the $650-million energy/power drink market in 2005, and its sales are growing at about 35% per year.3 The United States is the world’s largest consumer of EBs by volume, roughly 290 million gallons in 2007, or 3.8 qt per person per year.2 Consumption of EBs is most common among those aged 11 to 35 years, and 24% to 57% of this age group reported that they drank an EB within the past few months.4

Regulation of EBs, including content labeling and health warnings, differs across countries, with some of the laxest requirements existing in the United States.5 For instance, no EBs are banned in the United States, and EB companies can say whatever they want regarding energy and performance effects. This is in stark contrast to countries in which some EBs have been banned, and companies are not allowed to outline the performance effects that their products may or may not provide.4 This absence of oversight has resulted in aggressive marketing of EBs, targeted primarily toward young men and openly promoting psychoactive, performance-enhancing, and stimulatory effects.1,4

Alarming, EB consumption has been shown to be positively associated with high-risk behavior, including marijuana use, sexual risk taking, fighting, failure to use seat belts, and taking risks on a dare, as well as with smoking, drinking, problems stemming from alcohol abuse, and illicit drug use.5,6

In an era in which Gatorade and Powerade, termed sport drinks (SDs) for the purposes of this article, have paved the way as optimal hydration fluids that boast superiority to water, uncertainty is growing with regard to where EBs fit and how they are consumed.7 Convenience stores now display EBs next to the SDs, which can mislead the consumer into thinking that they are similar products. Whereas SDs can indeed provide hydration and replenishment of electrolytes and carbohydrates, the elevated levels of caffeine in EBs have diuretic effects, more pronounced in the first-time user, that increase urinary output and natriuresis.8 Additionally, EBs may have thermogenic effects.9 Moreover, EBs supply an amount of carbohydrate far beyond that recommended for physically active people, which can slow the rate at which fluid is absorbed into the bloodstream or lead to gastrointestinal distress.10 Finally, the effects due to the interaction of substances on which little research has been done (eg, glucuronolactone) are not well understood.

This review of EBs describes the various ingredients, discusses their safety, and provides recommendations regarding their use. Although most research studies and observational data have come from athletics, our research query included studies and information involving nonathlete consumers. Also, the review differentiates between these populations and offers recommendations specific to each group.

METHODS

A search of the English-language scientific literature was performed primarily by searching the MEDLINE and EMBASE databases and using the Google Internet search from the Division of Cardiology, Lyndon B. Johnson General Hospital, and Memorial Hermann Sports Medicine Institute, The University of Texas Medical School at Houston (J.P.H.); Noninvasive Cardiovascular Laboratory, University of Texas Health Science Center at Houston (T.D.T.); and School of Human Movements Studies, University of Queensland, St Lucia, Queensland, Australia (C.L.H.).

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energy for the period January 1976 through May 2010. For this search, we used keywords found in lead peer-reviewed articles and research outlining the current body of knowledge on EBs. Keywords used in the search are energy beverage, energy drink, power drink, exercise, caffeine, red bull, bitter orange, glucose, ginseng, guarana, and taurine. The bibliographies of articles were searched for relevant articles; links on Web sites containing published articles were searched for pertinent information. This review endeavored to bring the most pertinent research to light in order to present real-world recommendations to both the athletic community and the general population.

INGREDIENTS OF EBs

The most common ingredient in EBs is caffeine, which is often combined with taurine, glucuronolactone, guarana, and B vitamins to form what manufacturers have called an “energy blend.” When higher doses of caffeine are combined with these other substances currently blended in EBs, the subsequent effect cannot always be predicted; adverse effects have been reported, including cardiac arrest.11,12 The components of the 4 best-selling EBs are given in Table 1, and the specific ingredients are subsequently discussed in more detail.

Well-performed research indicates that some of these substances are important for proper body function, but this does not mean that a person has a deficiency. Moreover, important questions regarding overall intake and subsequent adverse effects should come to mind as the reader progresses through this review. We have also included information on the bioavailability of each substance if the necessary research has been completed.

CAFFEINE

Caffeine, an adenosine receptor antagonist, is a stimulant that can influence the activity of neuronal control pathways in the central and peripheral nervous systems.13 It is the most common stimulant in EBs, with most EBs containing between 70 and 200 mg of caffeine per 16-oz serving. In comparison, an 8-oz cup of coffee contains 110 to 150 mg for drip, 65 to 125 mg for percolated, and 40 to 80 mg for instant; caffeinated beverages contain about 50 to 100 mg of caffeine.14 Caffeine is known as an ergogenic compound that raises the heart rate and blood pressure. Adverse effects typically manifest with ingestion higher than 200 mg of caffeine and include insomnia, nervousness, headache, tachycardia, arrhythmia, and nausea.14,15 The ergogenic effects of caffeine on athletic performance have been shown, and its broad range of metabolic, hormonal, and physiologic effects has been described.16,17 Caffeine has been shown to be an effective ergogenic aid for endurance athletes when ingested before and/or during exercise in moderate quantities (3-6 mg/kg of body mass); abstaining from caffeine for at least 7 days before use will optimize the effect.16 Of note, caffeine appears on the list of substances banned by the International Olympic Committee.18 Of all the compounds analyzed in this review, caffeine is by far the most-researched in the literature.

The absolute bioavailability of orally administered caffeine was investigated in 10 healthy adult male volunteers aged 19 to 30 years.19 Participants received a 5-mg/kg dose of caffeine as either an aqueous oral solution or an intravenous infusion on separate occasions about 1 week apart in a randomized crossover fashion. Plasma samples were collected during the 24-hour period after each dose and assayed for their caffeine content using a high-performance liquid chromatographic technique. The oral absorption was very rapid, with caffeine reaching a peak plasma concentration after 29.8 minutes, and the variation in the maximum plasma concentration was low at 10.0 µg/mL. The absolute bioavailability was assessed by comparing the areas under the plasma concentration vs time curves for the intravenous and oral doses of caffeine. The rapid absorption resulted in essentially complete bioavailability of the oral caffeine, with a plasma half-life varying from 2.7 to 9.9 hours, indicating substantial variability in its elimination between participants.19

Caffeine mobilizes fat stores and stimulates working muscles to use fat as a fuel, which delays depletion of muscle glycogen and allows for prolonged exercise.20 The critical period in glycogen sparing appears to occur during the first 15 minutes of exercise, when caffeine has been shown to decrease glycogen utilization by as much as 50%. Thus, glycogen saved at the beginning is available during the latter stages of exercise. Although the exact mechanism is still unclear, caffeine caused sparing in all the human studies in which muscle glycogen levels were measured. The effect on performance, which was observed in most experimental studies, was that participants were able to exercise longer before exhaustion occurred.20

Caffeine binds to the adenosine class of G protein–coupled receptors on the surface of heart muscle cells, which begins a second messenger system with cyclic adenosine monophosphate inside the cells and mimics the effects of epinephrine.21 The rate of glycolysis increases, which increases the amount of adenosine triphosphate available for muscle contraction and relaxation.22 This can result in a positive inotropy and chronotropy, ie, a stronger and faster heartbeat. Caffeine immediately increases blood pressure and peripheral vascular resistance, in part because of sympathetic stimulation. One group of investigators showed a significant effect of drinking caffeinated coffee on arterial tone and function, suggesting that caffeine immediately
### Table 1. Comparison of Ingredients in Energy Beverages*

<table>
<thead>
<tr>
<th></th>
<th>Red Bull</th>
<th>Rockstar</th>
<th>Monster</th>
<th>Full Throttle</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Calories</strong></td>
<td>220</td>
<td>280</td>
<td>200</td>
<td>220</td>
</tr>
<tr>
<td><strong>Carbohydrates</strong></td>
<td>54 g    Sucrose, glucose</td>
<td>62 g Sucrose, glucose</td>
<td>54 g Sucrose, glucose, sucralose, high-fructose corn syrup, maltodextrin</td>
<td>57 g High-fructose corn syrup, sucrose</td>
</tr>
<tr>
<td><strong>Sodium</strong></td>
<td>Only listed as sodium citrate</td>
<td>80 mg sodium citrate</td>
<td>360 mg 16% RDA Sodium citrate, sodium chloride</td>
<td>160 mg Sodium citrate</td>
</tr>
<tr>
<td><strong>Caffeine</strong></td>
<td>160 mg 200 mg Part of a 1.35-g “energy blend”</td>
<td>Only listed as part of a 5000-mg “energy blend”</td>
<td>141 mg Part of a 3000-mg “energy blend”</td>
<td></td>
</tr>
<tr>
<td><strong>Taurine</strong></td>
<td>2000 mg 2000 mg Part of a 1.35-g “energy blend”</td>
<td>2000 mg Part of a 5000-mg “energy blend”</td>
<td>Only listed as part of a 3000mg “energy blend”</td>
<td></td>
</tr>
<tr>
<td><strong>Glucuronolactone</strong></td>
<td>Only listed (1200 mg)b</td>
<td>None listed</td>
<td>Only listed as part of a 5000-mg “energy blend”</td>
<td>None listed</td>
</tr>
<tr>
<td><strong>Niacin (B3)</strong></td>
<td>20% RDA Niacinamide (40 mg)b</td>
<td>40 mg 200% RDA Niacinamide</td>
<td>40 mg 100% RDA Niacinamide</td>
<td>None listed</td>
</tr>
<tr>
<td><strong>Inositol (B6)</strong></td>
<td>Only listed 50 mg Part of a 1.35-g “energy blend”</td>
<td>Only listed as part of a 5000-mg “energy blend”</td>
<td>None listed</td>
<td></td>
</tr>
<tr>
<td><strong>Pyridoxine hydrochloride (B6)</strong></td>
<td>500% RDA (10 mg)b</td>
<td>4 mg 200% RDA</td>
<td>4 mg 200% RDA</td>
<td>None listed</td>
</tr>
<tr>
<td><strong>Cyanocobalamin (B12)</strong></td>
<td>160% RDA Listed as vitamin B12 (10 µgb)</td>
<td>12 µg 200% RDA</td>
<td>12 µg 200% RDA</td>
<td>None listed</td>
</tr>
<tr>
<td><strong>Riboflavin (B2)</strong></td>
<td>None listed</td>
<td>6.8 mg 400% RDA</td>
<td>3.4 mg 200% RDA</td>
<td>None listed</td>
</tr>
<tr>
<td><strong>Pantothenic acid (B5)</strong></td>
<td>100% RDA Calcium pantothenate (10 mg)b</td>
<td>20 mg 200% RDA Calcium pantothenate</td>
<td>None listed</td>
<td>None listed</td>
</tr>
<tr>
<td><strong>Ginseng extract</strong></td>
<td>None listed 50 mg Part of a 1.35-g “energy blend”</td>
<td>400 mg Only listed as part of a 3000-mg “energy blend”</td>
<td>None listed</td>
<td></td>
</tr>
<tr>
<td><strong>Guarana extract</strong></td>
<td>None listed 50 mg Part of a 1.35-g “energy blend”</td>
<td>Only listed as part of a 5000-mg “energy blend”</td>
<td>None listed</td>
<td></td>
</tr>
<tr>
<td><strong>Ginkgo biloba leaf extract</strong></td>
<td>None listed 300 mg Part of a 1.35-g “energy blend”</td>
<td>None listed</td>
<td>None listed</td>
<td></td>
</tr>
<tr>
<td><strong>Milk thistle extract</strong></td>
<td>None listed 40 mg Part of a 1.35-g “energy blend”</td>
<td>None listed</td>
<td>None listed</td>
<td></td>
</tr>
<tr>
<td><strong>L-carnitine</strong></td>
<td>None listed 50 mg Part of a 1.35-g “energy blend”</td>
<td>Only listed as part of a 5000-mg “energy blend”</td>
<td>Only listed as part of a 3000-mg “energy blend” Carnitine fumarate</td>
<td></td>
</tr>
<tr>
<td><strong>Sorbic acid</strong></td>
<td>None listed Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>Sodium benzoate</strong></td>
<td>None listed Yes</td>
<td>Yes Benzoic acid</td>
<td>Yes Benzoic acid</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Citric acid</strong></td>
<td>None listed Yes</td>
<td>Benzoic acid</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Natural flavors</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Artificial flavors</strong></td>
<td>Yes</td>
<td>None listed</td>
<td>None listed</td>
<td>None listed</td>
</tr>
<tr>
<td><strong>Coloring</strong></td>
<td>“Colors” Blue 1, Red 40</td>
<td>“Caramel” Blue 1, Red 40</td>
<td>“Color added” Blue 1, Red 40</td>
<td></td>
</tr>
</tbody>
</table>

* As listed on 16-oz can unless otherwise noted. RDA = recommended daily allowance.

b This amount is not listed on the can; the corporate office was called and this was all the information given.
increases arterial stiffness, with the effect being more pronounced on aortic systolic and diastolic blood pressures than on the brachial artery.23

The discussion on caffeine and hydration continues to evolve. A number of studies have examined the effects of ingesting a large dose of caffeine and found that urine output was increased.24 Yet, the long-held conclusion that caffeine increases 24-hour urine output has been challenged, because most of the studies that established this conclusion involved persons who refrained from caffeine consumption before the analysis.25,26 The human body develops a tolerance to caffeine quickly, usually 3 to 5 days after regular use, and when this happens, it noticeably weakens the already limited diuretic effect of caffeine.26,27 Moreover, the human kidneys are masters at ensuring that proper homeostatic conditions are maintained. Recent research now leans toward the ability of the body to maintain proper water levels and overcome the mild diuretic effects of caffeine in long-term users.25,28

As the rest of the ingredients are reviewed herein, please note that in comparison with caffeine, each has had far fewer research studies performed.

**Taurine**

Taurine, a sulfur-containing amino acid, is the most abundant intracellular amino acid in humans and a normal constituent of the human diet.29

A study of the pharmacokinetics of taurine was conducted in 8 healthy male volunteers with a median age of 28 years. After oral administration of 4 g of taurine in the fasting state, blood samples were taken at regular intervals, and plasma taurine concentration was measured by a modified high-performance liquid chromatographic technique. Maximum plasma taurine concentration was 86 mg/L 1.5 hours after administration. Plasma elimination half-life and the ratio of clearance to bioavailability were 1.0 hour and 21 L/h, respectively.30

Taurine modulates skeletal muscle contractile function and may attenuate exercise-induced DNA damage, with some evidence showing the ability to improve exercise capacity and performance; however, this has not been definitively demonstrated.4 Taurine has numerous other biological and physiologic functions, including bile acid conjugation and cholestasis prevention; antiarrhythmic, inotropic, and chronotropic effects; central nervous system neuromodulation; retinal development and function; endocrine or metabolic effects; and antioxidant and anti-inflammatory properties.31 Taurine also assists in cell membrane stabilization, osmoregulation, and detoxification.32 However, the amounts of taurine found in popular EBs are far below the amounts expected to deliver either therapeutic benefits or adverse events.14

**Glucuronolactone**

This is a naturally occurring substance produced in small amounts within the body. Supplementation with D-glucarates, including glucuronolactone, may favor the body’s natural defense mechanism for eliminating carcinogens and tumor promoters and their effects.33 Toxicokinetic data on glucuronolactone in rats, which show bioavailability and lack of accumulation, with peak plasma levels 1 to 2 hours after oral administration, are in accordance with the limited human data.34 Unfortunately, little research has been done in humans, and the current body of knowledge on this substance is scant. Therefore, conclusions on whether this compound is harmful or beneficial cannot be made.

**B Vitamins**

The B vitamins are water-soluble vitamins required as coenzymes for proper cell function, especially mitochondrial function and energy production.35 B vitamins include thiamine, riboflavin, niacin, pantothenic acid, pyridoxine hydrochloride, biotin, inositol, and cyanocobalamin. Because EBs contain large amounts of sugar, these vitamins are touted as ingredients necessary to convert the added sugar to energy. Hence, the B vitamins are the “key” needed to unlock all the energy provided by the simple sugars in EBs, and this is the extra energy that EB companies claim their product can provide. An expansive amount of research has been done on the B vitamins, and we outline the specific functions each major B vitamin plays in the human body.

Thiamine (vitamin B1) is essential for the oxidative de-carboxylation of the multienzyme branched-chain α-keto acid dehydrogenase complexes of the citric acid cycle and thus serves as a coenzyme precursor of some key enzymes of carbohydrate metabolism.36 Riboflavin (vitamin B2) is required for the flavoenzymes of the respiratory chain and thus also supports energy metabolism involving fats, carbohydrates, and proteins.37

The reduced form of nicotinamide adenine dinucleotide (NADH) is synthesized from niacin (vitamin B3). This coenzyme is required to supply protons for oxidative phosphorylation and plays a major part in energy production in cells.38 It also stimulates the production of such neurotransmitters as L-dopa, dopamine, serotonin, and norepinephrine.

Pantothenic acid (vitamin B5) is required for coenzyme A, α-ketoglutarate, and pyruvate dehydrogenase formation, as well as fatty acid oxidation.35 Pyridoxine hydrochloride (vitamin B6) is a coenzyme involved in amino acid and homocysteine metabolism, glucose and lipid metabolism, neurotransmitter production, and DNA and RNA synthesis.39 Specifically, pyridoxine hydrochloride is involved in protein and red blood cell me-
tabolism, is important for immune system function, and is needed to convert tryptophan to niacin.

Biotin (vitamin B₉) is the coenzyme of decarboxylases required for gluconeogenesis and fatty acid oxidation. Inositol (formerly vitamin B₁₄ but declassified as a vitamin because it is synthesized by the human body) exists in 9 possible stereoisomers, of which the most common form is myo-inositol. It is part of cell membranes, plays a role in helping the liver process fats, and contributes to the function of muscles and nerves.

Cyanocobalamin (vitamin B₁₂) helps maintain nerve cell function, is needed for production of DNA, and is important in red blood cell formation.

GUARANA
Also known as guaranine, Paullinia cupana, and Sapindaceae, guarana is a rainforest vine that was domesticated in the Amazon for its caffeine-rich fruits and has long been used by the Amazonians to increase awareness and energy. Guarana seeds contain more caffeine than any other plant in the world, with levels ranging from 2% to 8%; guarana also contains the stimulants theobromine and theophylline. The amounts of guarana found in popular EBs are below the amounts expected to deliver therapeutic benefits or cause adverse events. However, some young adults have been admitted to emergency departments with overdoses of caffeine after overindulging in guarana-based EBs.

GINSENG
Ginseng is one of the most popular herbal supplements in the world and is used for treatment and prevention of many ailments. This adaptogen (a natural herb product said to increase the body’s resistance to stress, trauma, anxiety, and fatigue) is purported to increase energy, relieve stress, and increase memory by stimulating the hypothalamic and pituitary glands to secrete corticotropin. Athletes use ginseng for its alleged performance-enhancing attributes; however, a recent review concluded that enhanced physical performance after ginseng administration remains to be demonstrated. Adverse effects associated with ginseng include hypotension, edema, palpitations, tachycardia, cerebral arteritis, vertigo, headache, insomnia, mania, vaginal bleeding, amenorrhea, fever, appetite suppression, pruritus, cholestatic hepatitis, mastalgia, euphoria, and neonatal death. However, the amounts of ginseng found in EBs are far below the amounts expected to deliver therapeutic benefits or cause adverse events.

GINKGO BILOBA
Ginkgo biloba extract is derived from the leaves of the Ginkgo biloba tree and has been used in traditional Chinese medicine for centuries. Ginkgo biloba extract has been reported to have antioxidant properties, modify vasomotor function, reduce adhesion of blood cells to endothelium, inhibit activation of platelets and smooth muscle cells, affect ion channels, and alter signal transduction. However, to date, no large, well-conducted randomized controlled trials have shown that it has important clinical effects in healthy or ill persons.

L-CARNITINE
This amino acid is made predominantly by the liver and kidneys to increase metabolism. Dietary supplementation with L-carnitine has been shown to increase maximal oxygen consumption and lower the respiratory quotient, indicating stimulation of lipid metabolism. Recent evidence indicates that L-carnitine plays a decisive role in preventing cellular damage and favorably affects recovery from exercise stress. Uptake of L-carnitine by blood cells may promote (1) stimulation of hematopoiesis, (2) inhibition of collagen-induced platelet aggregation, and (3) prevention of programmed cell death in immune cells. There is evidence of a beneficial effect of L-carnitine supplementation in training, competition, and recovery from strenuous exercise and in regenerative athletics. No advantage appears to exist in giving an oral dose greater than 2 g at one time, because absorption studies indicate saturation at this dose.

SUGARS
Sugars are the basic currency for energy in the body, with glucose being the key carbohydrate that can readily be oxidized by skeletal muscle for energy production. Often, EBs contain sugar (high-fructose corn syrup or sucrose). Administration of glucose or other carbohydrates before, during, and after prolonged exercise (>1 hour) has been shown to postpone fatigue, conserve muscle glycogen, and improve performance. Ingestion of moderately concentrated carbohydrate solutions (4%-8%) with the aim of achieving a carbohydrate intake of 60 to 70 g/h enhances prolonged exercise performance and is appropriate for optimizing energy and fluid delivery without causing adverse effects. However, the ergogenic effects of carbohydrate ingestion on performance during intermittent exercise such as competitive sports are less well established, although the evidence to date suggests diminished performance when carbohydrates are limited. The amount of sugar provided in one can (or 500 mL) of an EB is typically about 54 g. A teaspoon of sugar weighs about 4 g, so a typical EB contains about 13 teaspoons, or just more than ¼ cup, of sugar.

Long-term exposure of the body to excesses of simple sugars is associated with the development of obesity and insulin resistance. Pancreatic beta cells increase insulin
secretion in response to this reduction in insulin sensitivity. Over time, in many individuals, the beta cells become unable to secrete sufficient insulin to maintain normal blood glucose levels, leading to the development of diabetes.51

**Antioxidants**

During exercise, inflammation and oxidative stress are linked by means of muscle metabolism and muscle damage.52 Antioxidants are purported to aid the body in the recovery phase and reduce damage to muscle cells.53 However, there is no convincing evidence that short-term or long-term exercise modifies antioxidant requirements, nor have significant effects been shown for supplementation in well-trained athletes.54

**DO EBs WORK AND ARE THEY SAFE?**

**Research Issues**

A number of factors relating to EB consumption may make good morbidity and mortality data difficult to ascertain. These include the following.

- The target market for EBs is people between 15 and 30 years of age. This population is typically healthy and involved in activities and includes a higher proportion of sports enthusiasts and high-risk takers.
- Because of the many ingredients in EBs, cause and effect is difficult to assign to one specific ingredient. Indeed, it may be the combination of ingredients that augments the effect.
- Most of the ingredients are available over the counter and are unregulated in the United States. Thus, EBs are not under the jurisdiction of the US Food and Drug Administration and therefore are not subject to the stringent requirements for safety and efficacy data before approval for human use.
- Binge drinking of EBs combined with alcohol is common practice, again making causation difficult to assign.
- Many people consume more than the recommended daily allowance of EBs. (Recommended daily allowance is one can.)

**Short-term Effects**

In addressing the question of safety, it is important to consider both short-term and long-term effects of ingestion of EBs. Physiologic effects occur immediately after drinking the first dose.

In one study, 15 healthy persons aged 18 to 40 years consumed 2 cans (500 mL) of a commercially available EB containing 1000 mg of taurine and 100 mg of caffeine, as well as vitamins B₃, B₆, and B₁₂, glucuronolactone, and niacinamide, daily for 1 week; effects of the EB on their blood pressure, pulse, and electrocardiogram (ECG) were measured.55 The key effects were as follows.

- Within 4 hours of EB consumption, the maximum systolic blood pressure increased by 8% on day 1 and 10% on day 7.
- Within 2 hours of EB consumption, the maximum diastolic blood pressure increased by 7% on day 1 and 8% on day 7.
- Heart rate increased by 8% on day 1 and 11% on day 7.
- Throughout the study, heart rates increased between 5 and 7 beats/min, and systolic blood pressure increased by 10 mm Hg after EB consumption.
- No clinically important ECG changes were observed.

The authors concluded that, although no clinically important ECG changes occurred, there were significant increases in heart rate and blood pressure, and thus patients with hypertension should not consume this type of drink.55

In a double-blind crossover study, 13 endurance-trained participants performed an exhaustive bout of endurance exercise at 3 different times.56 Before the exercise, they ingested the original Red Bull drink, a similar drink without taurine but containing caffeine, and a placebo drink without caffeine or taurine. Echocardiography was performed before ingestion of the drinks, before exercise, 40 minutes after ingestion, and in the recovery period after exercise. Stroke volume was significantly influenced only in the Red Bull group (80±21 mL before ingestion vs 98±26 mL in the recovery period), mainly because of reduced end-diastolic volume. Thus, this study shows that the original Red Bull increases cardiac contractility.56

A double-blind study of 68 healthy college-aged students showed that Red Bull reduced the changes in blood pressure during a stressful experience (cold pressor test) and increased participants’ pain threshold.57

In a study of the effects of EBs on high-intensity run time-to-exhaustion in physically active university students (n=17; 9 men; mean age, 21±4 years), sugar-free Red Bull did not influence high-intensity run time-to-exhaustion.58 However, in a study of 6 male and 6 female trained cyclists (mean age, 27.3 years), improved cycling time-trial performance was noted after ingestion of a caffeine-containing EB.59

In addition to these studies, several case reports on the immediate effects of EB ingestion have been published.

- Four documented cases of caffeine-associated death have been reported, as well as 5 separate cases of seizures associated with consumption of energy/power drinks.4,14
- An otherwise healthy 28-year-old man had cardiac arrest after a day of motocross racing.11
- A healthy 18-year-old man died playing basketball after drinking 2 cans of Red Bull.60
• Postural tachycardia syndrome associated with a vasovagal reaction was recorded in a young volleyball player after an excess intake of Red Bull as a refreshing energy/power drink, leading experts to suspect the drink as a possible cause of orthostatic intolerance.61

• Four cases of psychiatric effects on patients with known psychiatric illness were reported.4

• One case of suspected anaphylaxis was reported.62

Regarding fluid replacement in persons who do not typically ingest large amounts of caffeine, EBs deliver a considerable amount of caffeine, which can stimulate the kidneys to produce more urine. Thus, EBs can have a net dehydrating effect.63

LONG-TERM EFFECTS

Unfortunately, there are no long-term studies of the effects of caffeine, taurine, and glucuronolactone on the body.4 Energy beverages may exacerbate risk factors for heart disease because studies suggest that EBs may serve as a gateway to other forms of drug dependence.1 Norway, Denmark, and France have banned the sale of Red Bull, partly in response to a study on rats that were fed taurine and exhibited bizarre behavior, including anxiety and self-mutilation.64

Whether caffeine can cause hypertension and coronary artery disease is still controversial, but questions have been raised about its safety in patients with heart failure and arrhythmia.65 However, no clear association between coffee and the risk of hypertension, myocardial infarction, or other cardiovascular diseases has been demonstrated.66

COMBINING EBs AND ALCOHOL

Many consumers are combining EBs with alcoholic drinks. These individuals are typically white men and intramural athletes, a group now termed “toxic jocks.”74 The consequences can be tragic, as illustrated recently by Cleveland Browns wide receiver Donté Stallworth, who told police that he drank up to 4 shots of premium tequila and a can of Red Bull while partying with friends at a Miami Beach club, but did not feel drunk in the hours before he struck and killed a pedestrian with his car.65 The combination of EBs with alcohol can impair cognitive function and reduce symptoms of alcohol intoxication, including the depressant effects, thus increasing the probability of accidents and/or favoring the possibility of development of alcohol dependence.68,69 The combination might also increase important arrhythmia in patients with underlying heart disease.70

In late 2009, 100 scientists and physicians signed a petition that was delivered to the US Food and Drug Administration asking for more regulation of increasingly popular EBs because their high caffeine content puts young drinkers at possible risk of caffeine intoxication and higher rates of alcohol-related injuries.2,5

Clearly, more research is needed concerning the combined effects of the substances listed in this report and, specifically, at the doses EBs provide. Teens and young adults, both athletes and nonathletes, are consuming EBs at an alarming rate; thus, we need to determine whether long-term use of EBs by this population will translate into deleterious effects later.

RECOMMENDATIONS

On the basis of this review, we make the recommendations listed in Table 2.

In summary, one can of an EB during one session is safe for most healthy individuals. However, excess consumption and consumption with other caffeine-containing beverages or alcohol may lead to adverse effects and possibly death. Patients with clinically relevant underlying medical conditions, including heart disease and hypertension, should consult with their physician before drinking EBs.

CONCLUSION

Coaches and athletic departments should take the initiative in addressing the issue of EBs with student athletes and in educating them about the effects and risks. The main ingredients of energy/power drinks are caffeine, taurine, glucuronolactone, B vitamins, guarana, ginseng, ginkgo bi-
lora, l-carnitine, sugars, antioxidants, and trace minerals. The negative effects of excess caffeine have been proven, but the positive effects of many of the other additives, such as taurine and glucuronolactone, remain unproven, as does the combined effect of these ingredients in EBs.

Ingestion of EBs before an event or during training can have serious adverse effects, most notably restlessness and irritability; can increase blood pressure; and may result in dehydration. The long-term effects of EBs on the human body have not been established.

Limited ingestion of EBs by healthy people is not likely to cause major adverse effects, but binge consumption or consumption with alcohol may lead to adverse events. Individuals with medical illnesses, especially underlying heart disease, should check with their physician before using EBs, because they may exacerbate their condition.

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**DAY 2, SESSION 2: USE OF CAFFEINATED PRODUCTS**


13 Caffeine
Practical Implications

Andrew P. Smith

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INTRODUCTION

There have been many articles written about caffeine, and the present chapter has the aim of discussing some of the practical implications of caffeine ingestion. This first section gives some brief information about caffeine and then describes the structure of the chapter.

WHAT IS CAFFEINE?

Caffeine (1,3,7-trimethylxanthine) is a member of a class of naturally occurring substances termed methylxanthines (two other methylxanthines found in food are theobromine, which is present in cocoa, and theophylline, which is found in small amounts in coffee and tea).

WHERE DOES IT COME FROM?

Caffeine occurs naturally in a number of foods, is employed as a food additive, and is added to medications. It occurs naturally in coffee, tea, and cocoa, and the exact amount present will depend on growing conditions and preparation. Some rough approximations are shown below:

- Filter coffee: 100–150 mg (5 oz cup)
- Instant coffee: 50–60 mg
- Tea: 35–45 mg
- Chocolate: milk, up to 15 mg; dark, up to 35 mg

It is also added to soft drinks (e.g., cola has 40 mg in a 12 oz serving). Higher amounts are added to energy drinks (70 mg+). Caffeine is also added to over-the-counter (OTC) medications (usually in the region of 30–50 mg per tablet). Caffeine tablets can also be purchased, and the recommended dose to increase alertness is 100–200 mg.

HOW DOES IT ACT ON THE BODY?

Caffeine acts by blocking the effects of the naturally occurring neuromodulator adenosine. This produces an increase in central nervous system (CNS) activity which is associated with changes in many neurotransmitter systems (e.g., an increased turnover of central noradrenaline). Caffeine has other effects (e.g., influencing blood flow to the brain) but many of these (e.g., calcium mobilization and prostaglandin antagonism) are unlikely to occur with the amounts consumed by humans.
HOW MUCH CAN WE HAVE?

Average intake varies as a function of country and demographic factors. Several estimates suggest that average intake is about 200 mg per day. Problems usually only occur when excessive amounts are consumed (500 mg a day plus), and these problems usually take the form of an increase in anxiety. Some individuals are very sensitive to effects of caffeine, and even small amounts can cause adverse reactions. Most individuals control their consumption of caffeine. For example, consumption usually occurs when alertness is reduced (e.g., early in the morning, after prolonged work, and after lunch) and is reduced at times when high alertness is undesirable (e.g., before going to sleep).

WHAT EXTERNAL FACTORS AFFECT ITS METABOLISM?

Plasma levels of caffeine peak 15–45 minutes after ingestion, and the half-life is between five and six hours. A variety of factors influence the metabolism. For example, in pregnant women the half-life can increase to eighteen hours. Oral contraceptive use increases the half-life to eleven hours. In contrast, in cigarette smokers the half-life is only about three hours, which may account for the high level of consumption found in this group.

BEHAVIORAL EFFECTS OF ACUTE CAFFEINE INGESTION

There has been extensive research on the effects of caffeine on human behavior (for reviews, see Lieberman 1992; Smith 2002, 2005). In general, there is good agreement about the behavioral changes that occur following caffeine ingestion. Like most topics that have been studied extensively, there is variation in the reported effects. This often reflects methodological features of the studies (e.g., sample size, design and analysis, procedure, and choice of tasks) and there is a need to examine the literature so that findings can be weighted according to their methodological rigor. Once this has been achieved, secondary analyses based on large data sets can determine which effects are going to have a significant impact on real-life behavior. Similarly, meta-analyses, based on these robust findings, can address this issue in another way. What is presented below is a less formal approach to the topic based on years of reviewing the topic and conducting studies in the laboratory.

A PROFILE OF THE EFFECTS OF CAFFEINE ON MOOD AND COGNITIVE PERFORMANCE

Substantial research has shown that ingestion of moderate doses of caffeine (typically between 100 and 300 mg) increases alertness and the ability to respond to signals occurring at unexpected times or in unknown places. Smith et al. (2003) distinguish two different types of effect: the first occurs in low-alertness situations (e.g., when circadian alertness is low, after prolonged work, when the person is sleep deprived, or when the person has a cold), and caffeine improves performance on tasks known to be impaired by reduced alertness (e.g., variable fore-period simple reaction time tasks, vigilance tasks, and self-paced serial
response tasks). A second type of effect can be seen even in alert individuals and involves caffeine increasing the speed of encoding of new information. This faster processing can then lead to benefits in choice reaction time tasks or detection tasks involving detection of stimuli which are degraded (Smith et al. 1999) or presented rapidly.

Effects of caffeine on other functions, such as episodic memory, are rarely observed. However, caffeine may improve working memory (e.g., performance of a logical reasoning task; Smith et al. 1992, 1994) and retrieval from semantic memory (Smith et al. 1992, 1994, 1999). These effect sizes are usually smaller than those found using selective and sustained attention tasks. Similarly, it is unclear whether the effects are really due to changes in memory or just to a change in the speed of encoding the information.

While the main effect of caffeine is to increase alertness (especially at the end of a test battery when volunteers are fatigued), some studies have also shown that caffeine increases hedonic tone (feeling happier, feeling more sociable). Caffeine can also increase anxiety when given either in high doses (over 300 mg; Lieberman 1992) or to sensitive individuals. It has been suggested that this may lead to impairments in fine motor performance, but other research (e.g., Lieberman et al. 1987) has failed to confirm this. Given that caffeine increases alertness, it is not surprising that large amounts consumed late in the evening can interfere with sleep (prevent individuals from going to sleep and reduce sleep duration). Indeed, most individuals control their consumption to avoid this. While it is quite easy to demonstrate effects of late-night caffeine on sleep, it is more difficult to find evidence that high consumption per se will affect sleep. Indeed, Sanchez-Ortuno et al. (2005) conducted a survey of 1,498 French workers and found that habitual use of up to seven cups of coffee (600 mg caffeine) per day was not associated with a decreased duration of sleep.

A number of studies have shown that beneficial effects of caffeine can be observed using low doses typically found in commercial products (e.g., Lieberman et al. 1987; Durlach 1998; Smith et al. 1999). A linear dose–response curve has also been shown in a number of studies (Amendola et al. 1998; Brice and Smith 2001), although others suggest that the dose–response curve is flat at the low-dose end (Smit and Rogers 2000).

The effects described above appear to be robust in that they can be observed when caffeine is given to nonconsumers, withdrawn consumers, or those who have recently consumed caffeine. Most studies of caffeine have involved administration of a single large dose which is often not the way we consume it. Brice and Smith (2002) found that the enhanced mood and performance seen after a single dose of 200 mg caffeine were also obtained if volunteers consumed four separate doses of 65 mg over the day (which resulted in an identical final caffeine level to the 200 mg dose).

**CAFFEINE AND PHYSICAL PERFORMANCE**

This issue has often been considered in relation to sports performance (its relation to sustained operations will be discussed in this chapter). A recent position paper on this topic from the International Society of Sports Nutrition (Goldstein et al. 2010) can be summarized as follows:
1. Caffeine is effective for enhancing sport performance in trained athletes when consumed in low-to-moderate dosages (~3–6 mg/kg) and overall does not result in further enhancement in performance when consumed in higher dosages (≥ 9 mg/kg).

2. Caffeine exerts a greater ergogenic effect when consumed in an anhydrous state as compared to coffee.

3. It has been shown that caffeine can enhance vigilance during bouts of extended exhaustive exercise, as well as periods of sustained sleep deprivation.

4. Caffeine is ergogenic for sustained maximal endurance exercise, and has been shown to be highly effective for time-trial performance.

5. Caffeine supplementation is beneficial for high-intensity exercise, including team sports such as soccer and rugby, both of which are categorized by intermittent activity within a period of prolonged duration.

6. The literature is equivocal when considering the effects of caffeine supplementation on strength–power performance, and additional research in this area is warranted.

7. The scientific literature does not support caffeine-induced diuresis during exercise, or any harmful change in fluid balance that would negatively affect performance.

The next section considers whether there are plausible mechanisms for the observed effects of caffeine.

MECHANISMS UNDERLYING THE BEHAVIORAL EFFECTS OF CAFFEINE

Mechanisms underlying the behavioral effects of caffeine can be considered at several different levels (see Fredholm et al. 1999). If one starts with effects on the CNS, one finds that most of the data suggest that caffeine, in the doses that are commonly consumed, acts primarily by blocking adenosine A1 and A2A receptors. Even though the primary action of caffeine may be to block adenosine receptors, this leads to very important secondary effects on many classes of neurotransmitters, including noradrenaline, acetylcholine, dopamine, serotonin, glutamate, and GABA. Such effects show that caffeine has the ability to increase alertness, a possible reason underlying why people consume caffeine-containing beverages. There are other effects of caffeine on the CNS (e.g., direct release of intracellular calcium, and effects on alkaline phosphatase), but many of these only occur at doses well above the range of human consumption.

Studies from humans show that mechanisms underlying the beneficial effects seen in mood and performance may reflect changes in a number of neurotransmitter systems. The effects seen in low-alertness situations have been mimicked using drugs that reduce the turnover of central noradrenaline (Smith et al. 2003) or increase sedation through changes in the GABA/benzodiazepine system (File et al. 1982). The faster encoding of new information produced by caffeine may reflect cholinergic...
changes, and caffeine has been shown to reduce the impairments produced by scopolamine (Riedel et al. 1995).

Recent studies have examined effects of caffeine on blood flow to the brain. It is likely that caffeine exerts a complex pattern of effects, but the net effect is reduced cerebral blood flow. This has been demonstrated using positron emission tomography (PET), magnetic resonance imaging (MRI), and trans-cranial Doppler methodologies. The relevance of these changes for the behavioral effects requires further investigation.

Another approach has tried to elucidate the stages of processing influenced by caffeine. For example, Lorist and Snel (1997) have shown that target detection and response preparation are enhanced by caffeine, and Ruijter et al. (1999) have demonstrated that the quantity of information processed is greater after caffeine. Smith et al. (1999) have shown that caffeine increases the speed of processing new stimuli, confirming results reported by Streufert et al. (1997). In contrast to its effects on encoding and sustained attention, caffeine has not been shown to reduce resistance to distraction (Kenemans and Verbaten 1998). Similarly, caffeine appears to have little effect on output processes (e.g., movement time; Lorist 1998), although occasional reports of caffeine-induced impairments on hand steadiness can be found (e.g., Bovim et al. 1995). One interpretation of effects of caffeine in low-alertness situations is that caffeine reduces the occasional long responses which may be the result of error correction or stimulus uncertainty. This means that the tail of the reaction time distribution will be much more sensitive to effects of caffeine as will responses to stimuli where the fore-period is long (or, in the case of vigilance tasks, targets occurring at irregular rather than regular intervals). Effects of caffeine seen when individuals are alert have been interpreted in terms of faster encoding of new information. This can be examined by considering responses which are the same as the one made on the previous trial (little new encoding needed, little effect of caffeine) or different responses (new stimuli and new response, large improvement with caffeine). Similarly, degraded stimuli which are hard to encode show greater benefits after caffeine than those which are easy to encode (Smith et al. 1999).

Another type of explanation has been to suggest that caffeine has no direct effects but reverses the negative effects of withdrawal (James 1994; James and Rogers 2005). Smith (2005) has questioned this view and argued that it is unlikely to be correct for the following reasons. First, it cannot account for the behavioral effects seen in animals or nonconsumers (Addiccott and Laurienti 2009; Childs and de Wit 2006; Haskell et al. 2005; Hewlett and Smith 2006; Smith et al. 2006), where withdrawal cannot occur. Second, caffeine withdrawal cannot account for behavioral changes following caffeine consumption after a short period of abstinence (Warburton 1995; Smith et al. 1994) or the greater effects of caffeine when arousal is low. Finally, claims about the negative effects of caffeine withdrawal require closer examination as they can often be interpreted in ways other than caffeine dependence (e.g., expectancy; see Smith 1996; Rubin and Smith 1999). Indeed, in most of the studies that have demonstrated increases in negative affect following caffeine withdrawal, the volunteers have not been blind but have been told or even instructed to abstain from caffeine. This is clearly very different from the double-blind methodology typically used to study effects of caffeine challenge.
Other studies (e.g., Comer et al. 1997) suggest that effects of withdrawal are restricted to mood and that performance is unaltered. Like many areas of caffeine research, some of the effects that have been attributed to withdrawal are open to other interpretations. For example, Lane (1997), Phillips-Bute and Lane (1997), and Lane and Phillips-Bute (1998) compared days when midmorning coffee was either caffeinated or decaffeinated and found that caffeine consumption was associated with better performance and mood. The authors interpret this as a negative effect of caffeine withdrawal, whereas one could interpret it as a positive effect of caffeine. Other studies of caffeine withdrawal effects have methodological problems such as the lack of predrink baselines (e.g., James 1998; Robelin and Rogers 1998) or failure to consider possible asymmetric transfer when using within-subject designs (e.g., James 1998).

In summary, there is good agreement about the behavioral effects of caffeine. Plausible mechanisms have been put forward to account for these results, and it is likely that caffeine induces changes through more than one pathway. Given robust effects and plausible underlying mechanisms, it is now appropriate to consider the practical implications of ingesting caffeine. The next section examines the extent to which caffeine can remove or alleviate low alertness due to factors such as sleep deprivation, working at night, prolonged work, circadian dips (early morning and after lunch), and having a minor illness like the common cold.

REVERSAL OF IMPAIRMENTS DUE TO LOW LEVELS OF AROUSAL

SLEEP DEPRIVATION

It is well established that caffeine increases alertness and that this can interfere with sleep (for a review, see Bonnet and Arand 1994). Given this effect, it appears plausible that caffeine can reduce the effects of lack of sleep, an issue that has relevance to shift work and sustained operations. Sleep deprivation is both common and critically relevant in society. Sleep loss is common in a broad range of occupations and occurs in normal healthy individuals. Sleepiness poses an increased risk when driving and when carrying out other safety-critical activities. For those for whom sleep loss is inevitable (e.g., emergency services, and military personnel in prolonged operations), judicious use of stimulants such as caffeine is warranted. The American Academy of Sleep Medicine (Bonnet et al. 2005) has examined the efficacy and safety of caffeine use during sleep loss, and their main conclusions are summarized below.

Maintenance of Wakefulness Test (MWT) and Multiple Sleep Latency Test (MSLT)

The most commonly reported measure used to study this topic is the ability to stay awake (as measured by the MWT) or fall asleep (as measured by the MSLT). Fourteen out of fifteen studies reviewed have shown increased wakefulness measured by sleep latency tests following ingestion of caffeine by sleep-deprived volunteers.

Performance and Subjective Alertness

Choice reaction time performance of sleep-deprived individuals has been improved by caffeine in eight studies. Similarly, working memory performance of
sleep-deprived individuals (e.g., digit symbol substitution or grammatical reasoning) has been shown to be improved by caffeine in over ten studies. The effects of caffeine during sleep loss have been examined over a dose range from 75 to 1200 mg per twenty-four hours. Recommended doses are usually in the range of 200–300 mg, mainly because side effects are more prevalent with higher doses (e.g., 600 mg may lead to tachycardia, particularly in women; Lagarde et al. 2000). Caffeine administration typically improves performance during sleep loss as compared with placebo, but performance and alertness often continue to decline even when caffeine is given due to further sleep loss, circadian variations, and caffeine half-life.

Self-reported alertness decreases with sleep loss, and ratings of fatigue increase. Studies that have monitored mood typically show that caffeine ameliorates these changes with a similar time course to that seen for performance variables. However, some studies have not obtained these results, and it has been suggested that some “subjective tolerance” to caffeine may develop with prolonged testing.

Studies have generally shown that doses of 200–300 mg caffeine produce few side effects, whereas higher doses (600 mg+) may increase mild symptoms (e.g., gastrointestinal upset, nervousness, and muscle twitching). Based on these findings, the review concluded that caffeine can increase alertness and improve performance at doses of 75–150 mg after acute restriction of sleep and at doses of 200–600 mg after a night or more of total sleep loss. Caffeine is unlikely to have major disruptive effects on sleep that follows eight hours or longer after administration. Prolonged administration is not recommended due to the increasing likelihood of side effects with high doses.

This topic has continued to be studied since the above review. Results show that similar effects can be observed in rats (Alhaider et al. 2010) and monkeys (van Vliet et al. 2008), which suggests that benefits do not reflect removal of effects of caffeine withdrawal (Keane and James 2008). Recent studies have shown that caffeine does not remove all of the negative effects of sleep loss (e.g., caffeine may not prevent detrimental effects of sleep on more complex cognitive functions [Gottlieb et al. 2006] or the integration of emotion and decision making [Killgore et al. 2007]). Research has also suggested individual differences in the effects of caffeine on sleep-deprived volunteers (e.g., females given caffeine were worse than males; Killgore et al. 2008). Others (e.g., Anderson and Horne 2008) have shown that similar effects to caffeine may be produced by telling a person given a placebo that they have had caffeine. Other research has focused on individuals with pathological sleepiness and shown that caffeine can reduce performance impairments seen in sleep apnea (Norman et al. 2008).

Another recent development has been examination of how caffeine influences a model of fatigue, sleep deprivation, and circadian rhythms (Benitez et al. 2009). This model highlights patterns in data suggesting that there is a performance inhibitor that increases and saturates over a period of continuous wakefulness. Caffeine produces competitive inhibition of this inhibitor, whereas there is a multiplicative relationship between circadian rhythm and the performance inhibitor. Finally, another study (Jay et al. 2006) has extended our knowledge of caffeine and sleep loss by examining functional energy drinks. The results showed that energy drinks may be effective in reducing sleepiness associated with a single night shift.
The next section considers applied situations and the first addresses whether caffeine can prevent injuries and errors in shift workers.

**A REVIEW OF RANDOMIZED TRIALS OF CAFFEINE AND COGNITIVE PERFORMANCE**

Ker et al. (2010) have reviewed the effects of caffeine for preventing injuries, errors, and cognitive problems caused by impaired alertness in persons with jet lag or doing shift work. Thirteen trials were included, but none measured injury, two measured error, and the remaining trials assessed cognitive performance. The trials assessing the impact on errors found that caffeine significantly reduced the number of errors compared to placebo. Caffeine improved concept formation and reasoning, memory, orientation, attention, and perception. No benefit was found for language skills or verbal functioning. The results were largely from studies involving young participants under simulated conditions, and further research is needed on older workers and real-world shift work. Ker et al. conclude “Based on the current evidence, there is no reason for healthy individuals who already use caffeine within recommended levels to improve their alertness to stop doing so” (2010: ).

**CAFFEINE AND CIRCADIAN TROUGHS**

**Night Work**
Sleep deprivation has been the most widely studied low-arousal state in caffeine studies. Other research (e.g., Smith et al. 1993) has examined beneficial effects of caffeine over the course of the night. The results again show that caffeine can reduce the decline in alertness and psychomotor performance, although a similar circadian pattern is observed in caffeine and no-caffeine groups.

**Postlunch Dip**
Another time of day associated with reduced alertness is the early afternoon. Again, studies involving ingestion of caffeine after lunch have shown that caffeine can reduce the postlunch dip in alertness and sustained attention (Smith et al. 1991).

**Early Morning**
Many laboratory studies of the behavioral effects of caffeine have been carried out in the early morning. This is a time of day when alertness is below optimum, and this may be partially responsible for some of the observed benefits.

**Evening**
Circadian alertness starts to decline in the evening, and studies have shown that caffeine may improve performance and alertness at this time (Smith et al. 1993). However, fatigue in the evening is likely to be due to a combination of endogenous rhythms and fatigue due to the activity over the day. Indeed, research that has examined effects of prolonged work in the evening has been able to demonstrate both effects of caffeine seen in alert individuals and those effects that are only observed when alertness is reduced (Smith et al. 2005).
The next section reviews the effects of caffeine in counteracting the effects of prolonged work.

**CAFFEINE AND SUSTAINED WORK**

One source of reduced alertness is prolonged work. Indeed, even in short laboratory studies, alertness usually decreases over the course of the task battery. This shows that even when we are studying reasonably alert individuals, we are often studying effects of caffeine on reduced alertness. Caffeine has a much bigger effect when alertness is reduced by work, and this can be seen by comparing effects on rating of alertness taken at the start of a test battery and those at the end (caffeine has a much bigger effect on the posttask ratings). It also explains why studies with pre-caffeine baselines can be more sensitive as the volunteer may be fatigued by completing the baseline test battery.

Laboratory studies of prolonged work have shown that caffeine can reduce fatigue. For example, Smith et al. (1994) examined effects of caffeine given after eight hours of performing laboratory tasks on performance over the subsequent four hours. The results showed that caffeine reduced the fatigue seen in the placebo group (after twelve hours, the caffeine group was at the same level of performance as it had been six hours earlier). Smith et al. (2005) examined effects of caffeine after a day of normal consumption on prolonged performance in the evening. The results showed initial benefits of caffeine consumption that became more widespread as the session continued.

Other research has examined effects of caffeine in simulations of extreme situations (usually military operations), and these are described in the next section.

**SUSTAINED MILITARY OPERATIONS**

Lieberman et al. (2002) investigated whether caffeine would reduce the adverse effects of sleep deprivation and exposure to severe environmental and operational stress. They studied U.S. Navy Sea-Air-Land trainees and found that even in the most adverse circumstances, moderate doses of caffeine improved vigilance, learning, memory, and mood state. A dose of 200 mg appeared to be optimal under such conditions. Lieberman et al. conclude, “When cognitive performance is critical and must be maintained during exposure to severe stress, administration of caffeine may provide a significant advantage” (2002: ).

Other research has examined the beneficial effects of caffeinated tube food on pilot performance during a nine-hour simulated U-2 mission (Doan et al. 2006). The results showed that the caffeinated tube food (200 mg caffeine consumed every four hours) maintained cognitive performance at baseline levels over a nine-hour overnight period. Research has considered both cognitive and physical performance measures in sustained operations. McClellan et al. (2005) investigated performance during twenty-seven hours of sustained wakefulness in Special Forces personnel. They found that caffeine (200 mg caffeinated gum administered on three occasions) maintained performance of a reconnaissance vigilance task and also improved running times compared to placebo. However, caffeine had no effect on marksmanship. McClellan
et al. (2007) conducted a similar study over a period of four days and three nights of sustained operations. The results showed caffeine maintained both vigilance and physical performance during sustained operations that require periods of overnight wakefulness and restricted opportunities for daytime sleep.

Alertness can be reduced in many different ways, and one factor that is frequent and widespread is having a minor illness such as the common cold. The next section summarizes the effects of the caffeine in removing the malaise associated with the common cold.

**Caffeine and the Common Cold**

There is considerable evidence that minor illnesses, such as the common cold, are associated with reduced alertness and impaired performance (see Smith [in press] for a review). Smith et al. (1997) examined whether caffeine (1.5 mg/kg) would remove this behavioral malaise. Measures taken prior to the ingestion of caffeine showed that those with a cold reported reduced alertness and had slower reaction times on a simple reaction time task and a five-choice serial response task. The volunteers were then retested following ingestion of caffeine or placebo. Volunteers with colds given caffeine reported a similar level of performance to healthy volunteers, whereas those with colds in the placebo condition continued to show reduced alertness and impaired psychomotor performance.

One must now ask whether beneficial effects of habitual caffeine consumption can be seen in other aspects of everyday life.

**BENEFICIAL EFFECTS OF HABITUAL CONSUMPTION OF CAFFEINE**

There has been far less research on the effects of regular caffeine consumption than on acute effects. However, a number of papers suggest that high consumers of caffeine demonstrate better performance (e.g., Loke 1988, 1989). The strongest evidence for beneficial effects of regular caffeine consumption comes from a study by Jarvis (1993). He examined the relationship between habitual coffee and tea consumption and cognitive performance using data from a cross-sectional survey of a representative sample of over 9,000 British adults. Participants completed tests of simple reaction time, choice reaction time, incidental verbal memory, and visuo-spatial reasoning, in addition to providing self-reports of usual coffee and tea intake. After controlling extensively for potential confounding variables, a dose–response trend for improved performance with higher levels of coffee consumption (best performance associated with about 400 mg caffeine per day) was found for all tests. Estimated overall caffeine consumption showed a dose–response relationship to improved cognitive performance that was strongest in those who had consumed high levels for the longest time period (the fifty-five years plus age group). Studies by Hogervorst et al. (1998) and Rogers and Dernoncourt (1998) have failed to replicate these effects using acute caffeine challenges, suggesting that the above effects reflect regular consumption patterns rather than recent intake of caffeine.

Other research has demonstrated that effects of caffeine on artificial laboratory tasks extend to simulations of real-life activities (e.g., driving; Horne and Reyner
Diet, Brain, Behavior: Practical Implications

A study of simulated assembly line work (Muehlbach and Walsh 1995) also demonstrated significant improvements after caffeine on five consecutive nights and showed no decrements when caffeine was withdrawn.

CAFFEINE AT WORK

Smith (2005) examined the impact of habitual caffeine consumption on performance and safety at work. In the first study, volunteers, all of whom were regular caffeine consumers, rated their alertness and carried out a simple reaction time task before and after work on a Monday and Friday. Caffeine consumption during the day was recorded, and volunteers were subdivided into low and high consumers based on a median split (220 mg/day). The results showed that those who consumed higher levels of caffeine reported significantly greater increases in alertness over the working day and a significantly smaller slowing of reaction time.

The second study involved secondary analyses of a database formed by combining the Bristol Stress and Health at Work and Cardiff Health and Safety at Work studies. In the first analyses, associations between caffeine consumption and frequency of cognitive failures were examined in a sample of 1,253 white-collar workers. The second set of analyses examined associations between caffeine consumption and accidents at work in a sample of 1,555 workers who were especially at risk of having an accident. The results from the second study demonstrated significant associations between greater caffeine consumption and fewer cognitive failures and accidents at work. After controlling for possible confounding factors, it was found that higher caffeine consumption was associated with about half the risk of frequent or very frequent cognitive failures and a similar reduction in risk for accidents at work. Overall, the results from the three analyses confirmed that caffeine consumption may have benefits for performance and safety at work.

CAFFEINE OUTSIDE OF WORK

Smith (2009) conducted secondary analyses of a large epidemiological database to examine associations between caffeine consumption and cognitive failures (errors of memory, attention, and action) in a nonworking sample. Associations between caffeine consumption and physical and mental health problems were also examined. After controlling for possible confounding factors, significant associations between caffeine consumption and fewer cognitive failures were observed. Overall, the results show that caffeine consumption may benefit cognitive functioning in a nonworking population. This confirms earlier findings from working samples. This beneficial effect of caffeine was not associated with negative health consequences.

CAFFEINE AND SOCIAL BEHAVIOR

Most of the studies of caffeine have examined behavioral changes of an individual. Tse et al. (2009) examined the effects of caffeinated coffee on cooperative behavior. The results showed that caffeine improved social support and reduced negative affect. Animal studies show that caffeine can increase aggression in the rat (Wilson
et al., 2000), whereas the only laboratory study of acute caffeine and aggression has shown a decrease in aggressive responding after caffeine (Cherek et al., 1984). Similarly, there is a lack of information on caffeine and risk taking. Recent studies have suggested a link between high consumption of energy drinks and risky or anti-social behaviors (Miller, 2008; O’Brien et al., 2008; Jones and Lejuez, 2005). These cross-sectional studies do not rule out the influence of other possible confounders or reverse causality (e.g., individuals who habitually take risks choose to consume energy beverages). Further research is needed on this topic.

CAFFEINE AND DRIVING

It has been frequently shown that sleepiness is a major cause of road traffic accidents. The previous section showed that caffeine can reduce some of the negative effects of low alertness, and this has been examined using simulated driving (e.g., De Valck et al., 2003). For example, Horne and Reyner conducted a number of studies looking at (1) the efficacy of 200 mg caffeine with sleep-restricted and completely sleep-deprived drivers (Reyner and Horne, 2000), (2) an energy drink containing caffeine with sleep-restricted drives (Horne and Reyner, 2001), and (3) “a functional energy drink” and sleep-restricted drivers (Reyner and Horne, 2002). The results from these studies showed that caffeine generally reduced the impaired driving performance seen in sleepy drivers given placebo.

Philip et al. (2006) extended these results by examining the effects of sleepiness and caffeine on real-life driving. Extended driving and sleepiness resulted in an increase in lane crossing, which was reduced by 200 mg of caffeine. As shown in previous sections of this chapter, fatigue can be induced in a number of ways. Brice and Smith (2001) conducted a study which involved one hour of simulated driving before and after either caffeine or placebo. Volunteers were also given a battery of tasks measuring subjective alertness and sustained attention. Caffeine reduced steering variability, which in real-life driving may lead to lane crossing, and increased subjective alertness and improved cognitive vigilance. This suggests that results found after caffeine with artificial laboratory tasks may be applicable to real-life activities involving similar functions.

Driving performance can be impaired by a number of factors, the most widely studied being alcohol. Liquori and Robinson (2001) examined whether caffeine would reduce an alcohol-induced impairment of simulated driving. The results suggested that caffeine may increase alertness and improve reaction time after alcohol use but will not completely counteract the alcohol impairments seen in driving.

James and Keane (2007) argue that many of the effects of caffeine seen in studies of driving can be interpreted in terms of reversal of the effects of caffeine withdrawal. One method of distinguishing a benefit of caffeine from a reversal of caffeine withdrawal is to compare caffeine consumers with nonconsumers. This could be studied using an epidemiological approach examining associations between caffeine consumption and road traffic accidents. For example, Smith (submitted) examined a community sample from South Wales (N = 6,648). The respondents provided information on involvement in road traffic accidents; 3.6 percent of nonconsumers of caffeine were involved in a road accident requiring medical attention, whereas
only 2.2 percent of caffeine consumers were. Logistic regression analyses, including demographic, lifestyle, and psychosocial characteristics, showed that consumption of caffeine nearly halved the risk of being in a road accident (OR = 0.58 CI 0.35, 0.98). This result confirms previous research showing that caffeine reduces the risk of accidents (at work) and supports the existing literature and information campaigns about the positive benefits of caffeine for road safety.

**Prevention of Cognitive Decline in the Elderly**

Animal studies suggest that habitual caffeine consumption may prevent memory decline (Cunha and Agostinho 2010). Several epidemiological studies have examined associations between consumption of caffeine and dementia. A recent systematic review and meta-analysis (Santos et al. 2010) considered nine cohort and two case control studies. The outcomes examined were Alzheimer’s disease (four studies), dementia or cognitive impairment (two studies), and cognitive decline (three studies). The summary relative risk for the association between caffeine intake and the different cognitive measures was 0.84 [95 percent CI: 0.72–0.99]. This suggests a trend toward a protective effect of caffeine, but the large methodological heterogeneity across a small number of studies precludes more definitive conclusions. Further research is clearly needed in this area.

**Mental Health**

Anecdotal evidence suggests that when individuals have consumed an excessive amount of caffeine, they may become anxious. Similarly, some psychiatric patients attribute their problems to consumption of caffeine, which has led to a diagnosis of “caffeinism.” Other patients, especially those with anxiety disorders, report that caffeine may exacerbate their problems. The validity of these statements will now be assessed by consideration of the literature on these topics.

Lieberman stated that it appears that caffeine can increase anxiety when administered in single bolus doses of 300 mg or higher, which is many times greater than the amount present in a single serving of a typical caffeine-containing beverage. However, in lower doses it appears to have little effect on this mood-state or, under certain circumstances, it may even reduce anxiety levels. It has also been observed that caffeine reduces self-rated depression when administered in moderate doses (Lieberman, 1988). (1992: )

The literature supports Lieberman’s view since only a small proportion of the studies reviewed show increases in anxiety following administration of caffeine. Overall, these results suggest that increases in anxiety following caffeine are often only found following consumption of amounts that would rarely be ingested by the majority of people.

It is important to assess whether caffeine leads to mood problems when the person ingesting it already has a high level of anxiety. It has been claimed that some people abstain from caffeinated drinks because of the accompanying jitteriness and
nervousness (Goldstein et al. 1969). Other authors have even gone as far as to suggest that caffeine acts as a “fairly convincing model of generalised anxiety” (Lader and Bruce 1986). Caffeinism refers to a constellation of symptoms associated with very high caffeine intake that are virtually indistinguishable from severe chronic anxiety (Greden 1974). Caffeinism is usually associated with daily intakes of between 1000 and 1500 mg. However, it appears to be a rather specific condition, and there is little evidence for correlations between caffeine intake and anxiety in either nonclinical volunteers (Lynn 1973; Hire 1978) or psychiatric outpatients (Eaton and Mcleod 1984). Other research has investigated whether caffeine is capable of increasing the anxiety induced by other stressors. Shanahan and Hughes (1986) found that 400 mg of caffeine increased anxiety when paired with a stressful task. However, other research (e.g., Hasenfratz and Battig 1992; Smith et al. 1997b) has not been able to provide any evidence of interactive effects of caffeine and stress.

Recent research has shown an association between ADORA2A and DRD2 polymorphisms and caffeine-induced anxiety (Childs et al. 2008). Adenosine receptors functionally interact with dopamine receptors in the brain. Functional polymorphisms in the genes for either adenosine or dopamine receptors may, therefore, affect responses to caffeine. Childs et al. (2008) found that 50 mg caffeine didn’t increase anxiety in any individuals, whereas 450 mg caffeine increased it in the majority of the volunteers. With a dose of 150 mg caffeine, anxiety was associated with ADORA2A and DRD2 polymorphisms.

In contrast, moderate caffeine intake has been associated with fewer depressive symptoms and a lower risk of suicide (see Lara 2010 for a review). This effect of caffeine on depression may have other knock-on effects with regard to health. Smith (in preparation) conducted secondary analyses of a large epidemiological database ($N = 2,750$) to examine associations between caffeine and both chronic and acute health outcomes. Many of the initial associations between caffeine and health were no longer significant when potential confounders were examined. However, caffeine consumption was still significantly associated with reduced depression in the final regressions. Caffeine consumption was also associated in a dose–response fashion with fewer upper respiratory tract symptoms. This suggests that caffeine may influence the immune system, either directly or by reducing depression (a well-established risk factor for immunosuppression).

CAFFEINE AND CHILDREN AND ADOLESCENTS

Ingestion of caffeine from naturally occurring sources has been largely restricted to adults, but it is now added, sometimes in large quantities, to drinks that are consumed by children. Our knowledge of the effects of caffeine on the behavior of children needs to be extended by further research. The current position on this topic can be briefly summarized as follows (for a more detailed account, see Temple 2009).

Older studies of the behavioral effects of caffeine on children have shown similar effects to those observed in adults (Bernstein et al. 1994; Elkins et al. 1981; Rapoport et al. 1981). Effects in children are often smaller than those observed in adults, which may reflect the smaller doses consumed.
It is generally agreed that caffeine intake by pregnant women should be kept at a low level (below 200 mg) (CARE Study Group 2008). However, there is no evidence showing that caffeine consumption during pregnancy or childhood influences brain development.

HEALTH EFFECTS OF CAFFEINE CONSUMPTION

It is important to conduct a cost–benefit analysis when considering the effects of caffeine. Benefits with respect to caffeine usually refer to behavioral outcomes, and costs reflect possible long-term health effects. Caffeine has been linked with a range of possible health problems, but most of these associations are not significant when confounding factors are adjusted for (Nawrot et al. 2003). Indeed, in recent years there have been suggestions that caffeine consumption may have health benefits. Daly (2007) argues that studies of caffeine have played a key part in defining the role of adenosine receptors, phosphodiesterases, and calcium release channels in physiological processes. Caffeine and various analogs, the latter designed to enhance potency and selectivity toward specific biological targets, are potential therapeutic agents for intervention in Alzheimer’s disease, asthma, cancer, diabetes, and Parkinson’s disease.

CONCLUSIONS

Research on the behavioral effects of caffeine has provided an established profile of the changes in mood and performance that occur after ingestion. A systems neuroscience approach provides plausible mechanisms for these effects. For example, the alerting effects seen in fatigued individuals can be accounted for by blockade of adenosine receptors. In addition, explanations based on changes in cognition can explain behavioral effects of caffeine in terms of faster encoding of new information and changes in the tail of the reaction time distribution. A systems neuroscience approach should also look at effects at the level of real-life behavior, both of the individual and in society. The research reviewed here has shown that impairments in real-life activities due to fatigue can be reduced by caffeine. Such effects may be especially important for safety-critical activities such as driving.

There is also evidence that caffeine may prevent or reduce the impact of diseases associated with behavioral problems (e.g., depression and Alzheimer’s disease). Further research is required to determine effects of caffeine on well-being rather than removal of functional deficits. Similarly, more research is needed on the effects of caffeine on children and on a wider range of social behavior.

REFERENCES


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Academic Press
Caffeine and Caffeinated Energy Drinks

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BACKGROUND

The effects of caffeine have been widely studied and a number of recent reviews cover different outcome measures. Indeed, the present author has written a number of reviews of the behavioral effects of caffeine and has suggested that a cost–benefit analysis is an appropriate way of organizing the literature. The first part of this article will summarize research which demonstrates beneficial effects of caffeine. Earlier reviews suggested that the behavioral effects of caffeine are often positive except when one considers very large doses and sensitive individuals. The issue of dependence and negative effects associated with withdrawal clearly represent an area where one needs to assess possible costs of caffeine consumption. Consumption of energy drinks by children has become an important recent issue. These drinks often have high levels of caffeine and are being consumed by a potentially vulnerable sample. An overview of our current knowledge of these two topics will be given in the second part of the article.

Considerable detail about sources of caffeine, the pharmacology of caffeine, and levels of consumption is available elsewhere. The next section provides a very brief summary of these topics. Caffeine (1,3,7-trimethylxanthine) is a member of a class of naturally occurring substances termed methylxanthines. Plasma levels of caffeine peak 15–45 min after ingestion and the half-life is between 5 and 6 h. A variety of factors influence the metabolism. For example, in pregnant women the half-life can increase to 18 h. Oral contraceptive use
increases the half-life to 11 h whereas in cigarette smokers the half-life is only about 3 h which may account for the high level of consumption found in this group.

Caffeine acts by blocking the effects of the naturally occurring neuromodulator adenosine. This produces an increase in central nervous system (CNS) activity which is associated with changes in many neurotransmitter systems. Caffeine has other CNS effects (e.g., influencing blood flow to the brain). Many of these CNS effects of caffeine are unlikely to occur with the amounts consumed by humans.

Caffeine occurs naturally in a number of foods, is employed as a food additive, and is added to medications. It occurs naturally in coffee, tea, and cocoa and the exact amount present will depend on growing conditions and preparation. Some rough approximations of the caffeine content of products are: filter coffee – 100–150 mg (5 oz cup); instant coffee – 50–60 mg; tea – 35–45 mg; milk chocolate – up to 15 mg; dark chocolate – up to 35 mg. It is also added to soft drinks (e.g., Cola 40 mg in a 12 oz serving). Higher amounts are added to energy drinks (70 mg+). Caffeine is also added to over-the-counter (OTC) medications (e.g., to analgesics, usually in the region of 30–50 mg per tablet). Caffeine tablets can also be purchased and the recommended dose to increase alertness is 100–200 mg. Several estimates suggest that average intake is about 200 mg per day. Problems usually only occur when excessive amounts are consumed (500 mg a day plus) and these problems usually take the form of an increase in anxiety. Some individuals are very sensitive to effects of caffeine and even small amounts can cause adverse reactions. Most individuals control their consumption of caffeine. For example, consumption usually occurs when alertness is reduced (e.g., early in the morning; after prolonged work; after lunch) and is reduced at times when high alertness is undesirable (e.g., before going to sleep).

**BENEFICIAL EFFECTS OF CAFFEINE**

In a recent review of the literature on possible beneficial effects of human caffeine consumption, Michael Glade concludes that moderate amounts of caffeine lead to the following benefits:

1. Increased energy availability.
2. Increased daily energy expenditure.
3. Decreased fatigue.
4. Decreased sense of effort associated with physical activity.
5. Enhanced physical performance.
8. Increased alertness, wakefulness, and feelings of energy.
9. Decreased mental fatigue.
10. Faster reactions.
11. Increased accuracy of reactions.
12. Increased ability to concentrate and focus attention.
14. Increased ability to solve problems requiring reasoning.
15. Increased ability to make the correct decisions.
16. Enhanced cognitive functioning capabilities and neuromuscular coordination.

**CAFFEINE AND SPORTS PERFORMANCE**

A recent position paper on caffeine and sports performance from the International Society of Sports Nutrition can be summarized as follows:

1. Caffeine is effective for enhancing sport performance in trained athletes when consumed in low-to-moderate dosages (~3–6 mg kg\(^{-1}\)) and overall does not result in further enhancement in performance when consumed in higher dosages (≥9 mg kg\(^{-1}\)).
2. Caffeine exerts a greater ergogenic effect when consumed in an anhydrous state as compared to coffee.
3. It has been shown that caffeine can enhance vigilance during bouts of extended exhaustive exercise, as well as periods of sustained sleep deprivation.
4. Caffeine is ergogenic for sustained maximal endurance exercise, and has been shown to be highly effective for time-trial performance.
5. Caffeine supplementation is beneficial for high-intensity exercise, including team sports such as soccer and rugby, both of which are categorized by intermittent activity within a period of prolonged duration.
6. The literature is equivocal when considering the effects of caffeine supplementation on strength-power performance, and additional research in this area is warranted.
7. The scientific literature does not support caffeine-induced diuresis during exercise, or any harmful change in fluid balance that would negatively affect performance.

**CAFFEINE AND SLEEP DEPRIVATION**

The American Academy of Sleep Medicine has examined the efficacy and safety of caffeine use during sleep loss and their main conclusions are summarized below:
The most commonly reported measure used to study this topic is the ability to stay awake or fall asleep. Fourteen out of 15 studies reviewed have shown increased wakefulness measured by sleep latency tests following ingestion of caffeine by sleep-deprived volunteers.

Choice reaction time performance of sleep-deprived individuals has been improved by caffeine in eight studies. Similarly, working memory performance of sleep-deprived individuals has been shown to be improved by caffeine in over 10 studies. The effects of caffeine during sleep loss have been examined over a dose range from 75 to 1200 mg per 24 h. Recommended doses are usually in the range of 200–300 mg, mainly because side effects are more prevalent with higher doses. Caffeine administration typically improves performance during sleep loss as compared with placebo, but performance and alertness often continue to decline even when caffeine is given due to further sleep loss, circadian rhythms, and caffeine half-life.

Subjective alertness decreases with sleep loss and ratings of fatigue increase. Studies that have monitored mood typically show that caffeine ameliorates these subjective changes with a similar time course to that seen for performance variables. However, some studies have not obtained these results and it has been suggested that some “subjective tolerance” to caffeine may develop with prolonged testing.

Studies have generally shown that doses of 200–300 mg caffeine produce few side effects whereas higher doses (600 mg+) may increase mild symptoms (e.g. gastrointestinal upset, nervousness, muscle twitching). Based on these findings, the review concluded that caffeine can increase alertness and improve performance at doses of 75–150 mg after acute restriction of sleep and at doses of 200–600 after a night or more of total sleep loss. Caffeine is unlikely to have major disruptive effects on sleep that follows 8 h or longer after administration. Prolonged administration is not recommended due to the increasing likelihood of side effects with high doses.

CAFFEINE AND SUSTAINED MILITARY OPERATIONS

A number of studies have examined effects of caffeine in sustained military operations. Harris Lieberman and colleagues conclude that “When cognitive performance is critical and must be maintained during exposure to severe stress, administration of caffeine may provide a significant advantage”.

Other research has examined beneficial effects of caffeinated tube food on pilot performance during a 9-h simulated U-2 mission. The results showed that the caffeinated tube food (200 mg caffeine consumed every 4 h) maintained cognitive performance at baseline levels over a 9-h overnight period. Research has considered both cognitive and physical performance measures in sustained operations (e.g. performance during 27 h of sustained wakefulness in Special Forces personnel). The results showed that caffeine (200 mg caffeinated gum administered on three occasions) maintained performance of a reconnaissance vigilance task and also improved running times compared to placebo. A similar study was conducted over a period of 4 days and three nights of sustained operations. The results showed caffeine maintained both vigilance and physical performance during sustained operations that require periods of overnight wakefulness and restricted opportunities for daytime sleep.

CAFFEINE AND SHIFT WORK

A recent review has considered the effects of caffeine for preventing injuries, errors, and cognitive problems caused by impaired alertness in persons doing shift work. Thirteen trials were included but none measured injury, two measured error, and the remaining trials assessed cognitive performance. The trials assessing the impact on errors found that caffeine significantly reduced the number of errors. Caffeine improved concept formation and reasoning, memory, orientation, attention, and perception. The results were largely from studies involving young participants under simulated conditions and further research is needed on older workers and real world shift work. The authors conclude that “Based on the current evidence, there is no reason for healthy individuals who already use caffeine within recommended levels to improve their alertness to stop doing so.”

CAFFEINE AND SAFETY AT WORK

Research by the author has examined the impact of habitual caffeine consumption on performance and safety at work. In the first study volunteers, all of whom were regular caffeine consumers, rated their alertness and carried out a simple reaction time task before and after work on a Monday and Friday. Caffeine consumption during the day was recorded and volunteers were sub-divided into low and high consumers on the basis of a median split (220 mg day−1). The results showed that those who consumed higher levels of caffeine reported significantly greater increases in alertness over the working day and a significantly smaller slowing of reaction time.
The second study involved secondary analyses of a database formed by combining the Bristol Stress and Health at Work and Cardiff Health and Safety at Work studies. In the first analyses associations between caffeine consumption and frequency of cognitive failures were examined in a sample of 1253 white-collar workers. The second set of analyses examined associations between caffeine consumption and accidents at work in a sample of 1555 workers who were especially at risk of having an accident. The results from the second study demonstrated significant associations between caffeine consumption and fewer cognitive failures and accidents at work. After controlling for possible confounding factors, it was found that higher caffeine consumption was associated with about half the risk of frequent/very frequent cognitive failures and a similar reduction in risk for accidents at work. Overall, the results from the three analyses confirmed that caffeine consumption may have benefits for performance and safety at work.

**CAFFEINE AND HUMAN ERROR**

Other research by the author has involved secondary analyses of a large epidemiological database to examine associations between caffeine consumption and cognitive failures (errors of memory, attention, and action) in a non-working sample. Associations between caffeine consumption and physical and mental health problems were also examined. After controlling for possible confounding factors significant associations between caffeine consumption and fewer cognitive failures were observed. Overall, the results show that caffeine consumption may benefit cognitive functioning in a non-working population. This confirms earlier findings from working samples. This beneficial effect of caffeine was not associated with negative health consequences.

**CAFFEINE AND DRIVING**

A number of studies by Jim Horne and colleagues have examined (1) the efficacy of 200 mg caffeine with restricted and completely sleep-deprived drivers, (2) an energy drink containing caffeine with sleep-restricted drivers, and (3) “a functional energy drink” and sleep-restricted drivers. The results from these studies showed that caffeine generally reduced the impaired driving performance that was seen in sleepy drivers given placebo.

Others have extended these results by examining the effects of sleepiness and caffeine on real-life driving. Extended driving and sleepiness resulted in an increase in lane crossing, which was reduced by 200 mg caffeine. As shown in previous sections of this chapter, fatigue can be induced in a number of ways. One study involved 1 h of simulated driving before and after either caffeine or placebo. In addition, volunteers carried out a battery of tasks measuring subjective alertness and sustained attention. Caffeine reduced steering variability (which in real-life driving may lead to lane crossing) and led to an increase in subjective alertness and improved cognitive vigilance. This suggests that results found after caffeine with artificial laboratory tasks may be applicable to real-life activities involving similar functions.

Driving performance can be impaired by a number of factors, the most widely studied being alcohol. One study examined whether caffeine would reduce an alcohol-induced impairment of simulated driving. The results suggested that caffeine may increase alertness and improve reaction time after alcohol use but will not completely counteract the alcohol impairments seen in driving.

Jack James and colleagues argue that many of the effects of caffeine seen in studies of driving can be interpreted in terms of reversal of the effects of caffeine withdrawal. One method of distinguishing a benefit of caffeine from a reversal of caffeine withdrawal is to compare caffeine consumers with non-consumers. There is a need to do this using an epidemiological approach to examine associations between caffeine consumption and road traffic accidents.

Research by the author has examined a community sample from South Wales (N = 6648). These respondents provided information on involvement in road traffic accidents. 3.6% of non-consumers of caffeine were involved in a road accident requiring medical attention compared to only 2.2% of caffeine consumers. Logistic regressions, including demographic, lifestyle, and psychosocial characteristics showed that consumption of caffeine nearly halved the risk of being in a road accident (odds ratio [OR] = 0.58 confidence intervals [CI]: 0.35, 0.98). This result confirms previous research showing that caffeine reduces the risk of accidents (at work) and supports the existing literature and information campaigns about the positive benefits of caffeine for road safety.

**CAFFEINE AND DEMENTIA**

Several epidemiological studies have examined associations between consumption of caffeine and dementia. A recent systematic review and meta-analysis considered nine cohort and two case control studies. The outcomes examined were Alzheimer’s disease (four studies), dementia or cognitive impairment (two studies), and cognitive decline (three studies). The
CAFFEINE: AN ATYPICAL DRUG OF DEPENDENCE

CAFFEINE AND ANXIETY AND DEPRESSION

Caffeinism refers to a constellation of symptoms associated with very high caffeine intake that are virtually indistinguishable from severe chronic anxiety. Caffeinism is usually associated with daily intakes of between 1000 and 1500 mg. However, it appears to be a rather specific condition and there is little evidence for correlations between caffeine intake and anxiety in either non-clinical volunteers or psychiatric outpatients. Other research has investigated whether caffeine is capable of increasing the anxiety induced by other stressors. It has been found that 400 mg of caffeine increased anxiety when paired with a stressful task. However, other research has not been able to provide any evidence of interactive effects of caffeine and stress.

Recent research has shown an association between ADORA2A and DRD2 polymorphisms and caffeine-induced anxiety. Adenosine receptors functionally interact with dopamine receptors in the brain. Functional polymorphisms in the genes for either adenosine or dopamine receptors may, therefore, affect responses to caffeine. A recent study found that 50 mg caffeine did not increase anxiety in any individuals whereas 450 mg caffeine increased it in the majority of the volunteers. With a dose of 150 mg caffeine anxiety was associated with ADORA2A and DRD2 polymorphisms.

In contrast, moderate caffeine intake has been associated with fewer depressive symptoms and a lower risk of suicide. This effect of caffeine on depression may have other knock on effects with regards to health. The author conducted secondary analyses of a large epidemiological database ($N = 2750$) to examine associations between caffeine and both chronic and acute health outcomes. Many of the initial associations between caffeine and health were no longer significant when potential confounders were examined. However, caffeine consumption was still significantly associated with reduced depression in the final regressions. Caffeine consumption was also associated in a dose response fashion with fewer upper respiratory tract symptoms. This suggests that caffeine may influence the immune system, either directly, or by reducing depression (a well-established risk factor for immunosuppression). Other research by the author has shown that caffeine removes the malaise (fatigue, psychomotor slowing) associated with minor illnesses such as the common cold.

CHRONIC HEALTH EFFECTS OF CAFFEINE CONSUMPTION

It is important to conduct a cost–benefit analysis when considering the effects of caffeine. Benefits usually refer to behavioral outcomes and costs reflect possible long-term health effects. Caffeine has been linked with a range of possible health problems but most of these associations are not significant when confounding factors are adjusted for. Indeed, in recent years the trend has been for suggestions that caffeine may have health benefits. It has been suggested that studies of caffeine have played a key part in defining the role of adenosine receptors, phosphodiesterases, and calcium release channels in physiological processes. Caffeine and various analogs, the latter designed to enhance potency and selectivity toward specific biological targets, are potential therapeutic agents for intervention in Alzheimer’s disease, asthma, cancer, diabetes, and Parkinson’s disease.

CAFFEINE: AN ATYPICAL DRUG OF DEPENDENCE

Roland Griffiths and colleagues have suggested that caffeine is an excellent model compound for understanding drugs of abuse/dependence. Caffeine can be shown to act as a reinforcing agent, a criterion for dependence, under certain conditions. However, the level of responding is lower than that maintained by addictive drugs such as cocaine and amphetamine and there is little or no evidence for upward dose adjustment. Indeed, it is unclear about the contribution of desirable stimulatory effects and undesirable withdrawal symptoms in the reinforcing properties of caffeine.

It has been suggested that caffeine shares four behavioral pharmacological effects with classic drugs of abuse/dependence: reinforcing effects, discriminative/subjective effects, tolerance, and physical dependence. Similarly, it has been suggested that there are some people who report a compulsive pattern of caffeine use and are physiologically dependent on caffeine and there is evidence that just under 20% of caffeine users show some degree of dependence although this is small compared with nicotine (90% of users show dependence) and at a similar level to alcohol (where 14% show a lifetime prevalence of dependence). The most important issue is the severity of the harmful consequences associated with dependence. Compared to nicotine or alcohol the risks associated with moderate
caffeine consumption are generally low. Although caffeine fulfills some of the criteria for drug dependence and shares with amphetamines and cocaine a certain specificity of action on the cerebral dopaminergic system, the methylxanthine does not act on the dopaminergic structures related to reward, motivation, and addiction.

**CAFFEINE WITHDRAWAL**

Caffeine withdrawal is typically associated with symptoms of headache and drowsiness. These symptoms generally begin slowly, maximize after 1–2 days and are over within a few days. Many of the early studies of this topic used small samples and if one adjusted for the number of analyses carried out one would find few significant effects. The studies also have other undesirable features (e.g. in one study – the subjects were also the authors of the paper). The frequency of caffeine withdrawal has been examined in a population-based survey and in a controlled, blinded experiment. In the survey of over 11 000 people, 61% reported daily caffeine consumption and 11% of the caffeine consumers reported symptoms upon stopping caffeine. When volunteers were unaware that the focus of the study was caffeine withdrawal, reports of symptoms associated with withdrawal were less frequent. Indeed, in another double-blind study by the present author, caffeine withdrawal was associated with an increase in reports of headache but those who continued to consume caffeine also reported more headaches as the study progressed. Volunteers in this study were not very good at discriminating whether they were in the caffeine or no caffeine groups and this suggests that symptoms of caffeine withdrawal may only be apparent if volunteers know that caffeine has been withdrawn. Such a result was obtained in further research by the author which suggests that the dependence associated with caffeine may largely reflect the knowledge that caffeine has been withdrawn rather than a pharmacological dependence.

**BENEFICIAL EFFECTS OF CAFFEINE OR REMOVAL OF NEGATIVE EFFECTS OF WITHDRAWAL?**

Overall, the previous sections confirm that the effects of caffeine on performance are largely beneficial. However, this view has been questioned by Jack James who argues that the beneficial effects of caffeine are really only removal of negative effects produced by caffeine withdrawal. The author has argued against this general view of caffeine effects on a number of grounds. First, it cannot account for the behavioral effects seen in animals or non-consumers where withdrawal cannot occur. Second, caffeine withdrawal cannot account for behavioral changes following caffeine consumption after a short period of abstinence or the greater effects of caffeine when arousal is low. Finally, claims about the negative effects of caffeine withdrawal require closer examination as they can often be interpreted in ways other than caffeine dependence (e.g. expectancy). Indeed, in most of the studies that have demonstrated increases in negative affect following caffeine withdrawal, the volunteers have not been blind but have been told or even instructed to abstain from caffeine. This is clearly very different from the double-blind methodology typically used to study effects of caffeine challenge.

The view that beneficial effects of caffeine reflect degraded performance in the caffeine-free conditions crucially depends on the strength of the evidence for withdrawal effects. Jack James states that “there is an extensive literature showing that caffeine withdrawal has significant adverse effects on human performance”. If one examines the details of the studies cited to support this view one finds that some of them do not even examine performance, and that where they do, any effects are selective, not very pronounced, and largely unrelated to the beneficial effects of caffeine reported in the literature.

Peter Rogers and colleagues have reviewed a number of studies of caffeine withdrawal and performance. They conclude that “in a review of recent studies we find no unequivocal evidence of impaired psychomotor performance associated with caffeine withdrawal”. Indeed, they found that caffeine improved performance in both deprived volunteers and non-consumers. Furthermore, other studies which suggest that withdrawal may impair performance can be interpreted in other ways than deprivation (e.g. changes in state).

The effects of caffeine withdrawal are still controversial. One study showed that caffeine withdrawal impaired short-term memory performance but caffeine ingestion had no effect. In contrast, research by the author has shown that caffeine improved attention in both those who had been deprived of caffeine for a short period and those who had no caffeine for 7 days. Other studies suggest that effects of withdrawal are restricted to mood and that performance is unaltered. Like many areas of caffeine research, some of the effects that have been attributed to withdrawal are open to other interpretations. For example, some studies have compared days when mid-morning coffee was either caffeinated or de-caffeinated. Caffeine consumption was associated with better performance and mood. The authors interpret this as a negative effect of caffeine withdrawal whereas one could equally interpret it as a positive effect.
of caffeine. Other studies of caffeine withdrawal effects have methodological problems such as the lack of pre-drink baselines or failure to consider possible asymmetric transfer when using within subject designs.

Caffeine withdrawal has been widely studied because it is meant to provide crucial evidence on whether caffeine is addictive or leads to some kind of dependence. The most frequent outcome measure has been reporting of headache, but mood has been examined in other studies. Research has shown that caffeine deprivation led to increased reporting of stress by heavy coffee drinkers. This has been confirmed in another study which showed that caffeine withdrawal was associated with feelings of fatigue and decreased feelings of alertness. Indeed, results show that about 10% of volunteers with a moderate daily intake (235 mg day\(^{-1}\)) reported increased depression and anxiety when caffeine was withdrawn. Other research has examined the effects of varying time periods of caffeine deprivation (90 min, overnight and 7 days) on mood. The results showed that overnight caffeine deprivation produced dysphoric symptoms and these mood effects were reduced, but still present, after longer term abstinence. However, close examination of the results does not support this conclusion with only one of the 17 mood scales showing a significant effect.

Recent research in this area has been concerned with two main topics, namely what underlies the increase in symptoms following caffeine withdrawal, and, secondly, whether the positive effects of caffeine reflect removal of negative effects of withdrawal. Peter Dews and colleagues have considered factors underlying caffeine withdrawal and conclude that “non-pharmacological factors related to knowledge and expectation are the prime determinants of symptoms and their reported prevalence on withdrawal of caffeine after regular consumption”.

In contrast, some researchers still suggest that caffeine only has beneficial effects on performance when the person has had caffeine withdrawn. One study reported that caffeine improved performance on a sustained attention task and increased rated alertness when volunteers had been caffeine deprived but had no such effects when they were no longer deprived. However, the results showed an effect of order of treatments with those who received caffeine first continuing to show better performance even when subsequently given placebo.

Research by the author has examined effects of caffeine in the evening after a day of normal caffeine consumption. Caffeine improved performance which casts doubt on the view that reversal of caffeine withdrawal is a major component underlying effects on performance. Further evidence against the caffeine withdrawal explanation comes from recent studies of non-consumers. These studies not only detected few negative effects of withdrawal but also showed that caffeine improved the performance of both withdrawn consumers and non-consumers, a finding that argues strongly against the withdrawal reversal explanation.

Other research has compared the effects of caffeine following abstinence and normal caffeine use. Caffeine had a greater effect on mood in the abstained state. The authors also suggest that choice reaction time showed a similar effect although this would not be significant if adjustments were made for multiple statistical tests. Other aspects of performance showed significant effects of caffeine in both abstained and normal caffeine consumption conditions. Failure to adjust for multiple testing is a common problem in this area of research. In addition, it is often unclear why specific sample sizes or tests are used. Consideration of these factors leads to a very different interpretation of some of the literature. For example, Heatherley and colleagues claim that cognitive performance is only improved by caffeine after 8 h of abstinence. Adjustment for multiple testing shows that none of the effects of caffeine are significant which reflects the low power of the study and failure to covary baseline data. Similarly, claims that 9 to 11-year-old children show negative symptoms of withdrawal which are reversed by caffeine do not hold up when adjustments are made for the number of statistical tests conducted.

A review of caffeine withdrawal has been conducted by Roland Griffiths with a view to validate specific symptoms and signs to define important features of the syndrome. The review covered 57 experimental and 9 survey studies. Symptoms associated with caffeine withdrawal were: headache, fatigue, decreased energy, decreased alertness, drowsiness, depressed mood, difficulty concentrating, and irritability. The incidence of headache was 50% and the incidence of clinically significant distress was 13%. The onset of symptoms occurred 12–24 h after abstinence, with peak intensity at 20–51 h and for a duration of 2–9 days. Abstinence from even low doses (e.g. 100 mg day\(^{-1}\)) produced symptoms. Unfortunately, this review was selective and studies which suggested a different view of caffeine withdrawal were excluded. In addition, there was no attempt to distinguish between negative effects of withdrawal and positive effects of caffeine. For example, certain studies are interpreted in terms of negative effects of caffeine deprivation when they could actual be interpreted in terms of positive effects of caffeine ingestion. This review does not look at the details of the studies and, as stated above, many effects of caffeine deprivation are no longer significant when adjusted for multiple statistical tests.

Recent research has demonstrated that acute caffeine abstinence produces changes in cerebral blood flow...
velocity, EEG, and subjective effects. These vascular effects of caffeine withdrawal are clearly very different from behavioral effects of caffeine which are thought to reflect changes in a variety of neurotransmitter systems. Astrid Nehlig and colleagues present evidence that in animals caffeine does not trigger metabolic increases or dopamine release in brain areas involved in reinforcement or reward. A single photon emission computed tomography (SPECT) assessment of brain activation in humans showed that caffeine activates regions involved in the control of vigilance, anxiety, and cardiovascular regulation but did not affect areas involved in reinforcement and reward.

TOLERANCE

Developing tolerance is a hallmark of substance abuse and dependence. In adults, caffeine-induced tolerance has been shown for some, but not all, outcomes and only in a sub-set of consumers.

CROSS-SENSITIZATION

Cross-sensitization is the process by which taking one drug enhances the response to other drugs with the same neurobiological mechanisms. It has been suggested that caffeine may increase sensitization to nicotine although the correlation between caffeine use and smoking may reflect the faster metabolism of caffeine by smokers. There is no clear relationship between caffeine use and cocaine in humans and if anything, cocaine users are less likely to consume caffeine than non-cocaine users.

CAFFEINE AND CHILDREN AND ADOLESCENTS

Ingestion of caffeine from naturally occurring sources has been largely restricted to adults but it is now added, sometimes in large quantities, to drinks that are consumed by children. Our knowledge of the effects of caffeine on the behavior of children needs to be extended by further research. The current position on this topic can be briefly summarized as follows.

Older studies of the behavioral effects of caffeine on children have shown similar effects to those observed in adults. Effects in children are often smaller than those observed in adults which may reflect the smaller doses consumed.

It is generally agreed that caffeine intake by pregnant women should be kept at a low level (below 200 mg) because of the possible impact on birth problems and reduced body weight of the child. However, there is no evidence showing that caffeine consumption during pregnancy or childhood influences brain development.

A recent review has shown that caffeine containing drinks are now regularly consumed by children. Indeed, some caffeinated products are even marketed to children as young as 4 years old. Our knowledge of effects of caffeine on children is very limited and further research is needed in the area because children may be more sensitive to negative effects of caffeine than adults. This research should examine possible caffeine dependence and also caffeine intoxication. Caffeine intoxication is characterized by the following symptoms: restlessness, nervousness, excitement, insomnia, flushed face, diuresis, and gastrointestinal complaints. It is likely that low consumers of caffeine, such as children, may experience caffeine intoxication following consumption of a high dose (as found in some energy drinks). No empirical studies have been conducted to examine whether children and adolescents develop tolerance to the effects of caffeine. Surveys suggest that 41.7% of teenagers reported tolerance to caffeine and 77.8% reported symptoms of withdrawal. Consumption of caffeinated soft drinks is also associated with poor diet, excess weight, and dental caries.

There is a growing literature that suggests that caffeine use in adolescents and young adults is associated with impulsivity, risk taking, and sensation seeking. Unfortunately, due to the correlational nature of these studies, it is not possible to determine the direction of causality.

ENERGY DRINKS

Energy drinks represent the fastest growing sector in the beverage industry. These drinks often contain five times the amount of caffeine as soft drinks and may also contain taurine, riboflavin, pyridoxine, and various herbal derivatives. Most energy drinks also contain sugar in an amount that exceeds recommended daily allowances. Studies of the effects of energy drinks on behavior confirm that they increase alertness and attention, improve simulated driving when sleepy and can reduce sleepiness in night workers. However, energy drinks that also contain alcohol (6% by volume) have been shown to impair a global measure of cognitive functioning. Energy drinks have also been shown in laboratory studies to increase heart rate and blood pressure.

Energy drink consumption can lead to caffeine intoxication especially in children. Deaths attributed to energy drink consumption have been reported in Australia, Ireland, and Sweden. Health care providers
report the following effects after consumption of energy drinks: dehydration, accelerated heart rates, anxiety, seizures, acute mania, and strokes. The risk of caffeine intoxication may be greater for energy drinks than for other sources of caffeine due to inadequate labeling, advertising, and the consumer demographics.

Energy drinks are often combined with alcohol to increase the positive effects of alcohol ingestion and counteract the depressive effects. This can lead to increased alcohol intake and an increase in adverse events due to alcohol. Indeed, combining energy drinks with alcohol gives the person a false sense of control. Recent research has investigated the extent to which energy drink consumption was a risk factor for alcoholism. The results of a study of over 1000 university students showed that weekly or daily energy drink consumption was associated with more frequent and greater consumption of alcohol. This, of course, could be due to alcohol consumption influencing energy drink consumption rather than the other way around.

### CONCLUSIONS

In conclusion, there are many beneficial effects of caffeine and negative effects are restricted to consumption of high doses by susceptible individuals. Caffeine is almost certainly the most widely used drug of dependence in the world. Despite this, the evidence of morbidity associated with caffeine consumption is slight. Research on caffeine tells us little about the harmful effects of drugs of dependence and shows that caffeine dependence per se is not a problem.

### SEE ALSO

Tobacco, Food Addictions, Khat Addiction, The Biopsychosocial Model of Addiction, Tolerance and Withdrawal

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**List of Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>OTC</td>
<td>over-the-counter</td>
</tr>
<tr>
<td>SPECT</td>
<td>single photon emission computed tomography</td>
</tr>
</tbody>
</table>

**Further Reading**

- James, J.E., Rogers, P.J., 2005. Effects of caffeine on performance and mood: withdrawal reversal is the most plausible explanation. Psychopharmacology 182, 1–8.
- Temple, J.L., 2009. Caffeine use in children: what we know, what we have left to learn, and why we should worry. Neuroscience and Biobehavioral Reviews 33, 793–806.
Speaker Recommended References

DAY 2, SESSION 3: EXPLORING SAFE CAFFEINE EXPOSURE LEVELS

A risk analysis of in utero caffeine exposure is presented utilizing epidemiological studies and animal studies dealing with congenital malformation, pregnancy loss, and weight reduction. These effects are of interest to teratologists, because animal studies are useful in their evaluation. Many of the epidemiology studies did not evaluate the impact of the “pregnancy signal,” which identifies healthy pregnancies and permits investigators to identify subjects with low pregnancy risks. The spontaneous abortion epidemiology studies were inconsistent and the majority did not consider the confounding introduced by not considering the pregnancy signal. The animal studies do not support the concept that caffeine is an abortifacient for the wide range of human caffeine exposures. Almost all the congenital malformation epidemiology studies were negative. Animal pharmacokinetic studies indicate that the teratogenic plasma level of caffeine has to reach or exceed 60 μg/ml, which is not attainable from ingesting large amounts of caffeine in foods and beverages. No epidemiological study described the “caffeine teratogenic syndrome.” Six of the 17 recent epidemiology studies dealing with the risk of caffeine and fetal weight reduction were negative. Seven of the positive studies had growth reductions that were clinically insignificant and none of the studies cited the animal literature. Analysis of caffeine’s reproductive toxicity considers reproducibility and plausibility of clinical, epidemiological, and animal data. Moderate or even high amounts of beverages and foods containing caffeine do not increase the risks of congenital malformations, miscarriage or growth retardation. Pharmacokinetic studies markedly improve the ability to perform the risk analyses. Birth Defects Res (Part B) 92:152–187, 2011.

Key words: caffeine; spontaneous abortion; congenital malformations; growth retardation; toxicokinetics; biological plausibility
INTRODUCTION AND GOALS OF THIS REVIEW

We (Brent and Christian) received a request from the Caffeine Committee of the International Life Science Institute (ILSI) in 2008 to update our 2001 review since many publications dealing with the effects of caffeine had been published (Christian and Brent, 2001). A current literature review of human epidemiology studies, animal studies, and caffeine toxicology studies was performed using the Medline and Toxline data bases, articles in the author’s files, and publications containing important relevant information published earlier than 2001.

Goals of This Review

One of the reasons that epidemiologists have focused so much attention on the effects of caffeine is that caffeine is the most widely used CNS stimulant in the world. At doses achieved in normal human consumption, the main effect mediated by caffeine is interaction with the adenosine receptor, as well as with adrenergic, cholinergic, GABA, and serotonin receptors (Shi et al., 1993; Leon, 2005a,b).

We recognize that well-planned epidemiology studies are the most useful for performing accurate human risk assessment. When epidemiological studies are inconsistent, animal studies that utilize exposures that occur in humans can provide additional information that is necessary to perform a risk analysis. Animal studies are most useful if plasma and tissue blood levels of caffeine and/or caffeine metabolites are measured and can be compared with human exposures. We planned to use the same protocol for estimating the human risks of developmental and reproductive problems that were utilized in the 2001 caffeine review (Christian and Brent, 2001) (Table 1). The data reviewed in this manuscript are divided into three sections: Epidemiology studies, Animal and in vitro toxicology studies, and Pharmacokinetic studies.

EPIDEMIOLOGY STUDIES

We have reviewed human epidemiology publications that deal with

A. Pregnancy loss (miscarriage and spontaneous abortion [SAs])
B. Congenital malformations CMs, and
C. Fetal growth retardation (IUGR, SGA).

Although some of the epidemiological studies have examined more than one developmental effect, many of the studies have focused on one developmental endpoint. Two of the important studies cited and discussed in our 2001 caffeine review (Christian and Brent, 2001) were performed by Klebanoff et al. (1998, 1999). The reason for their importance is that the exposure to caffeine was determined pharmacokinetically by measuring serum caffeine and paraxanthine concentrations.

ETIOLOGY OF SA (MISCELLANEOUS CORRELATES)

Concern about the risk of SA from exposure to caffeine was one of the reasons for preparing this review. Many of the epidemiological studies fail to assess the factors that can alter the accuracy of epidemiological studies dealing with SA.

CAUSES OF SA. SAs, frequently referred to as miscarriages by the public, are common occurrences during pregnancy. According to the World Health Organization, 15% (with a large standard deviation) of women who know that they are clinically pregnant spontaneously abort. Research studies indicate that a higher percentage of embryos are spontaneously aborted before the first-missed menstrual period before the mothers know that they are pregnant (Tables 3 and 4). The lay population and the news media are under the impression that many SAs are due to exposures to some type of toxic agent during the woman’s pregnancy. This is an erroneous conclusion since most early SAs are due to chromosome abnormalities that are determined before conception because of chromosome aberrations that are

Table 1
Evaluating the Allegation of Teratogenicity

| Epidemiological Studies: Controlled epidemiological studies consistently demonstrate an increased incidence of a particular spectrum of embryonic and/or fetal effects in exposed human populations |
| Secular Trend Data: Secular trends demonstrate a positive relationship between the changing exposures to a common environmental agent in human populations and the incidence of a particular embryonic and/or fetal effect |
| Animal Developmental Toxicity Studies: An animal model can be developed, which mimics the human developmental effect at clinically comparable exposures. Since mimicry may not occur in all animal species, animal models are more likely to be developed once there is good evidence for the embryotoxic effects reported in the human. Developmental toxicity studies in animals are indicative of a potential hazard in general rather than the potential for a specific adverse effect on the fetus when there are no human data on which to base the animal experiments |
| Dose–Response Relationship: Developmental toxicity in the human increases with dose (exposure) and the developmental toxicity in animal occurs at a dose that is pharmacokinetically (quantitatively) equivalent to the human exposure |
| Biological Plausibility: The mechanisms of developmental toxicity are understood and the effects are biologically plausible |
| (a) Mechanisms |
| (b) Receptor agonistic or antagonistic studies |
| (c) Enzyme suppression |
| (d) Nature of the malformations |
| (e) Teratology principles |

Modified from Brent (1986, 1995a,b).
inherited, occur during the development of the sperm, or the mother’s ova (eggs). Some maternal diseases can also be responsible (Tables 3–5). Fifty to 60% of the early spontaneously aborted fetuses have chromosomal abnormalities (Bernirschke, 1974; Boue et al., 1975; Simpson, 1980). It has been estimated that up to 30–40% of all fertilized ova in the human are lost within the first three weeks of development (Hertig, 1967). This means that SAs are a common event and are due to many causes (Table 3). SAs can result from inherited or acquired chromosomal abnormalities, inherited diseases, medically or environmentally produced blighted (malformed) embryos, maternal illness, lupus anticoagulant factor (WHO, 1970; Stein et al., 1975; Kline and Stein, 1985; Beckman and Brent, 1986; Abenhaim and Lert, 1991). A more complete list of the causes of SAs is in Table 3.

Epidemiological investigations dealing with the causes of SAs must deal with formidable problems:

(1) A majority of SAs that occur early in pregnancy are due to chromosomal abnormalities that are unrelated to environmental exposures during pregnancy (Tables 2, 4, and 5).

(2) The risk of abortion changes with each day of pregnancy, so that it is essential to properly match controls, to eliminate the selection of two populations with different background SA rates (Table 2).

(3) Attempts to control for the hidden incidence of medical abortions have only limited success (Susser, 1983; Olsen, 1984). “The existence of high rates of medically induced abortion in the population may distort currently employed measures of the rate of SAs” (Susser, 1983). Susser indicated that women not infrequently would report medically induced abortions as SAs (“The Susser effect”).

### Table 2

<table>
<thead>
<tr>
<th>Time from conception</th>
<th>Percent survival to term</th>
<th>Percent loss during interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preimplantation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–6 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7–13 days</td>
<td>55</td>
<td>24.66</td>
</tr>
<tr>
<td>14–20 days</td>
<td>73</td>
<td>8.18</td>
</tr>
<tr>
<td>3–5 weeks</td>
<td>79.5</td>
<td>7.56</td>
</tr>
<tr>
<td>6–9 week</td>
<td>90</td>
<td>6.52</td>
</tr>
<tr>
<td>10–13 week</td>
<td>92</td>
<td>4.42</td>
</tr>
<tr>
<td>14–17 week</td>
<td>96.26</td>
<td>1.33</td>
</tr>
<tr>
<td>18–21 week</td>
<td>97.56</td>
<td>0.85</td>
</tr>
<tr>
<td>22–25 week</td>
<td>98.39</td>
<td>0.31</td>
</tr>
<tr>
<td>26–29 week</td>
<td>98.69</td>
<td>0.30</td>
</tr>
<tr>
<td>30–33 week</td>
<td>98.98</td>
<td>0.30</td>
</tr>
<tr>
<td>34–37 week</td>
<td>99.26</td>
<td>0.34</td>
</tr>
<tr>
<td>38 week</td>
<td>99.32</td>
<td>0.68</td>
</tr>
</tbody>
</table>

The etiology of these abortions is manifold and is listed in Table 3 (Kajii, 1980).

*Data from Kline et al. (1980). An estimated 50 to 70% of all human conceptions are lost in the first 30 weeks of gestation and 78% are lost before term.

*Modified from Schardein (2000).

(4) Reduction of coffee consumption and aversion to other odors and tastes is one of the earliest responses of the “pregnancy signal” that occurs in healthy pregnancies, during the pregnancy stages when chorionic gonadotropin (HCG) is at a high level. The “pregnancy signal” tends to separate the healthy pregnancies from the less healthy ones. Ignoring the importance of the pregnancy symptoms can seriously undermine the accuracy of SA and other reproductive toxicity studies.

### SA Articles That Will Be Discussed


In the Cnattinglus et al. (2000) and the Maconochie et al. (2007) studies there were no increased risks in the groups exposed to more than 500 mg/day of caffeine.

The Cnattinglus et al. (2000) publication was one of the few caffeine studies to consider the fetal karyotype. These authors also obtained information concerning nausea and vomiting symptoms; however, these data were insufficient to evaluate the pregnancy signal. In some of the studies there was no control for the presence or absence of the “pregnancy signal.” The authors attempted to control for many potential confounding factors, but the task is monumental and unending. While they measured cotinine levels to evaluate smoking exposure, the authors never measured the metabolic products of caffeine to determine the actual exposure to caffeine. These studies were sophisticated and time consuming; however, they provided conflicting answers to the question of whether caffeine ingestion represents a risk for SA.

Giannelli et al. (2003) studied the effect of caffeine consumption and nausea on the risk of miscarriage (SA). Cases were women in their first pregnancy who were interviewed about 3 weeks after their pregnancy loss on average, whereas controls were interviewed at the first prenatal care visit which typically occurred at a more advanced gestational age than the SAs. Thus, the burden of recalling caffeine exposure was not equivalent for cases and controls, which represents a defect in the study design. The fact that this was not a prospective study and the cases were interviewed earlier in pregnancy than the controls may account for the results. Daily consumption of >300 mg of caffeine per day resulted in and increased risk of SA (odds ratio, OR = 1.9 [1.0–3.6]). The OR was 2.2 in group consuming >500 mg per day. A much higher proportion of controls (no SA) reported nausea and vomiting during their pregnancy. There were other confounding factors that were not evaluated that prevented the study to definitively conclude that caffeine was causally related to the occurrence of SAs.

George et al. (2006) performed a case–control study of 108 women with SAs who had two or more SAs. Controls were obtained from a population of over 500 women who had two successful pregnancies and their last pregnancy was successful. The 108 women had two or more consecutive miscarriages (cases) and agreed to
participate. Mean caffeine consumption ≥300 mg/day was associated with a 2.7-fold increased odds of repeated miscarriage (95% CI 1.1–6.2) in nonsmokers, but not in smokers. After adjustment for many confounding factors, the odds of repeated miscarriage was no longer significantly increased in heavy caffeine users (≥300 mg/day OR 1.8, 95% CI 0.8–3.9). Lack of control for the pregnancy signal could have provided another explanation for the association between caffeine consumption of ≥300 mg/day and odds of repeated SA in nonsmokers. Studies have observed that smokers are less likely to experience nausea and vomiting during pregnancy than nonsmokers (Weigel and Weigel, 1989; Louik et al., 2006). Although the investigators had access to the nausea and vomiting data it was not utilized to determine the importance of the pregnancy signal. Selecting a small population of repeated aborters to study the risk of abortion from caffeine exposure during pregnancy complicates the planning and interpretation of these studies (Tables 3–5).

Greenwood et al. (2010) studied caffeine exposure during pregnancy, late miscarriage, and stillbirth.

### Table 3
#### Etiology of Abortion

1. Chromosomal abnormalities: pre-conceptional or periconceptional etiology
2. Embryos and fetuses with severe congenital malformations or growth retardation
3. Endometriosis
4. Lupus anticoagulant (antiphospholipid antibodies) and other immunological problems related to reproduction
5. Cervicitis; bacterial or viral infection (Kriel et al., 1970; Mead, 1989)
6. Uterine abnormalities: subserosal myoma or hematoma, infantile uterus, bifid uterus, IUD, etc. (8–10% of recurrent aborters)
7. Some teratogens, especially those with cytotoxic properties and endocrine disrupters (RU 486)
8. Maternal diabetes, alcoholism, hypothyroidism, illicit drug abuse, maternal phenylketonuria, hemorrhagic diatheses, and many other chronic and acute maternal diseases
9. Luteal phase hormonal deficiency
10. Trauma, IUDs, lightening and other rare miscellaneous events
11. Hypersecretion of LH
12. Hyperandrogenemia
13. Hyperprolactinemia
14. Autoimmune thyroid disease
15. Thrombophilic abnormalities other than antiphospholipid antibody
16. Vitamin B 12 deficiency
17. Elevated glutathione levels
18. Dietary factors; decreased with fruits and vegetable, increased with diet rich in fats
19. Twenty-seven percent of women with habitual abortion had a mutation G1691A in Factor V gene (Leiden mutation) of mutation C677T in the methylenetetrahydrololate reductase gene. The Leiden mutation may play a considerable role for women having primary recurrent abortions
20. Fourteen percent of women with unexplained recurrent abortion show highly skewed X-chromosome inactivation, which shows that they are carriers of X-linked lethal traits
21. IgG auto anti-laminin antibodies and recurrent abortion
22. HLA-G genotype and recurrent abortion
23. TH 1 type response associated with recurrent abortion (cytokines)

### Table 4
#### Etiology of Human Congenital Malformations Observed During the First Year of Life

<table>
<thead>
<tr>
<th>Suspected cause</th>
<th>Percent of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unknown</td>
<td>65 to 75</td>
</tr>
<tr>
<td>Polygenic</td>
<td></td>
</tr>
<tr>
<td>Multi factorial (gene-environment interactions)</td>
<td></td>
</tr>
<tr>
<td>Spontaneous errors of development</td>
<td></td>
</tr>
<tr>
<td>Synergistic interactions of teratogens</td>
<td></td>
</tr>
<tr>
<td>Genetic</td>
<td></td>
</tr>
<tr>
<td>Autosomal and sex-linked genetic disease</td>
<td>10 to 25</td>
</tr>
<tr>
<td>New mutations</td>
<td></td>
</tr>
<tr>
<td>Cytogenetic (chromosomal abnormalities)</td>
<td></td>
</tr>
<tr>
<td>Environmental</td>
<td>10</td>
</tr>
<tr>
<td>Maternal conditions: Alcoholism; diabetes; endocrinopathies; phenylketonuria; smoking and nicotine; starvation; nutritional, hyperthermia</td>
<td>4</td>
</tr>
<tr>
<td>Infectious agents: Rubella, toxoplasmosis, syphilis, herpes, cytomegalic inclusion disease, varicella, Venezuelan equine encephalitis, parvo virus B19</td>
<td>3</td>
</tr>
<tr>
<td>Mechanical problems (deformations): Amniotic band constrictions; umbilical cord constraint; disparity in uterine size and uterine contents</td>
<td>1 to 2</td>
</tr>
<tr>
<td>Chemicals, prescription drugs, high dose ionizing radiation</td>
<td>2 to 3</td>
</tr>
</tbody>
</table>

*Adapted from Brent (1976, 1985, 1999, 2004, 2008) and Brent and Holmes (1988).*
Exposure to teratogens follows a toxicological dose–response curve. There is a threshold below which no teratogenic effect will be observed, and as the dose of the teragen is increased, both the severity and frequency of reproductive effects will increase.

1. Exposure to teratogens follows a toxicological dose–response curve. There is a threshold below which no teratogenic effect will be observed, and as the dose of the teragen is increased, both the severity and frequency of reproductive effects will increase.

2. The embryonic stage of exposure is critical in determining what deleterious effects will be produced and whether any of these effects can be produced by a known teragen. Some teratogenic effects have a broad, and others, a very narrow period of vulnerability.

The most sensitive stage for the induction of mental retardation from ionizing radiation is from the 8th to 15th week of pregnancy, a lengthy period. Thalidomide’s period of vulnerability is approximately two weeks.

3. Even the most potent teratogenic agent cannot produce every malformation.

4. Most teratogens have a confined group of congenital malformations that result after exposure during a critical period of embryonic development. This confined group of malformations is referred to as the syndrome that describes the agent’s teratogenic effects.

5. While a group of malformations may suggest the possibility of certain teratogens, they cannot definitively confirm the causal agent because some teratogenic syndromes mimic genetic syndromes. On the other hand, the presence of certain malformations can eliminate the possibility that a particular teratogenic agent was responsible because those malformations have not been demonstrated to be part of the syndrome or because the production of that malformation is not biologically plausible for that particular alleged teragen.

According to the authors, “there are no large well-conducted effectiveness studies.” The study population included 2643 pregnant women, aged 18 to 45 years of age who were admitted to the study between 8 and 12 weeks gestational age. The pregnancies were monitored for late SAs and stillbirth. Total caffeine intake was estimated from all possible sources in the first trimester and throughout pregnancy. The adjusted data revealed a strong association between caffeine intake in the first trimester and subsequent late miscarriage between 12 and 24 weeks and stillbirth after 24 weeks. The cases ingested an average of 145 mg of caffeine per day, while the controls averaged 103 mg per day. All the OR were increased for the cases, and none of the increased OR’s were statistically significant. The authors support the conclusion that caffeine intake should be limited during pregnancy. Unfortunately, the investigators did not adjust the data for the pregnancy signal. The investigators provided no mechanism for caffeine exposure in the first trimester to produce a pregnancy loss many weeks later or even in the third trimester.

Karypidis et al. (2006) performed a case–control study comparing the risks of SA associated with CYP1B1 polymorphisms and a possible interaction of these polymorphisms with caffeine consumption. CYP1B1 is an enzyme that is known to take part in the metabolism of many steroid hormones as well caffeine. Caffeine consumption was assessed and categorized in mg/day as 0 to 99, 100 to 299, 300 to 499, and ≥500. Nausea was recorded by week of gestation and scored as never (0), sometimes but not daily (1), daily but not all day (2), and daily all day (3). Vomiting was recorded by week of gestation as never (0), sometimes but not daily (1), and daily (2). Mean weekly scores were calculated for each symptom. Smoking status was determined based on plasma cotinine levels, with smokers defined as those with levels >15 ng/ml. Overall, there was a significant interaction between homozygosity for Val and caffeine intake, such that compared to women who were homozygous for Leu and who consumed <100 mg of caffeine per day, the odds of miscarriage was significantly elevated only in women homozygous for Val and who consumed either 100 to 299 mg caffeine per day (OR = 2.36 [95% CI = 1.39–4.98]) or >500 mg/day (OR = 3.61; 95% CI = 1.36–9.61); for genotype strata Leu/Leu and Val/Leu, no significant associations were observed between increasing levels of caffeine consumption and the increased risk of miscarriage. No significant interaction was observed between caffeine ingestion and smoking. The many confounding issues that were evaluated in the analyses limited the ability to detect associations.

Khoury et al. (2004) conducted a cohort study within a prospective cohort of women with type 1 diabetes who were pregnant or planning a pregnancy. A total of 191 pregnancies were observed between 1978 and 1985. This is a small sample size for a SA study. Consumption of one or more cups of caffeinated beverages per day during the first trimester of pregnancy was reported by 54% of the women. Clinically recognized SAs ≤20 weeks were identified in 12%. Compared to no caffeine intake, the OR were 3.8 (95% CI = 0.8–16.9) for first trimester consumption of 1 to 2 cups of caffeinated beverages per day and 5.5 (95% CI = 1.2–22.0) for ≥3 cups per day. The difficulties with this study are that the investigators did not control for the pregnancy signal and their methodology for calculating caffeine consumption was imprecise.

Klonoff-Cohen et al. (2002) evaluated the risk of miscarriage in 221 couples undergoing in vitro fertilization (IVF) and gamete intra-Fallopian transfer (GIFT). There were no observed associations for miscarriage with first trimester caffeine use. The fact that there was an increased miscarriage risk for preconception exposure to caffeine makes little sense because caffeine has minimal mutagenic potential and is unlikely to result in an increase in chromosome aberrations resulting in pregnancy loss. Assisted Reproductive Technology (ART) patients are seeking these programs because they already have reproductive problems. The failure rate in these programs has a wide standard deviation. With this small population the task to determine the contribution of caffeine to the incidence of SA is very difficult.

Maconochie et al. (2007) studied risk factors for first trimester miscarriage (SA). The investigators determined that pregnant women who experienced nausea were strongly associated with a reduced odds for a miscarriage (OR = 0.3; 95% CI = 0.25–0.36) for mild or moderate nausea and (OR = 0.07; 95% CI 0.04–0.14) for severe nausea, defined as frequent vomiting. When nausea was controlled for exposures of >500 mg/day, with OR of 1.14 (95% CI = 0.79–1.66). The authors concluded that if you did not control for nausea and vomiting in the pregnant population, the studies that demonstrate a positive association of caffeine ingestion with SA may...
not be valid. While there are many problems in the design of this study, the investigators did demonstrate that caffeine exposure was not associated with the increased risk of SAs if the data were adjusted for the confounding effect of the Pregnancy Signal. In the Rasch (2003) studies, smoking, alcohol consumption, and caffeine ingestion were evaluated as risk factors for SA. Unfortunately, the investigator made no attempt to control for the pregnancy signal. An important and interesting concern is the possibility of evaluating fetal exposure following fetal demise. Since fetal demise may occur weeks before a SA is recognized, caffeine consumption may return to typical intake levels as pregnancy symptoms abate, artificially inflating estimates of caffeine use during the time period that may not be relevant. This was a large study of 303 women with documented SAs and 1168 controls. Almost half the women reported heavy caffeine consumption. SAs were increased in the group exposed to >375 mg of caffeine per day (OR = 2.2; [1.5–3.2]). Without controlling for nausea and vomiting symptoms it is not possible to verify a causal relationship to the caffeine exposure.

Sata et al. (2005) studied caffeine intake, CYP1A2 polymorphism, and the risk of recurrent pregnancy loss in a case–control study that reported no overall association between caffeine intake ≥300 mg/day and recurrent pregnancy loss (OR = 1.82; 95% CI = 0.72–4.58). The concept of the investigators was that polymorphism of CYP1A2 could result in populations with the ability to rapidly metabolize caffeine and therefore be able to tolerate higher exposures of caffeine. Unfortunately, the results were not decisive. No associations were observed among women with other CYP1A2 genotypes (CC), results were not decisive. No associations were observed between caffeine intake ≥300 mg/day and recurrent pregnancy loss (OR = 2.2; [1.5–3.2]). Without controlling for nausea and vomiting symptoms it is not possible to verify a causal relationship to the caffeine exposure.

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The risk of SA was increased in the exposure group compared to those who did not report nausea during the first trimester compared to those who did (29.6 vs. 7.2%). There was no statistically increased risk in the groups that ingested less than 300 mg/day and a very high RR of 5.4 in the group that ingested >300 mg/day. There was incomplete evaluation of confounding factors. Small number of SAs in all categories of caffeine consumption limited the ability to detect associations. There was lack of control for the pregnancy signal although the questionnaire did request information concerning the presence of nausea.

Weng et al. (2008) performed a prospective cohort study with data that had been utilized for several SA studies, so it is not clear whether the initial planning and collection of data had the intention to study the SA risk of caffeine exposure during pregnancy. One thousand and sixty-three (1063) women consented to be part of the study and completed the in-person interview soon after confirmation of pregnancy (median gestational age at interview was 10 weeks). Cox proportional hazards models were used to compare rates of miscarriage by caffeine exposure status, adjusted for maternal age, race, education, family income, marital status, previous miscarriage, nausea and vomiting since last menstrual period, smoking status, alcohol drinking, Jacuzzi use, and exposure to magnetic fields during pregnancy. The risk of SA was increased in the exposure group of >200 mg/day. It is interesting that these authors have already reported that magnetic fields increase the risk of miscarriage using the same population of pregnant patients (Li et al., 2002). In some instances the authors also determined that a miscarriage had occurred if the only source of the information was from the mother. In other words, they may not have medical documentation that a miscarriage had occurred. Exposures of >200 mg/day of caffeine had OR of 2.23 (95% CI = 1.34–3.69). However, when the subjects were identified as having the pregnancy signal and were in the group exposed to >200 mg/day, the risk of SA was not increased.

Signorello et al. (2001) studied the effect of caffeine consumption and nausea on the risk of miscarriage (SA).
This study was conducted utilizing the same case–control study population reported in Cnattingius et al. (2000). One hundred one (101) chromosomally normal SAs that occurred between 6 and 12 weeks of gestation were compared to the 953 controls that were matched by week of gestation and area of residence from the 562 control cases. With the goal of evaluating the variability in caffeine metabolism as a risk factor for SAs, the authors estimated the activity levels of two enzymes, cytochrome P450A2 (CYP1A2) and N-acetyltransferase 2 (NAT2) given both are involved in the metabolism or detoxification of many drugs including caffeine. This was a well planned and work intensive study to determine whether pregnant mothers with the ability to rapidly metabolize caffeine would have a lower risk for SA at all caffeine exposure levels. Using blood samples collected at the time of the SAs for cases and at the time of the interview for controls, polymorphisms of the NAT2 gene and CYP1A2 phenotypes were determined. It is not clear why the authors did not use the blood samples to determine the metabolic products of caffeine metabolism rather than the complicated indirect CYP1A2 analysis. The investigators reported that the women with high CYP1A2 activity had an increased risk for SAs in the 100 to 299 mg/day and the ≥300 mg/day groups, but no increase in SA risks among the subjects with low CYP1A2 activity. The results were not in the anticipated direction given that the authors’ hypothesis was that caffeine would be more strongly associated with SA among slow metabolizers due to slower caffeine clearance. While these studies were sophisticated and time consuming, they have provided conflicting answers to the question of whether caffeine ingestion represents a risk for SA.

Zusterzeel et al. (2000) performed a case–control study of recurrent early pregnancy loss that evaluated associations with polymorphisms in glutathione S-transferase (GST) and cytochrome P450 genes. The authors postulated that genetic polymorphisms in these genes may reflect impaired drug metabolism, resulting in an increased susceptibility to adverse outcomes from exposures to caffeine. The case included pregnant women who had at least two unexplained consecutive SAs occurring at <17 weeks of gestation. Coffee consumption was reported by the authors in the following categories, 1 to 5, 5 to ~10, and >10 cups of coffee per day. The data showed no observed associations between daily coffee intake and recurrent pregnancy loss for 1 to 5 cups and for >5 cups compared to noncoffee drinkers. Although the GSTP1b-1b polymorphism appeared to be more common among women with recurrent early pregnancy loss, the limited data presented in this paper offer no evidence to implicate a specific role for coffee intake via direct or interactive effects with GST polymorphisms.

Summary of caffeine exposure and the risk of SA (miscarriage). Since 2000, 17 epidemiological studies have been published dealing with the risk of SA from exposure to caffeine. Ten were case–control studies and the number of cases ranged from 58 to 953. There were six prospective cohort studies. One study was a nested control study. Only one of the studies measured the serum levels of caffeine or its metabolites to determine the actual caffeine exposure. With regard to the exposure that was evaluated, namely number of caffeine-containing beverages for various time periods, there was no increased risk of miscarriage in the majority of studies in women who drank three cups of coffee or less per day. However, there were a few studies with increased risks for miscarriage in the lowest exposure groups.

The most serious criticism of the studies dealing with SA is that 11 of the 17 studies failed to evaluate the importance of the Pregnancy Signal (Cnattingius et al., 2000; Wen et al., 2001; Klomoff-Cohen et al., 2002; Giannelli et al., 2003; Rasch, 2003; Tolstrup et al., 2003; Khoury et al., 2004; Sata et al., 2005; George et al., 2006; Weng et al., 2008; Greenwood et al., 2010). Evaluating the subjects in an epidemiology study with regard to the pregnancy signal allows the investigators to identify subjects with high and low reproductive risks (Weigel and Weigel, 1989; Lawson et al., 2004; Louis et al., 2006; Boylan et al., 2008).

Positive associations of maternal coffee drinking or caffeine ingestion during pregnancy and the increased risk of SAs have been reported in epidemiological studies or reviews (Christian and Brent, 2001; Leviton and Cowan, 2002; Signorello and McLaughlin, 2004; Infante-Rivard, 2007; CARE Study Group, 2008; Weng et al., 2008). Other reviews have not found such associations, and many of the associations observed may be attributable to confounding effects of maternal cigarette smoking or nutritional factors (Christian and Brent, 2001; Leviton and Cowan, 2002; Signorello and McLaughlin, 2004; Bech et al., 2007; Maconochie et al., 2007; Savitz, 2008).

The epidemiological studies evaluating the risk of SAs from caffeine exposure have been inconsistent. Reports of maternal consumption of caffeine at the level of <300 mg/day has been associated with an increased risk for SAs. Other studies have reported that exposures of 500 to 900 mg/day are not associated with and increased risk of SAs. Which result is correct? Unfortunately, none of the epidemiology studies cited the nonhuman mammalian studies dealing with caffeine exposure and SA. The animal studies reveal that the wide range of human exposures when utilized in animal reproductive studies do not result in increased pregnancy loss in mammalian reproductive studies. (See later sections.)

Congenital Malformations

The principles of teratology can be useful for planning epidemiology studies as well as interpreting the results (Table 5).

It is important to be cognizant of the fact that drugs and chemicals account for only a small percent of environmentally produced congenital malformations and that almost all teratogens produce a constellation of effects that is identified with the teratogen (Tables 4 and 5). This should indicate to physicians, epidemiologists, and scientists that determining whether a drug or chemical is responsible for increasing the risk for CMs is not a simple task. Statistical associations do not necessarily indicate causal associations! (Nelson and Forfar, 1971; Fedrick, 1974; Heinonen et al., 1977; Borlee et al., 1978; Linn et al., 1982; Rosenberg et al., 1982; Kurppa et al., 1983; James and Paull, 1985; Pieters, 1985; Olsen et al., 1991; Natsume et al., 2000; Torfs and Christianson, 2000; Browne, 2006; Bille et al., 2007; Browne et al., 2007; Mongraw-Chaffin et al., 2008).
Malformations were not more frequent than expected in these caffeine epidemiology studies performed before the year 2000 (Nelson and Forfar, 1971; Heinonen et al., 1977; Linn et al., 1982; Rosenberg et al., 1982; Kurppa et al., 1983; Olsen et al., 1991). In two other case-control studies, significant associations were observed with the consumption of caffeinated beverages during pregnancy among mothers of 464 anencephalic infants and 190 children with various malformations (Fedrick, 1974; Borlee et al., 1978).

In studies performed in 2000 and thereafter there were 11 epidemiological publications (Natsume et al., 2000; Torfs and Christianson, 2000; Browne, 2006; Bille et al., 2007; Browne et al., 2007; Mongraw-Chaffin et al., 2008; Collier et al., 2009; Johansen et al., 2009; Miller et al., 2009; Schmidt et al., 2009).

Bille et al. (2007) reported the association between oral clefts and first trimester maternal lifestyle factors utilizing the Danish record population that includes 100,000 pregnancies. There were 192 mothers in this cohort that gave birth to a child with an oral cleft. The investigators reported that first trimester smoking was associated with an increased risk of clefting, OR = 1.5 (95% CI 1.05–2.14). Evaluation of the risks of coffee, tea, and alcohol found OR > 1.0; however, the data were not statistically significant. The authors reported an association of drinking five or more cups of tea per day early in pregnancy among the mothers of 58 children with cleft palate only, OR = 2.9, 95% CI (1.1–5.6) for infants with isolated only cleft palate. No significant association was found with maternal coffee or cola drinking in this study among the mothers of children with cleft palate, and no associations were found among the mothers of 134 infants with cleft lip with or without cleft palate and consumption of any caffeinated beverage. Bille et al. concluded, “There is no solid evidence to support caffeine as a risk factor in humans for oral clefts” (Rosenberg et al., 1982; Levitan and Cowan, 2002; Nawrot et al., 2003). The authors also conducted sub-analyses restricted to nonsyndromic cases, which may be etiologically distinct from oral clefts that occur as part of a syndrome. In fact, this may be the incorrect approach because most teratogens produce syndromes and genetic abnormalities are an important contributor to the occurrence of isolated cleft lip and cleft palate.

Browne (2006) performed a systematic review of epidemiological studies published before 2006 and concluded that there is no evidence that maternal caffeine consumption during pregnancy increases the risk of congenital anomalies in infants.

Browne et al. (2007) reported no consistent association with maternal caffeine consumption early in pregnancy in a case-control study of 4,196 infants and 3,957 controls with various types of cardiac malformations utilizing the data from the National Birth Defects Prevention Study Program. In fact, in the analysis of the atrial septal defect incidence associated with coffee intake, the OR = 0.46 (CI = 0.28–0.75) indicated that there was a lower risk associated with caffeine exposure. The investigators concluded that the results indicated that caffeine is unlikely to be causally related to the occurrence of congenital heart malformations.

Collier et al. (2009) reported a significant association with maternal intake of 200 mg of caffeine per day or more, among the mothers of 175 infants with cleft lip with or without cleft palate and other congenital anomalies (OR = 1.7; 95% CI 1.0–2.9). For mothers who consumed 10 to 99 mg of caffeine per day, there was also a significant association with maternal intake of 100 mg of caffeine per day or more among the mothers of 657 infants with isolated cleft palate only (OR = 1.2; 95% CI 1.0–1.6). The lack of correction for multiple comparisons and lack of a dose effect with these associations makes a causal relationship less likely. Selecting isolated clefting malformations as potentially being produced by in utero exposure to caffeine is problematic. This malformation, which has an important genetic contribution and is frequently an isolated malformation, is unlikely to result from exposure to a teratogenic agent, since known causes of cleft palate from teratogens are syndromic (anticonvulsants, alcohol, amniop terin, and retinoids).

Johansen et al. (2009) reported an association with maternal caffeine consumption (for all beverages) during the first three months of pregnancy in a Norwegian case-control study of 573 children with isolated cleft lip with or without cleft palate (OR = 1.47; 95% CI 1.05–2.07). There were 763 randomly selected controls. For mothers who consumed >0 but <3 cups of coffee per day, OR = 1.39 (95% CI 1.01–1.92). For mothers who consumed three or more cups of caffeine containing beverages per day the OR = 1.59 (95% CI 1.05–3.59). There was no association of coffee consumption early in pregnancy among the offspring with cleft palate whose mother drank >3 cups day in this study, OR = 0.96, CI (0.55–1.67). There was a negative (i.e., a protective) association with maternal tea drinking among mothers of the children with isolated cleft lip with or without cleft palate (OD = 0.72; 95% CI 0.30–0.94) for mothers who consumed three or more cups of tea per day, and no association with maternal cola consumption or with estimated daily caffeine consumption from all sources in either group. The author’s conclusion was, “There was little evidence of an association between caffeine and clefts when all sources of caffeine were considered.”

Miller et al. (2009) studied “Maternal exposure to tobacco smoke, alcohol, and caffeine, and the risk of anorectal atresia.” The data utilized in this study are from the National Birth Defects Prevention Study (NBDPS). There were 464 infants with the diagnosis of anorectal atresia and 4,940 controls. There were three exposure categories: 10 to 99, 100 to 299, and >300 mg/kg/day. The OR for all three exposure groups were 1.4, 1.3, and 1.5 respectively and all three ORs were significant. There was no increasing risk with increasing exposure. The observed association of isolated anorectal atresia with caffeine is unlikely to be causally related to caffeine exposure (Table 5).

Mongraw-Chaffin et al. (2008) conducted a nested case-control study of cryptorchidism among children born to mothers enrolled in the Collaborative Perinatal Project between 1959 and 1967. The diagnosis had to persist beyond two years of age in order to be included in the study. The investigators found an association with maternal consumption of the equivalent of three or more cups of coffee per day (OD = 1.43, 95% CI 1.06–1.93). Selecting isolated cryptorchidism as a malformation that may be produced by in utero exposure to caffeine is problematic. This malformation, which has an important genetic contribution and is frequently an isolated malformation, is unlikely to result from exposure to caffeine.
Natsume et al. (2000) performed a case–control study of cleft lip and palate that included 306 cases of cleft lip, cleft palate, or both matched to 306 controls. The protocol of this report was lacking in detail. The investigators described the caffeine exposure in cups per week, which is inadequate. Although the analyses did not indicate that there was an increased risk of cleft lip and palate this study will not be included in the final analysis.

Slickers et al. (2008) studied maternal caffeine consumption and the risk of bilateral renal agenesis and renal hypoplasia. The data utilized in this study are from the National Birth Defects Prevention Study (NBDPS). Renal agenesis and hypoplasia has many etiologies, including genetic causes. The results were inconclusive, in that there was not a statistical increased risk with caffeine exposure. However, there were only 75 renal malformations in this case–control study, which makes any definitive interpretation problematic.

Schmidt et al. (2009) studied maternal caffeine consumption and the risk of neural tube defects (NTDs). The data utilized in this study are from the National Birth Defects Prevention Study (NBDPS). Total average daily caffeine dietary consumption was obtained during the year before pregnancy occurred for 768 mothers with children with NTDs and 4,143 control mothers and infants without NTDs. Positive associations were observed between caffeine consumption and spina bifida (OR = 1.4; 95% CI = 1.1–1.9). Interestingly, caffeinated tea consumption had a protective association (OR = 0.7, CI = 0.6–0.9). While most of the OR were greater than one, few were statistically significant. Furthermore, the mothers with the highest intake of caffeine (200–299 mg/day, >300 mg/day) did not have a statistically significant increased OR for NTDs. The discussion section of this publication is extensive and has numerous hypotheses as to why the findings indicate that caffeine causes NTDs. No mention is made of evaluating the “pregnancy signal” and its role in separating the at-risk from the low risk population. Since the vast majority of teratogenic drugs produce a teratogenic syndrome and not isolated malformations such as NTDs, their findings are not supported by one of the basic teratology principles (Table 5). The authors did not review the animal literature, which indicates that caffeine does not cause isolated NTDs. The first sentence in this publication states, “Animal studies demonstrate teratogenic effects of caffeine and human studies are inconclusive.” The first report of the teratogenicity of caffeine was published in 1960 and the dose administered was 250 mg/kg (Nishimura and Nakai, 1960). Animal studies result in teratogenesis (Christian and Brent, 2001), if the exposures are far above any possible human exposure from caffeine consumption and that epidemiological literature demonstrates that caffeine is unlikely to be a human teratogen from human dietary exposures. The authors do not report the folic acid levels in their patient populations and therefore cannot discuss the important nutritional data with regard to the role of nutrition as an etiological factor in the patients with NTDs in their study.

Torfs and Christianson (2000) examined some of the environmental risks for the occurrence of Down syndrome. The study was a population-based case–control study that identified 997 Down syndrome cases from the California Birth Defects Monitoring Program and 1,007 live born nonmalformed controls from the general population. Six months after delivery, the mothers were asked about their consumption of coffee, tea, and soft drink “around the time of conception.” Since Down syndrome is a chromosome abnormality due to the presence of an extra chromosome 21 during the maturation of the sperm or egg, caffeine, exposures during embryonic development cannot produce this abnormality. Preconception exposures to caffeine would be very unlikely to affect the maternal ova because caffeine is not considered to be mutagenic. A protective association between heavy coffee intake (≥ four or more cups per day) and Down syndrome was observed among nonsmokers (OR = 0.48; 95% CI = 0.28–0.82) but not smokers (OR = 1.6; 95% CI = 0.80–3.36). This study is of interest, but does not contribute to the evaluation of whether caffeine has a teratogenic effect. One of the several hypotheses generated by the investigators was that caffeine may have caused SAs of Down syndrome embryos, thus decreasing the incidence of Down syndrome in the high caffeine exposure group.

Summary of the risk of congenital malformations from dietary exposure to caffeine. It is very unlikely that the usual or even high exposures of dietary caffeine increases the risk of birth defects for pregnant mothers exposed to caffeine. Not one investigator has published the constellation of developmental abnormalities that constitutes the “caffeine teratogenic syndrome” in humans (Table 5). None of the epidemiologists have carefully examined the animal teratology or animal toxicokinetic literature to determine the magnitude of exposure necessary to produce congenital malformations. Schmidt et al. (2009) cited the original publication indicating that caffeine was teratogenic in the mouse (Nishimura and Nakai, 1960). These investigators administered 250 mg/kg i.p. to pregnant mice that resulted in vascular disruptive malformations at exposures that are never reached in humans from even high exposures of dietary caffeine.

Fetal Weight Reduction (Small for Gestational Age [SGA])

Before the year 2000, several studies were reported that indicated that caffeine exposure during pregnancy was associated with fetal growth retardation (Mau and Netter, 1974; Martin and Bracken, 1987; Fenster et al., 1991; Peacock et al., 1991; Vlajinac et al., 1997). Other investigators have indicated that smoking may be an important confounder in caffeine fetal growth studies (Beaulac-Baillargeon and Desroisiers, 1987). Studies have also reported that the results did not indicate that caffeine exposure during pregnancy reduced fetal growth (Linn et al., 1982; Cook et al., 1996; Committee on Toxicity, 2001).

During the years from 2000 to 2010, 17 articles were published evaluating the risk of maternal caffeine exposure and fetal weight reduction (Grosso et al., 2001, 2006; Claussen et al., 2002; Kiebanoﬀ et al., 2002; Balat et al., 2003; Bracken et al., 2003; Orskou et al., 2003; Vik et al., 2003; Parazzini et al., 2005; Santos et al., 2005; Tsubouchi et al., 2006; Bech et al., 2007; Diego et al., 2007; Infante-Rivard, 2007; Care Study Group, 2008; Xue et al., 2008; Bakker et al., 2010).
Bakker et al. (2010) examined the associations of maternal caffeine intake, based on coffee and tea consumption, with fetal linear growth and fetal weight measurements in each trimester of pregnancy and the risk of adverse birth outcomes. There were 7,346 pregnant women participating in a population-based prospective cohort study from early pregnancy onward in the Netherlands (2001–2005). Caffeine intake in the first, second, and third trimesters was on the basis of coffee and tea consumption and was assessed by questionnaires. Fetal linear growth measurements were repeatedly measured by ultrasound. Information about birth outcomes was obtained from hospital records. The investigators observed no consistent associations of caffeine intake with fetal head circumference or estimated fetal weight in any trimester. Higher caffeine intake was associated with smaller first-trimester crown-rump length, second- and third-trimester femur length, and birth length (p for trend <0.05). Offspring of mothers who consumed >6 caffeine units/day (540 mg) tended to have increased risks of small-for-gestational-age infants at birth. The authors concluded that caffeine intake of >6 units/day during pregnancy is associated with impaired fetal length. Caffeine exposure might preferentially adversely affect fetal skeletal growth and that further studies are needed. The actual data are more important than the conclusions. In the small group of 133 women who had the equivalent of 6 cups of coffee per day, the femur was smaller by 0.5 mm in the third trimester and the crown rump length (CRL) was reduced by 4.54 mm in the first trimester. There was no effect on birth weight, head circumference, or prematurity incidence. The CRL at term is not available in the publication. No mention is made of controlling for the pregnancy signal. The average birth weight was over 3,400 g. The only positive group for shortening of length (in millimeters) was the women who ingested 6 or more units, (540 mg) cups of coffee per day. It was not determined whether the reduced length was recoverable or of any clinical significance. This study is more important because of the negative findings, rather than the minimal positive findings. Caffeine exposure at every exposure had no effect on birth weight. There was no increase in SGA babies in this study.

Balat et al. (2003) recruited a group of smokers (n = 60) and nonsmokers (n = 63) who delivered at full-term (37–41 weeks) to evaluate the effect of caffeine intake on newborn and placental characteristics. The investigators obtained the caffeine intake based on the average number of cups of coffee and tea consumed per day. Based on the intake, the mothers were divided into two groups: <300 mg/day or >300 mg/day, assuming 107 mg of caffeine for each cup of coffee and 34 mg for each cup of tea. This study did not attempt to control for other important confounders such as maternal age, alcohol use, and gestational age at birth. The difference in birth weight between the >300 mg/day versus the <300 mg/day group was 128 g. The results do not definitively suggest that the caffeine was responsible for this very small, clinically insignificant weight difference.

Bech et al. (2007) randomly assigned women (n = 1,197) to caffeinated or decaffeinated instant coffee during the last half of pregnancy. The pregnancies were followed to evaluate differences in gestational age and mean birth weight. Participants were provided unlimited amounts of coffee, either caffeinated or decaffeinated as assigned. They were also free to consume other sources of coffee and caffeinated beverages. It would appear that this study should have been altered once the investigators realized that there were no real exposed and control groups. The possible interaction between smoking and caffeine consumption on birth weight is unconvincing without presentation of the results by compliance or, preferably, by actual caffeine consumption. The investigators reported a 263 g weight reduction in the newborns from exposure to caffeine, which is clinically significant.

Bracken et al. (2003): This prospective cohort study included 2291 pregnant women ≤24 gestational weeks from clinics and obstetric practices. Caffeine exposures were evaluated as urinary caffeine and self-reporting of caffeine ingestion during early and late pregnancy. The rates of IUGR (8.4%), low birth weight (4.7%), and preterm birth (7.0%) were lower in this cohort than in the general US population for the year 2000 (10, 6, and 11.6%, respectively) (León et al., 2002). There is minimal evidence in this publication to indicate that caffeine use during early or late pregnancy is related to low birth weight.

Care Study Group (2008) performed a prospective longitudinal observational study to examine the association of maternal caffeine intake with “fetal growth restriction.” During the 8th to 12th week of pregnancy 2635 women were recruited for the study. Assessments of (1) caffeine and (2) smoking and tobacco exposure were performed by self-reporting and by measuring caffeine and cotinine in the saliva. This was a large and ambitious project. There were four categories of exposure (<100, 100–199, 200–299, >300 mg of caffeine per day). The adjusted OR were calculated for the 12 groups that were evaluated. There were four groups that were not significant. The remaining OR’s were significant with five of the OR’s having a CI lower than 1.0. Placing this data into clinical perspective, the average difference in birth weight between the caffeine exposed and the controls in the 12 groups ranged between 21 and 89 g. Those differences in birth weight are the equivalent to less than one to three ounces, and are clinically insignificant. The authors describe these findings as associations; however, their clinical recommendations infer that the caffeine has a causal relationship and not just an association. Their recommendation is, “Sensible advice would be to reduce caffeine intake before conception and throughout pregnancy.” More appropriate advice would be to also stop smoking, limit alcohol consumption, limit vigorous exercise, limit calorie restriction, nutritional fads, and recreational drugs. This was a very large and comprehensive study; however, the investigators ignored the evaluation of the pregnancy signal for collating pregnancies into high risk and low risk categories for reproductive and developmental problems, which is a serious deficiency.

Claussen et al. (2002): A population of patients that had been part of a SA study (Cnattingius et al., 2000) was followed to evaluate the effects of caffeine use on the birth weight of the newborns. Caffeine intake was obtained for the first six weeks of gestation, second trimester, and for a portion of the third trimester. There were four exposure categories, 0 to 99, 100 to 299, 300 to
499, or ≥500 mg/day. There were no differences in birth weights in the five different, caffeine intake categories.

Diego et al. (2007): Birth weights were obtained from the medical records of 452 participants who were recruited between 20 and 28 weeks of gestation. The data pertaining to the caffeine exposure are unclear and there was no attempt to evaluate confounding factors. The exposures ranged between zero and six caffeine-containing drinks per day. The exposure assessment was problematic and therefore the weak association with weight reduction is of little value.

Grosso et al. (2001): There were over two thousand pregnant participants in this cohort study of IUGR. Cord blood samples were obtained for the analysis of caffeine metabolites from 1,606 participants. Pregnancy symptoms were not evaluated. The investigators measured cord serum caffeine, paraxanthine, theophylline, and theobromine concentrations as indicators of the amount of caffeine or its metabolites entering fetal circulation after crossing the placenta. There was no association between IUGR and caffeine intake during the first (OR = 0.91; 95% CI = 0.44–1.90) or seventh month of pregnancy (OR = 1.00; 95% CI = 0.37–2.70).

Grosso et al. (2006): The pregnancy signal was not included in their evaluation. Women in the lower caffeine exposure group delivered newborns with a reduced risk of IUGR. Women with the highest concentrations of paraxanthine had an increased risk of IUGR (OR = 3.3; 95% CI = 1.2–9.2). Fast metabolizers of caffeine and caffeine metabolic products were associated with an increased risk for IUGR (OR = 1.21; 95% CI = 1.07–1.37). The fast metabolizers should have the lowest caffeine levels and therefore, the lowest risk. This result is the opposite of what one would have predicted, which confuses the attempt to clarify the relationship of caffeine exposure and the risk of fetal weight reduction.

Infante-Rivard (2007): There were 451 cases and 451 controls born after the 24th week of gestation with no CMs in this SGA (10th percentile or less) case-control study that examined the association of caffeine exposure and the risk of fetal weight reduction. There were two exposure groups (<300 mg/day vs. ≥300 mg/day). Maternal and newborn blood samples were obtained for CYP1A2 and CYP2E1 polymorphisms genotyping. Growth retardation was not affected by the polymorphism in the mother or child. There was a very small reduction in birth weight associated with an increasing caffeine exposure in both the first and third trimester. For every 100 mg of caffeine consumed, the birth weight was reduced by 31 and 38 g for every 100 mg of caffeine consumed during the second and third trimester, respectively. These are very small reductions in newborn weights and of no clinical significance whether or not this is a causal association.

Klebanoff et al. (2002): This study measured paraxanthine, the major metabolite of caffeine, in third trimester serum samples banked for 2,515 women participating in the Collaborative Perinatal Project (CPP) between 1959 and 1966. Controls were selected from the CPP population (Klebanoff et al., 1999). SGA was defined as birth weight <10th percentile. The risk of delivering a SGA infant increased with rising serum paraxanthine concentrations, but only among smokers. Increased risk among smokers was modest (OR = 2.0 and lower) and only present for categories of paraxanthine concentrations exceeding 715 ng/ml. Apparently, there were no associations with serum caffeine concentrations. The pregnancy symptoms were not included in the evaluation, which detracts from the validity of the final analysis.

Orskou et al. (2003): This study determined risk factors for high birth weight (>4,000 g). In a large prospective cohort study, pregnant women were selected from a cohort of over 24,000 pregnant Danish women who were interviewed at approximately 16 weeks of gestation for the average daily consumption of cups of coffee, tea, cola, and cocoa, that was converted to total caffeine intake (mg/day). The women who consumed more than 200 mg/day of caffeine were associated with a decreased risk of giving birth to a high birth weight infant (>4,000 g). This study is not directly related to the concern regarding the risk of caffeine producing newborns with SGA.

Parazzini et al. (2005) selected 555 women delivering singleton, small for gestational age (SGA <10th percentile) babies and 1,966 controls who delivered healthy, term singletons for a case–control study. Caffeine consumption was listed as the number of cups per day before pregnancy and during each trimester. The pregnancy signal data were collected on 50% of the cases and 66% of the controls. The authors observed no associations between SGA and intake of three or more cups of coffee per day during pregnancy or >4 cups of coffee per day before becoming pregnant.

Santos et al. (2005): This retrospective cohort study of 5,189 singletons was evaluated for SGA from exposure to a caffeinated beverage consumed in South America called mate. All the mothers were interviewed within the 24 hr following delivery. The investigators estimated that the daily mate consumption was equivalent to a daily average caffeine intake of 300 mg (Santos et al., 1998). The investigators controlled for eight confounding factors and concluded that mate (300 mg/day) does not increase the risk of having a SGA newborn.

Tsoubouchi et al. (2006): This study is a physiological study of 10 pregnant women designed to measure whether caffeine affects maternal and fetal blood flow velocity using Doppler sonography. The pregnant women were given one cup of coffee (100 mg of caffeine) before determining maternal and fetal blood flow in the third trimester. The caffeine had no effect on blood flow in the uterine artery, fetal middle cerebral artery, or umbilical artery. This study only indicates that if fetal growth retardation is caused by exposure to caffeine, the mechanism is not via altering the blood supply to the fetus.

Vik et al. (2003): Caucasian pregnant women who were described as high risk based on their pregnancy histories were selected before the 20th week of gestation to participate in a caffeine exposure study. The high risk category included the following criteria:

1. Low birth weight,
2. Smoking,
3. Prepregnancy weight <50 kg,
4. Previous perinatal death and being chronically ill.

A complete dietary analysis was performed for each woman during various stages of pregnancy for 858
women. The mean caffeine intake at the 17th week was 232 and 205 mg/day at 33 weeks. For a 60 kg woman the caffeine exposure is 3.9 and 3.4 mg/kg, respectively. No association was observed between “high” caffeine intake at 17 weeks and giving birth to a SGA infant (OR = 1.1; 95% CI = 0.6–2.1), but high consumption at 33 weeks was associated with an increased OR for SGA (OR = 1.6; 95% CI = 1.0–2.5). Yet the findings in this study indicate that high caffeine intake did not result in an increased risk of newborns with SGA with caffeine exposures at midgestation (17 weeks). The 33-week group exposed to high exposures of caffeine did have a statistically increased risk for infants with SGA.

Xue et al. (2008): The Mothers of subjects in the Nurses’ Health Study (n = 34,063) were sent questionnaires to collect pregnancy and newborn data that occurred many years in the past. The mothers answered a questionnaire pertaining to events that occurred 40 to 60 years ago with regard to caffeine ingestion. This is an exceptionally long period of time to expect an accurate recall of the mother’s caffeine ingestion. It is a serious deficiency in this study. There were five categories of caffeine consumption corresponding to never, <1, 1 to 2, 3 to 4, and ≥5 cups per day. The authors report that birth weight was negatively associated with coffee consumption during pregnancy decreasing by 15, 34, and 54 g for consumption of 1 to 2, 3 to 4, and ≥5 cups of coffee per day during pregnancy. These weight reductions are clinically insignificant since it is one percent or less of the weight of a newborn baby. Pregnancy symptoms were not considered as confounders.

Summary of the growth retardation studies. The growth retardation studies were not consistent. In six of the studies the results were negative for an association of growth retardation due to exposures to caffeine. Seven of the studies were equivocal demonstrating a risk for growth retardation with increasing exposures to caffeine but with the inability to determine the role of confounding factors. Four of the studies did not evaluate the pregnancy signal. Two of the studies were not devoted to the caffeine exposure and the risk of fetal growth retardation. In some of the positive studies, the magnitude of the growth retardation was clinically insignificant. None of the epidemiology studies examined the growth retardation studies in animals that indicated pharmacokinetically that exposures had to be significantly above even the highest caffeine exposures to which pregnant women would be exposed to produce fetal growth retardation.

**ANIMAL REPRODUCTIVE, DEVELOPMENTAL AND IN VITRO STUDIES DEALING WITH EXPOSURES TO CAFFEINE**

Reproductive and Developmental Toxicology

Review of the animal studies has revealed some interesting as well as unexpected findings. None of the results of the oral administration of caffeine indicated that caffeine increased the risk of embryonic death. While a few manuscripts reported research conducted in consideration of US (FDA) or international (ICH) guidelines, most are conducted using inappropriate routes of exposure (only a few are relevant to normal human exposure). Most oral studies were conducted at toxic levels, that is, those in excess of the 30 mg/kg/day NOEL in rodents, and only a few of the studies are relevant to normal human exposures (in general, it was not possible to extrapolate nonclinical oral exposures to human exposures). The results of the review of all the papers are outlined in Supplemental Table 1, and Tables 6–9. Not all the mg/kg/day dosages are available (in some cases these can only be estimated, because of the route used). There is also inadequate information regarding concentrations, consumption, and animal body weights. Supplemental Table 1 represents a summary of the recent animal toxicology literature pertaining to caffeine.

Extrapolating the results of caffeine animal toxicology studies for human risk assessment:

1. Parental (i.v, i.p., and s.c.) administration in animal study makes it difficult to perform human risk assessment. Even once a day oral intubation presents difficulties in utilizing the animal toxicology results for human risk assessment.
2. Without human serum and animal serum levels of caffeine and its metabolites, risk assessment is problematic.
3. Most animal teratology studies exposed the animals to caffeine at the appropriate stages for comparing risks in the animal model with potential risks in the human.
4. Most human exposures were measured in cups of coffee per day. However, it is difficult to define a cup (1 cup = 8 fluid ounces); coffee makers measure in 5-ounce serving cups. A 10 cup coffee maker = 50 ounces (www.Starbucks.com), which by standard measure = 80 ounces, a discrepancy of 30 ounces or a 27.5% difference in intake. “Cup” was never defined in the publications reviewed.
5. Few studies reference International Regulatory Guidelines for pharmaceutical development (e.g., ICH, EG, or FDA guidelines). Very few studies were performed in compliance with current regulatory guidelines. Most studies cited various animal use guidelines (specified animal treatment/handling guidelines).

A previous review (Christian and Brent, 2001) of the developmental toxicology of caffeine in animals and humans identified a No Effect Level (NOEL) of approximately 30 mg/kg/day in rodents, the reproductive NOEL to be approximately 80 to 120 mg/kg/day and the teratogenic NOEL as 80 to 100 mg/kg/day based on the following studies (Knoche and Konig, 1964; Palm et al., 1978; Aeschbacher et al., 1980; Nolen, 1981; Nagasawa and Sakurai, 1986; Pollard et al., 1987; Purves and Sullivan, 1993). The 2001 publication essentially addressed the question of human teratogenicity of caffeine. The publication cautioned that although pregnant women who do not smoke or drink alcohol and who consume moderate amounts of caffeine (<5–6 mg/kg/day spread throughout the day) do not have an increase in any reproductive risks, individuals who consume large amounts of caffeine are at greater risk of being a smoker and of drinking alcoholic beverages to excess. Such an individual may have an increased risk of reproductive problems for other associated issues that have not yet been recognized as important reproductive and developmental toxic agents or behaviors.

If mammalian animal studies are to be utilized to estimate human risks, the oral route is the only
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<td>Klebanoff et al. (2002)</td>
<td>Humans</td>
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<tr>
<td>du Preez et al. (1999)</td>
<td>Humans</td>
<td>IV</td>
<td>4.0 to 7.7 mg/kg</td>
<td>The clearance rate and volume of distribution of theophylline in apneic premature neonates</td>
</tr>
<tr>
<td>Mazkereth et al. (1997)</td>
<td>Humans</td>
<td>IV</td>
<td>6 mg/kg</td>
<td>Effect of aminophylline dosage on urinary output in premature infants.</td>
</tr>
<tr>
<td>Maza et al. (2001)</td>
<td>Rats</td>
<td>IV</td>
<td>6 mg/kg</td>
<td>Effect of hepatic regeneration after partial hepatectomy on theophylline pharmacokinetics</td>
</tr>
<tr>
<td>Jorritsma et al. (2000)</td>
<td>Rats</td>
<td>IP</td>
<td>10 mg/kg</td>
<td>Induction of P4501A with caffeine in therapeutic model of hyperbilirubinemia in Gunn rats</td>
</tr>
<tr>
<td>Pelissier-Alicot et al. (2002)</td>
<td>Rats</td>
<td>SC</td>
<td>25 mg/kg</td>
<td>Effect of administration (AM vs. PM) of caffeine on daily rhythms of heart rate, body temperature, and locomotor activity</td>
</tr>
<tr>
<td>Schrader et al. (1999)</td>
<td>Rats</td>
<td>Analytical method</td>
<td>–</td>
<td>Development of reverse-phase HPLC method for analyzing caffeine + all eight metabolites simultaneously from rat urine</td>
</tr>
<tr>
<td>Buters et al. (1996)</td>
<td>Mice</td>
<td>IP</td>
<td>2 mg/kg</td>
<td>Confirmed involvement of CYP1A2 in PK and metabolism of caffeine in CYP1A2−/− and CYP1A2+/− mice</td>
</tr>
<tr>
<td>Derkenne et al. (2005)</td>
<td>Mice</td>
<td>IP</td>
<td>8 mg/kg</td>
<td>Replaced mouse Cyp1a2 (−/−) with human CYP1A2 gene to restore metabolism of caffeine and change it to human profile</td>
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<tr>
<td>Kolarovic et al. (1999)</td>
<td>Mice</td>
<td>IP</td>
<td>20 mg/kg</td>
<td>Use of caffeine as a biomarker for the estimation of xenobiotic biotransformation and possible hepatotoxicity</td>
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<tr>
<td>Labedzki et al. (2002)</td>
<td>Mice/human microsomes</td>
<td>In vitro comparison</td>
<td>–</td>
<td>In vitro comparison of murine and human CYP1A2-mediated metabolism of caffeine and quinolones</td>
</tr>
<tr>
<td>Janus and Antoszek (2000)</td>
<td>Cattle</td>
<td>IV</td>
<td>5 mg/kg</td>
<td>Effect of gender and age on the pharmacokinetics of caffeine in Holstein cattle</td>
</tr>
<tr>
<td>Janus et al. (2001)</td>
<td>Cattle (calves)</td>
<td>IV</td>
<td>5 mg/kg</td>
<td>Effect of 4-day starvation or water deprivation on the pharmacokinetics of caffeine in calves</td>
</tr>
<tr>
<td>Peck et al. (1997)</td>
<td>Horses</td>
<td>IV</td>
<td>2.5 mg/kg</td>
<td>Compared the pharmacokinetic disposition of caffeine and its metabolites in horses and donkeys</td>
</tr>
<tr>
<td>Todi et al. (1999)</td>
<td>Horses</td>
<td>IV</td>
<td>2 g or less</td>
<td>Detection of caffeine in serum and urine after doses of caffeine or theophylline in race horses</td>
</tr>
<tr>
<td>Wasfi et al. (2000)</td>
<td>Camels</td>
<td>IV</td>
<td>23.5 mg/kg</td>
<td>The pharmacokinetics, metabolism, and urinary detection time of caffeine was characterized in camels</td>
</tr>
<tr>
<td>Fort et al. (1998)</td>
<td>Frog (Xenopus)</td>
<td>In vitro</td>
<td>–</td>
<td>The developmental toxicities of caffeine and 13 metabolites were investigated in the FETAX Teratogenesis Assay</td>
</tr>
</tbody>
</table>

PO, oral; IV, intravenous; IP, intraperitoneal; SC, subcutaneous.
<table>
<thead>
<tr>
<th>Publication</th>
<th>Species</th>
<th>Type</th>
<th>Route</th>
<th>Gestation</th>
<th>Potential effect investigated</th>
<th>NOEL (noted only when identified)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asadifar et al. (2005)</td>
<td>Rats</td>
<td>Sprague–Dawley</td>
<td>Diet</td>
<td>Postnatal</td>
<td>Effect of Cu deficiency on heart</td>
<td></td>
</tr>
<tr>
<td>Hongu and Sachan (2000)</td>
<td>Rats</td>
<td>Sprague–Dawley</td>
<td>Diet</td>
<td>28 days from 7 week</td>
<td>Body weight changes</td>
<td>Postnatal study only</td>
</tr>
<tr>
<td>Burdan et al. (2002)</td>
<td>Rats</td>
<td>Wistar</td>
<td>Diet</td>
<td>GDs 8 to 14</td>
<td>Caffeine+OTC propyphenazon and paracetamol effect on fetal development</td>
<td></td>
</tr>
<tr>
<td>Burdan et al. (2004)</td>
<td>Rats</td>
<td>Wistar</td>
<td>Diet</td>
<td>GDs 8 to 14</td>
<td>Caffeine+OTC propyphenazon effect on fetal development</td>
<td></td>
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<tr>
<td>Nomura et al. (2004)</td>
<td>Rats</td>
<td>Not identified</td>
<td>Diet</td>
<td>In vitro</td>
<td>Effects on gene expressions</td>
<td></td>
</tr>
<tr>
<td>da Silva et al. (2005)</td>
<td>Rats</td>
<td>Not identified</td>
<td>Drinking water</td>
<td>Not identified</td>
<td>Effect of maternal caffeine intake on hyperlocomotion in pups</td>
<td></td>
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<tr>
<td>Bodineau et al. (2003)</td>
<td>Rats</td>
<td>Sprague–Dawley</td>
<td>Drinking water</td>
<td>Throughout gestation</td>
<td>Respiratory control in newborn</td>
<td>≥30 mg/kg/day</td>
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<tr>
<td>Aden et al. (2000)</td>
<td>Rats</td>
<td>Wistar</td>
<td>Drinking water</td>
<td>GD 2-postnatal</td>
<td>Adenosine receptors</td>
<td></td>
</tr>
<tr>
<td>da Silva et al. (2008)</td>
<td>Rats</td>
<td>Wistar</td>
<td>Drinking water</td>
<td>Mating gestation, part of lactation</td>
<td>Postnatal development</td>
<td></td>
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<tr>
<td>Iglesias et al. (2006)</td>
<td>Rats</td>
<td>Wistar</td>
<td>Drinking water</td>
<td>GDs 2-end of gestation</td>
<td>Heart receptors—maternal and fetal</td>
<td></td>
</tr>
<tr>
<td>León et al. (2002)</td>
<td>Rats</td>
<td>Wistar</td>
<td>Drinking water</td>
<td>GDs 2-end of gestation</td>
<td>Brain—adenosine A1 receptor in dams and fetuses</td>
<td></td>
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<tr>
<td>León et al. (2005a)</td>
<td>Rats</td>
<td>Wistar</td>
<td>Drinking water</td>
<td>GDs 2-end of gestation</td>
<td>Maternal and fetal brain receptors</td>
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</tr>
<tr>
<td>León et al. (2005b)</td>
<td>Rats</td>
<td>Wistar</td>
<td>Drinking water</td>
<td>GDs 2-end of gestation</td>
<td>Brain—adenosine A1 receptor in dams and fetuses</td>
<td></td>
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<tr>
<td>Gaytan et al. (2006)</td>
<td>Rats</td>
<td>Sprague–Dawley</td>
<td>Gavage</td>
<td>PND 2 to 6</td>
<td>Adenosine A1 receptor system</td>
<td></td>
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<tr>
<td>Burdan et al. (2000)</td>
<td>Rats</td>
<td>Wistar</td>
<td>Gavage</td>
<td>GDs 8 to 14</td>
<td>Caffeine acacetaminophen and isopropylalpyrine on pregnant liver</td>
<td></td>
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<tr>
<td>Burdan et al. (2001)</td>
<td>Rats</td>
<td>Wistar</td>
<td>Gavage</td>
<td>GDs 8 to 14</td>
<td>Determine effect of paracetamol and caffeine administered together</td>
<td></td>
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<tr>
<td>Burdan et al. (2003)</td>
<td>Rats</td>
<td>Wistar</td>
<td>Gavage</td>
<td>GDs 8 to 14</td>
<td>Effects on neonatal rat cornea</td>
<td></td>
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<tr>
<td>Evereklioglu et al. (2003)</td>
<td>Rats</td>
<td>Wistar</td>
<td>Intraperitoneal</td>
<td>GDs 9 to 21</td>
<td>Establish model for study of cataract development in rats</td>
<td></td>
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<tr>
<td>Evereklioglu et al. (2004)</td>
<td>Rats</td>
<td>Wistar</td>
<td>Intraperitoneal</td>
<td>GDs 9 to 20</td>
<td></td>
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<tr>
<td>Boyer et al. (2003)</td>
<td>Rats</td>
<td>Wistar-CRL</td>
<td>Intraperitoneal</td>
<td>Postnatal</td>
<td>Behavior-cafefe enhancing effect?</td>
<td></td>
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<tr>
<td>Chornostowska-Wynimko et al. (2004)</td>
<td>Mice</td>
<td>Bulb/c</td>
<td>Diet</td>
<td>During pregnancy and lactation</td>
<td>Fetal development and postnatal status of immune system</td>
<td></td>
</tr>
<tr>
<td>Björklund et al. (2007)</td>
<td>Mice</td>
<td>AI/AR; A2a/RK; WT</td>
<td>Drinking water</td>
<td>GD 7 PND 7</td>
<td>Adenosine receptors</td>
<td>Doses exceeded 30 mg/kg/day</td>
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<tr>
<td>Albina et al. (2002)</td>
<td>Mice</td>
<td>Swiss</td>
<td>Gavage</td>
<td>GDs 0 to 18</td>
<td>Stress and caffeine</td>
<td>≥30 mg/kg/day</td>
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<tr>
<td>Colomina et al. (2001)</td>
<td>Mice</td>
<td>Swiss</td>
<td>Gavage</td>
<td>GD 0 to 18</td>
<td>Stress and caffeine/aspirin</td>
<td>Not appropriate for human extrapolation</td>
</tr>
<tr>
<td>Bahi et al. (2001)</td>
<td>Mice</td>
<td>Swiss</td>
<td>Intraperitoneal</td>
<td>PND 0; PNDs 1 to 3</td>
<td>Effect on lesions of periventricular white matter</td>
<td></td>
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<tr>
<td>Publication</td>
<td>Species</td>
<td>Type</td>
<td>Route</td>
<td>Gestation</td>
<td>Potential effect investigated</td>
<td></td>
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<tr>
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<tr>
<td>Desfrere et al. (2007)</td>
<td>Mice</td>
<td>Not identified</td>
<td>Intraperitoneal injection</td>
<td>PNDs 3 to 10; PNDs 4 to 10</td>
<td>Effect on developing mouse brain</td>
<td></td>
</tr>
<tr>
<td>Lutz and Beck (2000)</td>
<td>Mice</td>
<td>C57BL/6J BK</td>
<td>Intraperitoneal injection</td>
<td>GD 9</td>
<td>Interaction between caffeine and Cd sulfate</td>
<td></td>
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<tr>
<td>Sahir et al. (2000)</td>
<td>Mice</td>
<td>Swiss</td>
<td>Intraperitoneal injection</td>
<td>GDs 8 to 10</td>
<td>Brain development and early encephalization</td>
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</tr>
<tr>
<td>Sahir et al. (2001)</td>
<td>Mice</td>
<td>Swiss</td>
<td>Intraperitoneal injection</td>
<td>GDs 8.5 to 10.5</td>
<td>Gene modulation in postimplantation mouse embryos</td>
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<tr>
<td>Momoi et al. (2008)</td>
<td>Mice</td>
<td>CD-1</td>
<td>Subcutaneous injection</td>
<td>GDs 9.5 to 18.5</td>
<td>Fetal cardiovascular function affected by maternal exposure</td>
<td></td>
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<tr>
<td>López and Alvariño (2000)</td>
<td>Rabbits</td>
<td>NZW—semen</td>
<td>In vitro</td>
<td></td>
<td>Effect of caffeine on semen</td>
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<tr>
<td>Clyman and Roman (2007)</td>
<td>Sheep</td>
<td>–</td>
<td>In vitro</td>
<td></td>
<td>Effects on preterm sheep ductus arteriosus</td>
<td></td>
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<tr>
<td>Tomimatsu et al. (2007)</td>
<td>Sheep</td>
<td>–</td>
<td>Intravenous administration</td>
<td></td>
<td>Brain-effect on fetal cerebral oxygenation</td>
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<tr>
<td>Tatham et al. (2003)</td>
<td>Buffalo-Cattle</td>
<td>–</td>
<td>In vitro</td>
<td></td>
<td>Effect of caffeine on buffalo sperm to overcome male infertility</td>
<td></td>
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<tr>
<td>Miscellaneous</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buttar and Jones (2003)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>Symposium on herbal remedies</td>
<td></td>
</tr>
<tr>
<td>Gilbert-Barness (2000)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>Letter to the editor</td>
<td></td>
</tr>
<tr>
<td>Keller et al. (2007)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>Overview—cardiovascular development</td>
<td></td>
</tr>
</tbody>
</table>

N/A, not applicable; GD, gestation day; PND, postnatal day.
appropriate route for evaluating human risks from exposure to caffeine in caffeinated beverages or naturally containing caffeinated drinks, food, or medication. The majority of animal caffeine studies did not use the oral route. However, analyses of all animal studies were performed regardless of the caffeine formulations, vehicles, route of administration, doses, or stages of pregnancy when exposure occurred. All recent animal toxicology publications were reviewed for relevance. Only those that included treatment during pregnancy, or the early postnatal period in rats, when the brain is similar in development to that of human fetuses, are included in this review. These publications are included by species and publication date in Supplemental Table 1, and Tables 6 and 7.

Unfortunately, the better designed and more comprehensive animal studies were performed before 2000. Palm et al. (1978) exposed Sprague–Dawley female rats before pregnancy and throughout pregnancy to 12.5, 25, or 50% brewed coffee in their drinking water, which was equivalent to 9, 19, or 38 mg/kg/day of caffeine. Even at the highest exposure there was no difference in the number of resorptions, litter size, fetal weight of sex ratio, or the offspring when compared to the control litters. On the 38th post partum day the animals that had been allowed to litter were comparable to the controls with regard to litter size, viable young, birth weight, and pup weight at 38 days. These results were in agreement with other investigators (Aeschbbacher et al., 1980; Nagasawa and Sakurai, 1986). Even relatively high

**Table 8**

<table>
<thead>
<tr>
<th>Method of administration</th>
<th>Exposure</th>
<th>Plasma caffeine level</th>
<th>Teratogenic effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 2 cups of coffee/day in humans; 1 to 2 mg/kg</td>
<td>100 to 200 mg of caffeine</td>
<td>1 to 3 μg/ml peak level</td>
<td>Not teratogenic</td>
</tr>
<tr>
<td>3-5 cups of coffee/day in humans; 3 to 5 mg/kg</td>
<td>500 to 600 mg of caffeine</td>
<td>5 to 6 μg/ml peak level</td>
<td>Not teratogenic</td>
</tr>
<tr>
<td>10 cups of coffee per day over a 10-hr period</td>
<td>&lt;1,000 to 1,200 mg of caffeine</td>
<td>Speculation; &lt;10 μg/ml peak level</td>
<td>Minimal data; unlikely to be teratogenic</td>
</tr>
<tr>
<td>Caffeine in the drinking water in the rat</td>
<td>80 mg/kg/day</td>
<td>5.7 ± 2.3 mg/kg/day</td>
<td>Not teratogenic</td>
</tr>
<tr>
<td>Caffeine in the drinking water in the rat</td>
<td>205 mg/kg/day</td>
<td>Peak ?</td>
<td>Not teratogenic</td>
</tr>
<tr>
<td>Caffeine by once a day gavage in the rat</td>
<td>80 mg/kg/day</td>
<td>Peak &gt;60 μg/ml</td>
<td>Teratogenic</td>
</tr>
<tr>
<td>Caffeine in the drinking water in the rat</td>
<td>330 mg/kg/day</td>
<td>Peak &gt;60 μg/ml</td>
<td>Teratogenic</td>
</tr>
<tr>
<td>Caffeine in drinking water in the rat</td>
<td>80 mg/kg/day</td>
<td>0.10 to 5.74 μg/ml</td>
<td>Not teratogenic</td>
</tr>
<tr>
<td>Caffeine bolus of 25 mg, 24 hr later in nonpregnant rat</td>
<td>25 mg/kg</td>
<td>2 μmol/l/0.4 μg/ml</td>
<td>A pharmacokinetic study</td>
</tr>
<tr>
<td>Caffeine bolus of 25 mg, 24 hr later in 20-day pregnant rat</td>
<td>25 mg/kg</td>
<td>20 μmol/l/1.4 μg/ml</td>
<td>A pharmacokinetic study</td>
</tr>
<tr>
<td>Human exposure during pregnancy of a mother who drank 9 to 24 cups of coffee/day (Khanna and Somani, 1984; Bodineau et al., 2003)</td>
<td>900 to 2,400 mg/day</td>
<td>80 μg/ml at birth, estimated 40.3 μg/ml at the 12th postpartum day. Maternal serum level on the 10th postpartum day, 18.4 μg/ml</td>
<td>No teratogenesis, growth retardation. Liveborn who is doing well and was weight-appropriate for the gestational age</td>
</tr>
<tr>
<td>Food and Drug Administration recommendation (1980)</td>
<td>Limit caffeine to &lt;400 mg/day (6.7 mg/kg/day for a 60-kg human)</td>
<td>Peak blood level will be very low</td>
<td>No data; Very unlikely to be teratogenic</td>
</tr>
</tbody>
</table>

**Table 9**

<table>
<thead>
<tr>
<th>Mechanisms of Action of Environmental Teratogens</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Cytotoxicity or mitotic delay beyond the recuperative capacity of the embryo or fetus (ionizing radiation, chemotherapeutic agents, alcohol)</td>
</tr>
<tr>
<td>2. Inhibition of cell migration, differentiation, and cell communication</td>
</tr>
<tr>
<td>3. Interference with histogenesis by processes such as cell depletion, necrosis, calcification, or scarring</td>
</tr>
<tr>
<td>4. Biologic and pharmacological receptor-mediated developmental effects (i.e., etretinate, isotretinoin, retinol, sex steroids, streptomycin, and thalidomide)</td>
</tr>
<tr>
<td>5. Metabolic inhibition (i.e., warfarin, anticonvulsants, and nutritional deficiencies)</td>
</tr>
<tr>
<td>6. Physical constraint, vascular disruption, inflammatory lesions, and amniotic band syndrome</td>
</tr>
<tr>
<td>7. Interference with nutritional support of the embryo by decreasing maternal food intake or affecting yolk sac or chorioplacental function or transport</td>
</tr>
</tbody>
</table>

exposures of caffeine or coffee in the water supply had minimal effects on birth weight, pup weight, and perinatal mortality in other studies (Knoch and Konig, 1964; Nolen, 1981; Pollard et al., 1987). Exposures of caffeine up to 60 mg/kg/day in rats and 74 mg/kg/day in mice did not alter the number of resorptions, conceptions, litter size, or births (Aeschbacher et al., 1980; Nagasawa and Sakurai, 1986; Pollard et al., 1987).

The FDA commissioned an “in-house” study using pregnant Osborne-Mendel rats that were administered caffeine by gavage from 0 to 19 days of pregnancy with 0, 6, 12, 40, 80, or 125 mg/kg of caffeine with each intubation (Collins et al., 1981). The highest dose was maternally toxic as evidenced by the fact that 6 of the 50 pregnant rats in the 125 mg/kg group died. CMs were increased in the two groups with the highest exposure. Ectodactyly occurred in 28.5% of the fetuses in the 125 mg/kg group. The NOEL for CMs for caffeine was determined to be 40 mg/kg/day. Many investigators had results that were similar to the FDA study (Bertrand et al., 1965; Leuschner and Schverdtfeger, 1969; Bertrand et al., 1970; Ikeda et al., 1982; Smith et al., 1987). It is important to emphasize that gavage or tube installation feeding will have a much lower teratogenic NOEL than when caffeine is placed in the water or food supply. In fact, in many studies investigators were unable to produce CMs by adding large amounts of caffeine to the water or food supply (Leuschner and Schverdtfeger, 1969; Gilbert and Pistey, 1973; Collins et al., 1983; Smith et al., 1987). Some investigators were able to produce malformations using caffeine in the water supply; however, it required an exposure of 330 mg/kg/day (Fuji and Nishimura, 1972).

Collins et al. (1981) demonstrated that a single oral gavage exposure of 80 mg/kg of caffeine was teratogenic, but 205 mg/kg/day in the water supply was not teratogenic. Stillbirths and miscarriages were observed with increased frequency among the offspring of macaque monkeys treated during pregnancy with caffeine in a dose equivalent to 5 to 7 or 12 to 17 cups of coffee per day (Gilbert et al., 1988). The cause for the stillbirths was not apparent at necropsy; no malformations were seen. Body weight of the male but not the female infants of treated monkeys were reduced (Gilbert and Rice, 1991).

An increased frequency of malformations, especially of the limbs and palate, has been observed among the offspring of rats or mice treated with caffeine during pregnancy in doses equivalent to human consumption of 40 or more cups of coffee daily (Purves and Sullivan, 1993; Nehlig and Debray, 1994). Behavioral alterations have also been observed among the offspring of monkeys born to mothers treated during pregnancy with caffeine in doses equivalent to 5 to 15 cups of coffee per day (Rice and Gilbert, 1990; Gilbert and Rice, 1994). The relevance of these observations to the risks in infants born to women who drink large amounts of caffeinated beverages during pregnancy is unknown.

High doses of caffeine influence the teratogenic activity of many other agents in animal studies (Nehlig and Debray, 1994; Sivak, 1994). Co-administration of caffeine often enhances the teratogenic action of other agents, but in some instances there is no interaction and in others, caffeine exhibits a protective effect. The relevance of these findings to humans is uncertain.

There are animal experiments that do assist in the evaluation of the human risks of caffeine exposure during pregnancy (Tables 8 and 9).

**Extrapolation of the Caffeine Animal Studies for Human Risk Assessment (Supplemental Table 1, and Tables 6–8)**

Purves and Sullivan (1993) classified caffeine’s teratogenic effect as a “peak blood level effect” and not an “area under the curve effect.” This is important because it emphasizes the importance of the method of administration in designing animal studies that are designed to evaluate the reproductive and developmental risks of caffeine in human populations. The peak exposure plasma level in animal models that is necessary to result in teratogenesis is equal to or > 60 μg/ml (Elmazar et al., 1982; Ikeda et al., 1982; Smith et al., 1987; Sullivan et al., 1987) (Table 10).

The results of properly planned animal studies can be helpful in solving some of the dilemmas created by inconsistent findings in epidemiological studies. An animal study reported in 1960 first focused our attention to the potential developmental effects of caffeine. However, the exposure reported by Nishimura and Nakai (1960) was from intraperitoneal injections of 250 mg/kg in the mouse, an extremely high dose that would result in a blood plasma level that could never be attained from consuming caffeine containing products in food or beverages. More recent animal studies have demonstrated that depending upon the method of administration and species, the developmental NOEL in rodents is approximately 30 mg/kg/day; the teratogenic NOEL is 80 to 100 mg/kg/day, and the reproductive NOEL approximately 80 to 120 mg/kg/day (Nash and Persaud, 1988; Nolen, 1989; Stavric, 1992; Dlugosz and Bracken, 1992).

Purves and Sullivan (1993) agreed with the information previously cited by the FDA, since their conclusions are in basic agreement with the FDA position (1986). However, Purves and Sullivan (1993) evaluated the pharmacokinetics of caffeine more extensively, which is important to estimate the risk. The cited studies and
comments convincingly demonstrate that the route of administration (bolus vs. administrating in drinking water or diet) and the timing of treatment during pregnancy (or development) are related to the serum blood levels attained in the specific species tested. As a result, this review indicates that such factors must be considered in any risk assessment process for caffeine, because under normal conditions of consumption, humans cannot attain serum blood levels comparable to those associated with the threshold for adverse effects from caffeine exposure in rats (Tables 8 and 9).

Although apparent differences exist because of the duration of administration, the study by Collins et al. (1983), in which caffeine was dissolved in drinking water, and the previous study described by Nolen (1981), in which caffeine was provided as brewed or instant coffee in drinking water, have remarkable similarities in the mode of caffeine administration (oral, drinking water) and the effects produced. Both these studies were conducted using adequate numbers of animals and well-defined protocols.

The relevance of the mode of exposure to resultant toxic effects was also confirmed by Smith et al. (1987). In this study Wistar rats were given 10 or 100 mg/kg/day of caffeine on p.c.d.s. 6 to 20, either as bolus oral doses (once daily), or as four 2.5 or 25 mg/kg doses given at three-hour intervals. Maternal body weight and feed consumption were reduced in both groups given total doses of 100 mg/kg of caffeine and in the group given 25 mg/kg of caffeine four times/day. Developmental effects in these groups included dose-related decreases in fetal weight, placental weight, crown-rump length and skeletal ossification. Major abnormalities, principally ectodactyly, occurred only in the group given the bolus 100 mg/kg dose, confirming the observations of Collins et al. (1983).

Colomina et al. (2001) exposed mice to caffeine (30 mg/kg) and aspirin (ASA). (250 mg/kg by gavage on the 9th post conception day.) There was no significant maternal or developmental toxicity in this group of animals and offspring. The studies also included stress-fuy restraint. However, the exposure and the stress in the mouse studies cannot be utilized to determine human developmental risks, especially since the developmental results were minimal and the exposure equivalency in the human is unknown.

Evereklioglu et al. (2003, 2004) administered caffeine i.p. to Wistar rats on post conception days 9 to 21. There were four groups: 0, 25, 50, and 100 mg/kg/day. There was no maternal toxicity but there were seven fetal deaths in two dams in the 100 mg/day group. The investigators attributed the embryonic deaths to the i.p. injections of a high dose of caffeine. Histopathologic lens opacities were noted in the 100 mg/kg group. The investigators were unable to determine the human risk for cataracts from those studies.

Leon et al. (2002, 2005a,b) exposed Wistar rats to caffeine in drinking water from day 2 until delivery. The estimated exposure was 83.2 mg/kg/day. The authors hypothesized that caffeine and theophylline could have harmful effects on the developing fetal brain. Based on their findings they hypothesized that caffeine and theophylline may be associated with potentially harmful effects on the developing fetal brain.

Lutz and Beck (2000) administered 1.0, 2.5, and 5.0 mg/kg of cadmium subcutaneously on post conception days 9 to 12 in C57 BL/6 J BK mice. They were simultaneously administered zero or 50 mg/kg of caffeine subcutaneously. The teratogenic effects of cadmium were ameliorated by the caffeine administration. Litter size, fetal weight, fetal mortality, and dam weight were not affected by the co-treatment of caffeine.

Sadaani-Makki et al. (2004) exposed pregnant Sprague–Dawley rats to 0.02% caffeine in their drinking water (postconception days of exposure not mentioned). The estimated caffeine consumption was 49.8 mg/kg/day. In utero exposure resulted in an increase in birth rate. There was also evidence for involvement of adrenergic A1 systems by the occurrence of respiratory perturbation in newborns. There was no discussion of human risk assessment of caffeine exposure based on these studies.

The critique of the animal studies may appear to negate their usefulness in estimating human reproductive and developmental risks. This conclusion may be due to the many animal studies utilizing parenteral or bolus administration of caffeine. The smaller percentage of animal studies that utilized the administration of caffeine in the food or drinking water has yielded important information summarized in Table 10. It indicates that the NOEL for teratogenesis necessitates a plasma level of caffeine > 60 µg/ml. This is unattainable without pregnant women ingesting large quantities of caffeine. For example, 10 cups of coffee over a period of 8 to 10 hr (1,000 mg of caffeine) would never be able to reach a plasma level of 60 µg/ml.

### PHARMACOKINETICS

#### Cross-Species Similarities in Metabolism

One reason that animal models are useful in the study of caffeine is that the pharmacokinetics of caffeine may be similar to humans in some animal species. In both animals and humans, oral administration of caffeine results in its rapid absorption, with peak plasma levels attained within 3 to 120 min (Perves and Sullivan, 1993). The absorption rates also increase with increased dosages due to the many animal studies utilizing parenteral or bolus administration of caffeine. The smaller percentage of animal studies that utilized the administration of caffeine in the food or drinking water has yielded important information summarized in Table 10. It indicates that the NOEL for teratogenesis necessitates a plasma level of caffeine > 60 µg/ml. This is unattainable without pregnant women ingesting large quantities of caffeine. For example, 10 cups of coffee over a period of 8 to 10 hr (1,000 mg of caffeine) would never be able to reach a plasma level of 60 µg/ml.

#### Table 10

<table>
<thead>
<tr>
<th>Effect</th>
<th>Risk</th>
<th>Quality of the data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congenital malformations</td>
<td>Unlikely</td>
<td>Good</td>
</tr>
<tr>
<td>Spontaneous abortion</td>
<td>Minimal</td>
<td>Fair to good</td>
</tr>
<tr>
<td>Fetal growth retardation</td>
<td>Unlikely</td>
<td>Fair to good</td>
</tr>
</tbody>
</table>

#### Conclusion

Consumption of caffeinated beverages during pregnancy is unlikely to increase the risk of congenital malformations and growth retardation; and poses a minimal risk for miscarriage, possibly at very, very high caffeine exposures. These risk estimates include the evaluation of inconsistent epidemiology studies and the utilization of animal (mammalian) reproductive studies.

tract is rapid in adult humans, with attainment of maximum caffeine concentrations within 15 to 60 min after oral ingestion (for dosages of 5 to 8 mg/kg, the plasma concentrations equating 8 to 10 μg/ml) Supplemental Table 8. Once absorption occurs, caffeine is rapidly distributed in body water, equilibrating between blood and tissues, including the embryo/fetus, as well as the brain and testes. It is also rapidly distributed to the breast milk. Caffeine in human breast milk contains approximately 75% of the plasma level, depending upon the maternal dosage (3.2–8.6 μg/ml of caffeine is found in human breast milk and 0.7–7.0 μg/ml in rat milk).

Consumption of caffeine in the milk results in only 1% of the maternal intake being consumed by human infants and 2% of the maternal intake consumed by rat pups (Perves and Sullivan, 1993).

Pregnancy alters the metabolism of caffeine, which, under normal conditions, is rapidly metabolically eliminated. Caffeine’s retention is increased during pregnancy in humans, late human fetuses, and neonates, with a half-life varying from 80 to 100 hr. Presumably this increase in retention is the result of deficient P-450 enzymes in the fetus and neonate. Human metabolism of caffeine reaches adult parameters after approximately 7 months of age, but the half-life can be affected by inducing agents. For example, the half-life in smokers is approximately half of that in nonsmokers (Christian and Brent, 2001).

The characterization of the enzymatic process of caffeine metabolism was also explored by Buters et al. (1996), who investigated the involvement of CYP1A2 metabolizing enzymes in the pharmacokinetics and metabolism of caffeine using mice lacking its expression (CYP1A2−/−). The mice were intraperitoneally administered 2 mg/kg of caffeine, a dosage that was reported to be equivalent to that of a human drinking one cup of coffee. The half-life of caffeine elimination from blood was seven times longer, AUC was increased eight times, and clearance was consequently eight times longer in these animals than in wild-type mice. Other P450 enzymes were not affected and the clinical pathology evaluations of the liver and kidney were unaffected. These data indicate that the clearance (elimination) of caffeine in wild-type mice is primarily determined by CYP1A2. Because human and mouse CYP1A2 resemble each other in cDNA-derived amino acid sequence, these data also suggest that humans have a similar elimination pattern.

Derkenne et al. (2005) confirmed the conclusions of previous investigators that mouse or human CYP1A2 is the predominant enzyme for theophylline metabolism. Seven blood samples were taken at intervals from 5 to 400 min after IP injection of 8 mg/kg theophylline in mice. Replacing mouse CYP1A2 (−/−) with a functional human CYP1A2 gene restored the ability to metabolize theophylline, and the metabolism changed to a human profile. Comparing the hCYP1A1_A2 Cyp1a2 (−/−) and wild-type mice with published clinical studies revealed that theophylline clearance to be approximately 5 × and 12 ×, respectively, greater than that reported in humans, which is due to the well-known fact that mice clear drugs more rapidly than humans. Metabolism of caffeine varies remarkably among species and within the same species, and it is highly dependent on variables such as sex, age, and pregnancy status. In human newborns, the plasma half-life of caffeine is 4 days, while in young children and teenagers (6–13 years old), the plasma half-life is 2.3 hr. In adult humans, the half-life averages 2-6 hr in healthy nonsmokers, but it is prolonged in pregnant women to 10 to 20 hr. In rats, a half-life of 2.12 hr is reported for 8-week-old Sprague–Dawley male rats given one oral dosage of 4 mg/kg of caffeine. The major metabolite in humans is paraxanthine, or 1,7-dimethylxanthine. In rats, the major metabolite is 1, 3, 7-diaminouracil, or 6-amino-5-[N-formylmethylamino]-1,3-dimethyluracil. Caffeine is demethylated in both rats and humans to three dimethylxanthenes (theophylline, theobromine, and paraxanthine), which suggests that rats are an appropriate model for use in risk assessment for humans.

The differences in caffeine and paraxanthine metabolism between human and murine CYP1A2 in liver microsomes were also explored by Labedzki et al. (2002). Results of the in vitro studies confirmed the important role of CYP1A2 in both murine and human metabolism of caffeine, despite formation of 1, 3, 7-trimethylurate as an in vitro “artifact” in both human and murine microsomal preparations. Both human and murine CYP1A2 enzymes have close similarities in the primary metabolic steps of caffeine. However, paraxanthine in vivo was not metabolized by murine CYP1A2 to a relevant extent, which is in contrast to the human situation. Also, results of this study confirmed the known reported inhibitory effects of the quinolones, norfloxacin, and pefloxacin on human CYP1A2, while in murine hepatic microsomes, quinolones did not exert an inhibition of caffeine 3-demethylation.

The authors concluded that murine models are important for understanding the metabolism of xenobiotics in humans, but that extrapolation of data may be inaccurate in certain cases, such as in cases where compounds have low affinity ligands to CYP1A2. Therefore, interspecies comparison may be required before the use of mouse models to predict toxicity and/or pharmacological activity in humans. However, the metabolic patterns in rats are more closely related to the human.

**Effect of Caffeine on the Neonate**

The capability to adequately metabolize xenobiotics are greatly reduced in neonatal or premature infants and animals due to an inadequately developed hepatic enzyme system, and often it is difficult to determine exact medicinal dosages during this age. In humans, intravenous theophylline is frequently administered to premature neonates during the first several days to reduce apnea, although there has been little emphasis in the literature on the pharmacokinetics in this segment of the population. Two clinical trials on this subject are presented below to describe some of the pharmacokinetic parameters.

The clearance rate (CL) and volume of distribution (V) of theophylline were studied by du Freez et al. (1999) in 105 apneic premature neonates (mean weight: 1.3 kg; age: 1.1 days) receiving intravenous loading dosages of 4 to 7.7 mg/kg aminophylline. Maintenance dosages ranged from 1.4 to 6 mg/kg/day in 2 to 4 divided doses. Data were analyzed using the nonlinear mixed effects model (NONMEM), and a one-compartment model with first-order elimination. The study differed from other cited premature neonatal references in that it was conducted in South Africa on all-black babies that had a 92% incidence of respiratory distress syndrome, and the described PK
related only to the first few postnatal days. Low CL values were recorded (0.0084 and 0.0561/hr/kg, respectively, for babies with and without oxygen support), while values of ≥ 0.0121/hr/kg have been cited by other investigators. As a result of the low CL, long half-lives (54 and 76 hr, respectively, for babies with and without oxygen support) were reported. The calculated value for V was 0.631/kg. Variability in both CL and V were high, and it was concluded that theophylline PK is highly variable in neonates because physiologic parameters are changing rapidly and theophylline clearance and urinary metabolite patterns apparently do not reach stable adult values until 55 weeks postconception.

Urinary output was also evaluated in 19 premature infants aged 45±4.0 days before and after a 20-min loading solution of aminophylline (6 mg/kg), which was followed by a maintenance therapy of 2 mg/kg every 12 hr (Mazkereth et al., 1997). The infants had a mean gestational age of 31.1±2.8 weeks and a birth weight of 1481±454 g. Marked diuresis occurred immediately after the loading dose, and the ratio of urinary output to water intake increased from 0.58±0.36 to 1.19±0.65. Fractional excretion of sodium and potassium increased, and urinary calcium and uric acid excretion was also enhanced. Tubular reabsorption of phosphorus was not affected. These effects were no longer evident after 24 hr, despite aminophylline maintenance therapy. The authors concluded that the aminophylline acted directly on tubular reabsorptive functions of the nephron. Neonatal patients afflicted with hyperbilirubinemia may also gain some benefit from a neonatal rat model that could be used to evaluate new therapeutic agents for this disease.

Induction of cytochrome P450 1A (CYP1A) may be a valuable therapeutic modality for reducing the hyperbilirubinemia of infants with Crigler–Najjar syndrome type 1 (CNS-I), a severe form of congenital jaundice. To evaluate inducers of CYP1A, a novel assay was established by Jorritsma et al. (2000), based on the comparison of the type of urinary pattern of caffeine metabolites in rats when 10 mg/kg of 1-Me-14C-caffeine is injected intraperitoneally before and 48 hr after injection of a potential CYP1A inducer, such as 5,6-benzo-flavone (BNF). The inducing effect of BNF on CYP1A activity was confirmed by the urinary pattern of caffeine metabolites in Wistar rats and was paralleled by a decrease in plasma bilirubin in male JJ Gunn rats.

It is interesting to note that in conjunction with the above study, a selective and sensitive reverse-phase liquid chromatographic method was developed by Shrader et al. (1999) for the simultaneous analysis of [1-methyl-14C]caffeine and its eight major radiolabel-led metabolites in rat urine. Separation of the complex mixture of metabolites was achieved by gradient elution with a dual solvent system using an endcapped C18 reverse-phase column, which, in contrast to commonly used C18 reverse-phase columns, also allows the separation of the two isomers of 6-amino-5-(N-formylmethylamino)-1,3-dimethyluracil (1,3,7-DAU), a metabolite of quantitative importance predominantly occurring in rats.

Impact of Various Factors on Altering the Pharmacokinetics of Caffeine

The effect of gender on the pharmacokinetics of caffeine (5 mg/kg, intravenously) was explored in 10 male and 10 female Holstein cattle during the ages 1, 2, 4, 6, 8, 12, and 18 months (Janus and Antoszek, 2000). The findings were compared to the results in other species, including humans. The volume of distribution (V) decreased significantly with age, as it does in pigs and humans; results were similar in males and females. A steady, significant decrease in mean residence time (MRT) also occurred in both sexes, although the MRT was significantly shorter in females after 8 months of age. Significant decreases over time also occur in dogs, pigs, and humans because caffeine clearance depends principally on intrinsic hepatic clearance. Total plasma clearance (Cl) of caffeine increased by nearly 100% between the first and 18th month of life (from 0.80 to 1.55 ml/min/kg in males; from 0.84 to 1.80 ml/min/kg in females). Similar changes occur in dogs and humans; the change is due to inadequate development of the hepatic microsomal enzyme system in the neonatal period. It was concluded that clear-cut sex differences in MRT and Cl occurred in cattle over eight months in age, the females being the more active metabolizers.

In a similar manner, Janus et al. (2001) investigated the effects of short-term (4 days) starvation or water deprivation on the pharmacokinetics of caffeine (5 mg/kg, intravenously) in three groups of ten 24- to 25-day-old Holstein calves. An automated enzyme-multiplied immunoassay technique was used to determine plasma caffeine concentration just before the administration of caffeine and four days later at the end of the deprivation period. Results from the caffeine study indicated that four days of starvation or water deprivation was associated with significant increases in MRT and Total Plasma Clearance (Clt) of 20 to 30%. V was slightly (not significantly) decreased. It was concluded that the results from this study were similar to the findings reported in sheep, horses, laboratory animals, and humans, and indicate that starvation and water deprivation lead to a general inhibition of the hepatic P450 enzyme system and may impair the elimination of drugs that undergo metabolism by these enzymes.

Pelissier-Alicot et al. (2002) investigated the effects of caffeine on the daily rhythms of heart rate, body temperature, locomotor activity, and caffeine pharmacokinetics (PK) in 10-week-old male Wistar rats in relation to time-of-day. The study was divided into three 7-day phases: a control period, a treatment period, and a recovery period. During the treatment period, 25 mg/kg of caffeine was administered subcutaneously to groups of rats (four rats/group) at 8:00 AM in the morning, and to other groups at 8:00 PM in the evening. Blood for PK parameters was drawn at periodic intervals of 0.25 to 24 hr postinjection on the 7th day of treatment. Telemetry was used in similarly treated rats to obtain pharmacodynamics data. Morning administration of caffeine suppressed locomotor activity and modified the diastolic–systolic amplitudes of heart rate and body temperature; evening administration did not alter locomotion, but altered the blood pressure elevations, amplitudes, and acrophases of the three rhythms, indicating a chronopharmacologic effect. PK data revealed that the area-under-the-curve (AUC) was significantly lower in rats medicated in the evening, compared to medication in the morning, due to an increase in total plasma clearance and volume of distribution. However, there was no significant time of administration-dependent difference in C_{max}, T_{max}, or half-life.
The influence of hepatic regeneration after partial hepatectomy (removal of median and lateral lobes) on theophylline (Th) pharmacokinetics in groups of five adult male Wistar rats was studied by Maza et al. (2001). At 12 and 24 hr and 3, 6, and 15 days after partial hepatectomy, Th was administered intravenously as a single dosage of 6 mg/kg, and plasma concentrations were determined at periodic intervals. Liver weights and clinical pathology parameters were also determined. Liver mass at the respective dates above were: 3.8, 5.0, 6.5, 7.1, and 9.4 g, compared to 12.1 g in nonhepatectomized rats. Liver function tests were increased significantly at 12 and 24 hr. Initial Th concentrations and volume at steady state varied during regeneration. The control elimination half-life of 4.30 ± 1.37 hr notably increased after hepatectomy (7.27 ± 1.38 hr), and then decreased with time to 5.17 ± 0.87 hr at 15 days. The increase in elimination half-life led to a decrease in mean residence time during the period of regeneration; however, the intrinsic clearance hardly varied.

**Appropriate Use of Animal Studies for Assessing Human Risk**

Although many metabolic and kinetic factors appear similar in rats and humans, only clinical studies in humans and intact animal pharmacokinetic studies in animals can be used to extrapolate risks from animal species to humans. There are few or no data regarding blood levels attained or the comparability of dosages administered. One of the most important considerations regarding comparability of blood levels is that humans consume caffeine over a period of time, rather than as a bolus dosage, and certainly not from an intraperitoneal injection. Humans consuming a 1 to 2 mg/kg dosage of caffeine attain a blood concentration of 1 to 2 µg/ml; a 3 to 5 mg/kg intake of caffeine results in a 5 µg/ml serum concentration. Thus, a 1 mg/kg intake produces a 1 µg/ml blood concentration over the range humans are likely to consume, fitting first-order kinetics for human metabolism of caffeine. The kinetics in rats is dose-dependent and zero order, indicating a saturable process, particularly at high dosages (Christian and Brent, 2001) (Tables 8 and 9).

Many animal studies in the previous review (Christian and Brent, 2001) and in this current review were conducted using bolus gavage dosages, rather than exposure over a period of time as the result of administration in the drinking water or diet. Such differences in the route of exposure often confound interpretation of data and results in inappropriate identification of the NOEL (no observable effect level). Most comparisons are made on the basis of mg/kg dosages, rather than attained blood levels, that are generally considered more useful in cross-species extrapolation, but which are rarely identified in human studies. For example, pregnant rats that were administered caffeine by gavage or via the drinking water for the first 11 days of pregnancy and then administered an 80 mg/kg dosage of radiolabeled caffeine on days 12 to 15 of gestation had blood serum concentrations of caffeine that were much greater after gavage dosage (60–63 µg/ml) than after drinking water exposure (0.10–5.74 µg/ml). However, the drinking water levels were more variable because of the remarkable variability in timing and consumption of drinking water. The half-life of an 80 mg/kg dosage of caffeine in pregnant rats in this study was approximately 1.7 to 2.6 hr (Christian and Brent, 2001) (Tables 8 and 9). When two bolus gavage dosage of caffeine, 5 and 25 mg/kg, were administered to Wistar pregnant rats, apparent enzyme saturation resulted in nonlinear kinetics at the higher dosage only, resulting in an increased half-life and/or an increased distribution phase. However, mean peak plasma concentrations in nonpregnant and pregnant gestation day 20 rats and in the placenta, amniotic fluid, and fetal blood were linear at approximately equivalent times for both dosages. At 24 hr after the 25 mg/kg dosage, plasma concentrations of caffeine were 2 µmol/l (0.4 µg/ml) and 20 µmol/l (4 µg/ml) in nonpregnant and pregnant rats, respectively, and the half-life was significantly longer in pregnant (8.9 hr) than in nonpregnant (3.8 hr) rats at the 5 mg/kg dosage but increased at the 25 mg/kg/day dosage, indicating saturation (Christian and Brent, 2001) (Tables 8 and 9). When given intravenously to pregnant sheep, as described by Tomimatsu et al. (2007), maternal intravenous administration of 3.5 mg/kg of caffeine resulted in a maternal plasma caffeine concentration of 5 µg/ml and fetal caffeine concentrations in excess of 80% of maternal concentration. Other authors cited that the metabolism of caffeine differs between rats and humans, with the half-life much shorter in rats. Using a correction factor, Tanaka et al. (1983) demonstrated that a dosage of 70 mg/kg/day ingested by pregnant rats is equivalent to a dosage of approximately 30 mg/kg/day for humans. Thus, Bodineau et al. (2003) considered a 49 mg/kg/day dosage of caffeine in drinking water to pregnant rats to be in the moderate range for a human model although all other authors consider this a high exposure. Newborns exposed to caffeine in utero exhibited apnea postnatally. In toto, these toxicokinetic experiments show that:

1. Serum and/or plasma concentrations of caffeine are much higher in rats after gavage treatment than after sipping treatment or continuous intravenous infusion; 2. Pregnancy alters pharmacokinetics in both humans and rats, and 3. The changes may be dose-dependent and species-specific.

Yet, pharmacokinetic studies with caffeine can serve a very useful purpose, especially when it is used as a biomarker for the estimation of xenobiotic biotransformation and possible hepatotoxicity. An example of such an investigation was conducted in adult mice (BALB/c mice) by Kolarovic et al. (1999). The test article was enflurane, a fluorinated volatile anesthetic, administered by inhalation in either anesthetic or subanesthetic doses, with/without prior intraperitoneal injection of 1 g/kg ethanol. Two control groups were administered only ethanol or saline. Anesthetic exposure occurred for 6 hr/day for 5 days. On the 6th day, half the mice were injected intraperitoneally with 20 mg/kg caffeine and 8-9 hr urine samples were collected for caffeine metabolite assay; remaining mice were used to determine liver function and cytochrome P450 analysis. Liver function tests were all normal, but liver P450 levels were higher in the group treated with enflurane and ethanol, compared to other groups. Excretion of caffeine and its metabolites was different among the groups. Quantities of caffeine metabolites that are predominantly metabolized by CYP450E1 were higher in urine of enflurane-treated mice, while quantities of caffeine
metabolites predominantly metabolized by CYP-4501A2 were significantly lower than in controls. Control values for the CYP-4501A2 enzymes were: 1,7-dimethyl uric acid (1,7-U) = 4.155 ± 1.956; 1,3,7-threemethyl uric acid (1,3,7-U) = 6.314 ± 2.992. It was concluded that use of caffeine as a biomarker is a highly sensitive test for estimating xenobiotic transformation and possible hepatotoxicity.

**Plasma Levels Versus Organ Exposure**

Plasma levels are not always indicative of exposure of a specific organ. The disposition of caffeine and its metabolites were evaluated in brains from adult and fetal rats on p.c.d. 20 after a single maternal dosage of 5 or 25 mg/kg of caffeine. Fetal and adult caffeine AUC values did not differ between brain and plasma at either dosage. However, the three primary metabolites of caffeine in rats accumulated in the fetal brain at both dosages, resulting in a 3-fold increase in brain metabolite exposure compared with fetal circulatory levels (Christian and Brent, 2001).

**Caffeine Studies Relevant to Teratogenicity or SA (Pregnancy Loss)**

Previous FDA (1980) conclusions and those described by Christian and Brent (2001) appear to provide sufficient precaution regarding consumption of caffeine, that is, that moderate consumption of caffeine (which was defined as ≤5–6 mg/kg/day) is unlikely to increase the risk of SA. These conclusions also appear to apply to the two additional human studies summarized below that were included in the present literature search conducted in 2008.

In a case–control study of 73 women with, and 141 women without SA, Fenster et al. (1998) determined the activity of the three principal caffeine-metabolizing enzymes (P4501A2, xanthine oxidase, and N-acetyltransferase) by measuring the levels of caffeine metabolites in urine. Caffeine was entered as a categorical variable in models with the following levels of caffeine consumption: no caffeine level; 1 to 150 mg/day (<2.5 mg/kg in a 60-kg woman); and >150 mg/day. Results established no association between caffeine consumption, caffeine metabolism, and risk of SA. However, due to small sample size, the study was not able to reliably estimate the risk for recurrent abortion in relation to caffeine consumption and the indices of enzyme activity.

Possible adverse effects of caffeine on pregnancy were also investigated by Klabanoff et al. (2002). They tested 2,515 women to determine whether third-trimester maternal serum concentration of paraxanthine, caffeine’s primary metabolite, is associated with the delivery of a small-for-gestational age infant (birth weight of <10th percentile for gender gestational age and ethnicity), and whether the magnitude of the association is affected by smoking. The subjects were selected from women who enrolled in the Collaborative Perinatal Project at 12 sites in the U.S.A. The mean serum paraxanthine concentration was greater in women who gave birth to small-for-gestational age infants (754 ng/ml) than to “normally” grown infants (653 ng/ml, p = 0.02). However, the linear trend for increasing serum paraxanthine concentration to be associated with increasing risk of small-for-gestational age birth was confined to women who also smoked (p = 0.03). There was no association between paraxanthine and fetal growth in nonsmokers (p = 0.48).

The Frog Embryo Teratogenesis Assay—Xenopus (FETAX) was used to test the 13 metabolites, including theophylline, paraxanthine, and a synthetic methylxanthine analogue (Fort et al., 1998). Frog embryos were exposed to two concentrations of each test article, with or without a metabolic activation system. Assay results indicated that the fetotoxic potencies of each of the di- and monomethylxantine metabolites were similar to that of caffeine. None of the caffeine metabolites tested was found to be significantly more potent than caffeine itself in the FETAX assay.

**Modulation of Teratogenic Properties of Other Agents**

It is well known that low dosages of caffeine can modulate the teratogenic effects of other agents in animal studies. As summarized in Lutz and Beck (2000), defects produced by ionizing radiation, chemical carcinogens, and pharmaceuticals, including anticonvulsants, all have been shown to be potentiated by nonteratogenic dosages of caffeine. In contrast, 5-azacytidine-induced digital defects in mice were suppressed by post-treatment with caffeine. Treatment with caffeine also reduced the teratogenicity of urethan, ethylnitrosourea, and 4-nitroquinoline-1-oxide. Although environmental exposures to Cadmium (Cd) are not considered to be a human teratogen, it has been shown to be teratogenic in rats, hamsters, and mice, with the predominant malformation being right-sided forelimb ectodactyly in mice. This malformation has also been reported in mice after exposure to carbonic anhydrase inhibitors; acetazolamide, ethoxzolamide, and dichlorphenamide. The results of a study by Lutz and Beck (2000) provide evidence that a nonteratogenic dosage of caffeine (50 mg/kg, s.c.) can ameliorate Cd-induced forelimb ectodactyly in this Cd-sensitive mouse strain (C57BL/6JK mice) injected intraperitoneally with 0, 1.00, 2.50, or 5.0 mg/kg of Cd on post conception day 9 and examined on post conception day 18 for ectodactyly and other gross morphological malformations.

**Caffeine Interaction with Stress**

A series of manuscripts were produced by researchers at the University of Seville, Spain and the University of Picardie Jules Verne, France (Bodineau et al., 2003; Saadani-Makki et al., 2004; Gaytan et al., 2006) regarding the potential effects of caffeine and other xyanthines as the result of their binding with adenosine receptors and their potential effect on respiration. Again, these studies were conducted because caffeine is used therapeutically to normalize breathing in apnea-affected infants. The authors stated that premature infants may be exposed to relatively high serum concentration of caffeine (10–15 µg/ml) for up to 8 weeks of treatment. They referenced Shi et al. (1993) who demonstrated that chronic caffeine exposure alters the density of adenosine, adrenergic, cholinergic, GABA, and serotonin receptors and calcium channels in the mouse brain, resulting in a reduction in the fetal cerebral weight. They also indicate that sustained maternal caffeine intake induces harmful physiologic effects on human newborns, including respiratory perturbations, citing a case report (Khanna and Somani, 1984) of a woman reported to have consumed 24 cups of coffee per day during pregnancy,
with a newborn who experienced apnea episodes attributed to methylxanthine withdrawal.

The first study by Bodineau et al. (2003) was conducted using the drinking water route (calculated consumed caffeine dosage = 49 ± 4 mg/kg/day). A subsequent study by the same group (Saadani-Makki et al., 2004) used tissues from the generated pups and evaluated brainstem–spinal cord preparations isolated from these newborn rats. In both studies, the authors noted an increase in pup weight, without any consideration for the mean number of pups per litter. Both these observations should be considered unrelated to caffeine (the increase in newborn weight (7.7 g) in the caffeine exposed group versus the control (6.7 g) was most probably the result of the fewer pups in the caffeine group (10.9 pups) versus the control (13.8 pups), a finding reflecting the relatively few litters evaluated (eight per group) and the normal variability in litter sizes). No historical data were provided.

In the Bodineau et al. (2003) study, the consequences of in utero caffeine exposure on respiratory output in normoxic and hypoxic conditions and related changes of Fos (binding protein involved in transcription regulation) expression were evaluated. The study was conducted using brainstem–spinal cord preparations isolated from newborn rats. Sprague–Dawley rats (control and caffeine groups = 8/group) were given water or 0.02% caffeine in water, with intake evaluated daily, presumably from conception until parturition, because the caffeine was removed after parturition. The experiments were conducted on brainstem–spinal cord preparations isolated from 37 control and 35 caffeine group rats. The authors claimed to know the exact dosage consumed (50.4 ml/day control, 62.3 ml/day—caffeine) with the consumption of 49 ± 4 mg/kg/day, estimated according to drinking fluid intake. The body weight was increased and litter size of the newborn caffeine group rats was reduced, compared with the control group. However, based on the standard deviation of the caffeine group, it is probable that one litter was affected (data were not provided; and the authors did not identify statistical significance). A later study (Saadani-Makki et al., 2004), using tissues from the same animals, further evaluated involvement of the adenosinergic A1 systems in the occurrence of respiratory changes in newborns after in utero caffeine exposure and the importance of the rostral pons in adenosinergic A1 modulation in respiratory control. As before, exposure was during pregnancy, via maternal drinking water, and caffeine fluid intake was estimated at 49.8 mg/kg/day, based on drinking fluid intake, a toxic level. The authors concluded that their work brought evidence of the involvement of the adenosinergic A1 systems in the occurrence of apnea in newborn infants after in utero caffeine exposure.

Further studies by this group (Gaytan et al., 2006) evaluated postnatal exposure to caffeine on the pattern of adenosine A1 receptor distribution in respiration-related nuclei of the rat brainstem. They evaluated the ontogeny of the adenosine A1 receptor system in the brainstem of the newborn rat after postnatal treatment with caffeine. This study identified that the previously reported results, with the main difference between control and caffeine administered rats being the transient increase (on postnatal day 6 only) in the parabrachial and Kölliker–Fuse nuclei, which are classically associated with the adenosine A1 receptor system. The authors concluded that the role of caffeine in decreasing the incidence of neonatal respiratory disturbances may be due to earlier than normal development of the adenosinergic system in the brain.

There was another group of publications originating in Spain regarding the potential interactions of caffeine and stress during pregnancy in mice (Colomina et al., 2001; Albina et al., 2002). In the manuscript by Colomina et al. (2001), a single oral dosage of caffeine or aspirin on p.c.d 9 was given to mice orally exposed to toxic levels of caffeine (30 mg/kg/day), aspirin (250 mg/kg), or a combination of caffeine and aspirin (30 and 250 mg/kg, respectively). Three additional groups were given the same doses and restrained for 14 hr. The pregnant mice were restrained 2 hr/day on p.c.d.s 0 to 18 by placing them in methacrylate cylindrical holders and keeping them in a prone position with the paws immobilized with elastic adhesive tape, a procedure the authors previously reported to produce stress in pregnant mice (Colomina et al., 1995; Scalli et al., 1995; Colomina et al., 1999). Other mice were given toxic dosages of caffeine by gavage at 30, 60, and 120 mg/kg/day on GDs 0 to 18, and another group was administered the same dosages of caffeine immediately followed by restraint stress for 2 hr/day on the same days (Colomina et al., 1999). No caffeine levels were recorded. Although the authors do not identify maternal toxicity, it is noteworthy that the weekly intervals measured for body weights are inappropriate (drug treatments and restraint occurred on one day; the intervals are evaluated for three or four days). Maternal toxicity was evident, with reductions or frank weight losses in body weight and feed consumption measurements. Regarding caffeine, these effects were most severe for the three groups of interest (restraint, 30 mg/kg caffeine and combined 30 mg/kg of caffeine and 14 hr of restraint), on p.c.d.s 9 to 11. Of these three groups, the effects were most severe for the combined caffeine and stress group. The 30 mg/kg plus restraint group also had an increase in postimplantation loss, including dead fetuses and late resorptions. An increase in early resorptions was seen in the restraint alone group, but the group with both restraint and 30 mg/kg of caffeine were increased compared with the restraint alone group. As would be expected, there was an increase in reduced ossification in the restraint group alone, the 30 mg/kg caffeine alone, and the combined caffeine and stress group. There was no increase in malformations in any group. The authors considered there to be some clinical relevance for the data because real life involves multiple simultaneous exposure to many chemicals. However, the duration of oral exposure to aspirin and caffeine on gestational day 9 in this study is not analogous to the type of stress experienced by pregnant women who drink coffee and take aspirin. Interspecies differences and pharmacokinetics and bioavailability are both important considerations.

Albina et al. (2002) reported a study by Nehling and Debry (1994) in which daily consumption of caffeine ranged from 203 to 283 mg, or 2.7 to 4.0 mg/kg/day of caffeine in adults (equivalent to 3.38–4.72 mg/kg for a 60 kg person). Albina et al. (2002) also refer to the FDA 1980 recommendation that pregnant women limit caffeine consumption to less than 400 mg/day (6.7 mg/kg/day for a 60 kg human), based on animal studies (FDA, 1980).
1980). These authors report that 30 mg/kg/day of caffeine administered with maternal stress is an effect level (they did not report that stress alone was an effect level). Nonetheless, the authors recommended that women under notable stress during pregnancy should reduce caffeine ingestion to reasonable levels; for example, a dosage of 10 mg/kg/day. For 60-kg women, 10 mg/kg/day would be a daily ingestion of 600 mg, or four cups of strong coffee or eight cups of weak coffee.

**Interaction of Caffeine as a Pharmaceutical**

A series of studies in rats was conducted by Burdan and his colleagues at the Experimental Teratology Unit of the Human Anatomy Department of the Medical University School in Lublin, Poland. The initial objective was to evaluate the effects of caffeine on skeletal development, when administered by gavage during gestation (Burdan et al., 2000). The later studies were designed to evaluate the effects of over-the-counter preparations of various mixtures of propyphenazone, caffeine, and paracetamol, with the purpose of determining liver toxicity (Burdan et al., 2001) and the prenatal risk of COX inhibitors administered with or without caffeine (Burdan, 2002, 2003, 2004). The studies were conducted in general conformance with evaluations performed for testing pharmaceuticals, but used fewer rats than are usually utilized in studies designed for regulatory use (generally 15 per group, rather than the recommended 16–20 litters), an abbreviated treatment period p.c.ds. 8 to 14, rather than the current usual interval, gestation days 7 to 17. As a result, the exposure period differs by one day from many studies published for regulatory use. Nevertheless, the manuscripts are well documented and easily interpreted. All the findings regarding caffeine’s maternally and developmentally toxic dosages do not indicate new concerns, even in combination with the interacting medications.

Burdan et al. (2000) did not observe adverse maternal or developmental effects at caffeine dosages up to 70 mg/kg administered on p.c.ds. 8 to 14, which is unusual. The Burdan et al. (2001) study showed that caffeine is toxic to the liver only at dosages greater than those tested in this study (the highest dosage of caffeine tested was 70 mg/kg/day), and when given for a prolonged period. The dosages tested in this study were mixtures prepared in 5:3:1 ratio (acetaminophen, isopropylantipyrine, and caffeine), with the caffeine dosages at 0.7, 7, and 70 mg/kg/day. Although the authors concluded that the administration of the mixture to nonpregnant rats at the maximum dosage tested in this study only slightly impaired liver function, hepatotoxic effects were observed in pregnant female rats at the high dosage. Thus, they also concluded that the pregnant rat’s liver was more vulnerable than the nonpregnant rat’s to the tested materials, although they cautioned that the studies were difficult to extrapolate to human exposure.

The next series of studies of combined drugs in over-the-counter products evaluated acetaminophen, isopropylantipyrine, and caffeine (Burdan, 2002). There were 29 control rats and 15 to 19 per group in those administered the caffeine mixture. Caffeine was given by gavage at 0.7, 7.0, or 70 mg/kg, in combination with the other drugs (acetaminophen:isopropylantipyrine:caffeine 5:3:1 ratio [A:I:C]). The authors concluded that this mixture of acetaminophen, isopropylantipyrine, and caffeine administered in a constant proportion of 5:3:1 for the entire second week of pregnancy was not teratogenic in rats but was maternally toxic at the mid and high dosages (35:21:4.7 and 350:214.70 mg/kg, respectively), and was embryotoxic only at the high dosage (350:214.70 mg/kg, respectively).

Burdan (2003) then administered dosages of 3.5:0.7, 35.0:7.0, and 350.70 mg/kg/day of paracetamol:caffeine, respectively, on p.c.ds. 8 to 14. All dosages were maternally toxic, producing reduced maternal weight gain and liver weight. The mid and high dosages also reduced kidney weights, observations that were attributable to paracetamol. At the maternally toxic mid and high dosages, reduced fetal body weight/growth and placental weight occurred, previously described reversible effects of gavage dosages of caffeine, but there was no increase in fetal malformations.

Finally, Burdan (2004) administered maternal dosages of 2:1.0:7, 21:7, or 210:70 mg/kg propyphenazone:caffeine on p.c.ds. 8 to 14. The only evidence of maternal toxicity was decreased liver weight at the high dosage. Fetal body weight was reduced in groups given the middle (21.7 mg/kg propyphenazone:caffeine) and high (210:70 mg/kg propyphenazone:caffeine) dosages of the propyphenazone:caffeine mixtures and the middle dosage of the propyphenazone:paracetamol mixture. The effects on fetal body weight were not dose-dependent, possibly because of the increase in resorption that also occurred at the high dosage. These results are similar to the previously described parallel studies in which all three compounds were given separately or in a mixture. Dose-dependent liver injury was seen in dams given propyphenazone and caffeine and the mixture with all three ingredients, with a hepatotoxic effect and decrease in maternal body weight in the middle and high dosage groups. The authors concluded that co-administration of propyphenazone and caffeine or propyphenazone and paracetamol caused growth retardation but no teratogenic effects and that the results supported the prenatal safety of low dosages of caffeine.

**In Vitro Study on Placental Gene Expression**

Nomura et al. (2004) studied whether caffeine alters gene expressions in human cytotrophoblast-like cell line, Be Wo, using cDNA microarray technology. Tissues were obtained from pregnant rats fed a 20% protein diet or the same 20% protein diet supplemented with caffeine 2 mg/100 g body weight (20 mg/kg) from day 1 (fertilization) until day 20 of gestation, when the placentas were removed by Caesarean-section. Placental blood flow decrease has the potential to lead to intrauterine growth retardation. The present findings demonstrated that caffeine caused a decreased level of Bcl-2 expression in a human trophoblast cell line and placentas removed from caffeine-administered pregnant rats. The exposure of 20 mg/kg is very high and it would be problematic to apply these findings to human pregnancies.

**Caffeine Studies Regarding Adenosine Receptor Interaction and Adenosine Effects**

As noted in some of the studies previously discussed, caffeine interacts with the adenosine receptor, and it is the most widely known adenosine receptor antagonist.
The biochemical mechanism underlying the effects of caffeine is the blockade of adenosine receptors, which is an antagonist for adenosine modulation. Although adenosine receptor interaction with caffeine may not result in teratogenicity, caffeine may affect neuronal growth and neuron interconnections during gestation and the neonatal period. It would be important to determine the NOAEL for deleterious effects on neuronal growth and neuron synapse formation.

Caffeine modulation of adenosine receptor and ontogeny was tested in the following studies. Snyder (1984) provided an extensive review of adenosine as a potential modulator of the behavioral effects of xanthines, approximately 20 years after it was identified that phosphodiesterase was an enzyme that degraded cyclic AMP (Sutherland and Rall, 1958; Butcher and Sutherland, 1962; Salmi et al., 2007). According to Igelias et al. (2006), adenosine, a nucleoside, is widely distributed in the peripheral and central nervous systems and acts through G-protein coupled receptors. Four types of receptors have been identified: A1, A2A, A2B, and A3. A1 and A3 receptors inhibit adenylyl cyclase activity through Gi protein. A2A and A2B receptors act by stimulating adenylyl cyclase activity through Gs protein. A1 and A2A receptors have a greater affinity with adenosine and are blocked by caffeine. Adenosine, working through the A1 receptors, inhibits glutamate release, thus acting as a neuromodulator and neuroprotector. Snyder (1984) also noted that phosphodiesterase was inhibited by the xanthines, including caffeine and theophylline, and that via this mechanism, xanthines could elevate cyclic AMP levels. However, to substantially inhibit phosphodiesterase, millimolar concentrations of caffeine were required, approximately 100 times the levels of caffeine found in the human brain after ingestion of typical dosages in humans. In addition, it was noted that some inhibitors of phosphodiesterase were 100 to 1,000 times more potent than caffeine but without behavioral effects.

Adenosine has many effects, including dilation of blood vessels, especially in the coronary and cerebral circulation, inhibition of platelet aggregation, and inhibition of hormone-induced lipolysis. It also has a variety of actions on central neurons, usually inhibiting spontaneous neuronal firing (Phillis and Wu, 1981; Stone, 1981). Adenosine inhibition of the release of excitatory neurotransmitters is the predominant presynaptic activity, although postsynaptic effects are also present. Many studies were conducted testing the hypothesis that in utero exposure altered adenosine receptors and their activities, including postnatal functional activity in the brain and heart. All the studies appear to have been performed at dosages that either were toxic, were reversible in effect, or not sufficiently well documented for use in human risk assessment.

The first biochemical analysis of adenosine receptor activity was by Sattin and Rall (1970) who demonstrated that adenosine can increase the accumulation of cyclic AMP in brain slices without conversion of adenosine to cyclic AMP, an action on extracellular receptors. The effects of adenosine on the enzyme adenylyl cyclase, which synthesizes cyclic AMP, revealed two distinct subtypes of adenosine receptors, designated A1 and A2 (van Calker et al., 1979; Burnstock and Brown, 1981; Londos et al., 1981). Depending upon the system, adenosine increases or decreases adenylyl cyclase activity, with the enhancing actions occurring at micromolar concentrations via A2 receptors. Nanomolar concentrations of adenosine cause the A1 receptors to inhibit adenylyl cyclase activity. Marked stereospecific effects of phenylisopropyladenosine (PIA) occurs at the A1 receptors. L-PIA is remarkably more potent than D-PIA, although the two isomers are relatively similar in effect at the A2 receptors. Most xanthines have similar potencies blocking both A1 and A2 receptors.

Direct binding studies have demonstrated that in all species studied, adenosine receptors labeled with [3H]DPX, a xanthine derivative, binding showed that nanomolar potency was present for adenosine derivatives and sterosepecificity for PIA isomers. However, binding studies identified heterogeneity of adenosine receptors beyond the A1 and A2 distinction. Another xanthine derivative (DPX) was about 250 times more potent in competing for [3H]CHA sites in calf than in guinea pig and human brain. As summarized by von Borstel and Wurtman (1984), considerable evidence has been accumulated that competitive antagonism at cell surface adenosine receptors may be the most important molecular action for methylxanthines, including caffeine. Administration to animals can produce sedation, bradycardia, hypotension, hypothermia, and attenuation of the response of the heart, vascular, and adipose tissue to sympathetic stimulation and are generally opposite to those produced by caffeine or theophylline alone. Methylxanthines competitively antagonize these and other adenosine actions at concentrations similar to those found in plasma after consumption of one to three cups of coffee (5–30 μM) (Rall, 1980).

A series of new manuscripts identified in this review describe studies designed to evaluate the effect of caffeine on adenosine receptor ontogeny. One group of investigators (Adén et al., 2000) identified that administration of caffeine at dosages resembling those consumed by humans does not significantly influence the development of receptors known or believed to be affected by caffeine. The results, described below, in contrast to other publications, indicate that caffeine can modify adenosine receptors and/or behavior. However, it is unclear what dosages were used or what postnatal blood levels of caffeine were attained. Adén et al. (2000) reported that maternal caffeine intake has minor effects on the adenosine receptor ontogeny in the rat brain. Caffeine was provided in the drinking water given to pregnant rats, beginning on p.c.d. 2 and continuing throughout gestation and postnatal life of the offspring. Although the authors noted that only a low dosage of caffeine was administered, estimated to be up to 3 cups of coffee/day, or what a woman might drink during pregnancy, it must be noted that mg/kg/day consumed dosages vary throughout gestation and lactation. This is further confounded by the pup’s consumption of the maternal drinking water, which contained caffeine. They reported that low-dosage caffeine-exposure during gestation and postnatal life had minor effects on the development of adenosine A1 and A2 receptors and GABA receptors in the rat brain.

Other studies were often designed to evaluate whether caffeine affected excitotoxic brain lesions in mice, because it is often given to human pre-term newborns. Bahi et al. (2001) examined the effects of caffeine on neonatal excitotoxic lesions of the periventricular white
matter. This study was designed to mimic caffeine exposure of human preterm infants in neonatal intensive care units. Most of this study is inappropriate for inclusion in this review because it addresses postnatal evaluations, rather than in utero exposure. It has been included because it had two sets of experiments, one performed postnatally and the other with in utero exposure, unfortunately by the intraperitoneal route (5 mg/kg caffeine citrate administered IP to 3 pregnant dams on p.c.d.s. 8–18 and another group injected IP with 12.5 mg/kg caffeine on p.c.d.s. 8–11). Although no mechanism was shown, it appeared that caffeine had a neuroprotective effect in mice.

An interaction study in knock-out mice was performed by Björklund et al. (2007) to investigate whether the response of the adenosine receptor system to a low perinatal exposure to methylmercury (MeHg) would be altered by caffeine treatment or eliminated by genetic modification (A1R and A2AR knock-out mice). Pregnant mice were administered 1 μM MeHg and/or 0.3 g/l caffeine (>30 mg/kg) in the drinking water. The consequences of MeHg toxicity during gestation and lactation were reduced by adenosine A1 and A2a receptor inactivation, either by genetic deletion or treatment with their antagonist, caffeine. This work also showed a protective effect of a high caffeine dosage of (>30 mg/kg/day).

In a 2008 study, da Silva et al. evaluated maternal caffeine intake to determine whether it affected acetylcholinesterase in the hippocampus of neonatal rats. The control group was given tap water and the caffeine group given 1.0 g/l caffeine diluted in tap water. Experiments were performed using 30 male and 30 female pups at 7, 14, and 21 days of age. Caffeine did not alter the age-dependent increase of acetylcholinesterase activity or the age-dependent decrease of acetylcholinesterase expression. However, it resulted in a 42% increase in acetylcholinesterase activity, without changing the level of acetylcholinesterase mRNA transcripts in 21-day-old rats. These results further demonstrate the ability of maternal caffeine intake to interfere with cholinergic neurotransmission during brain development.

A series of studies conducted by investigators in Spain considered the effects of down regulation of Adenosine A1 receptors and other receptors in the brain and heart that are affected by caffeine (León et al., 2002, 2005a; Iglesias et al., 2006). They reported caffeine intake as 83.2 mg/kg/day (administered at 1 g/l in the drinking water from p.c.d.s. 2 throughout pregnancy [sperm = gestation day 1]). The reported estimated dosage appears to be correct, because a 250 g rat would consume at least 20 ml/day of drinking water, although this value is somewhat low for a pregnant rat. These investigators considered this dosage equivalent to approximately 80 to 180 mg caffeine in a cup of coffee, or consumption of one cup of coffee by a pregnant woman. This calculation appears inappropriate because 180 mg consumed by a 60-kg human would be equivalent to only 3 mg/kg/day, much lower than the 83.2 mg/kg/day dosage consumed by the rats.

In the León et al. (2002) publication, it was reported that caffeine consumption during gestation caused down-regulation of adenosine A1 receptors in both the maternal and fetal brain. The later publications noted that it also inhibited A1 receptor function in the maternal rat brain and down regulation of metabotropic glutamate receptors in the brain from both mothers and fetuses (Leon et al., 2005a,b). The results of this study, evaluating isolated rat heart membranes, immunodetection of mGluR1, indicate down-regulation of different components of the mGluR 1/PLC pathway in the maternal and fetal heart, and loss of receptor responsiveness in fetuses that can alter the physiological function of the heart, especially in fetal tissue mGluRs.

Iglesias et al. (2006) demonstrated that chronic intake of caffeine during gestation in rats down regulates metabotropic glutamate receptors in maternal and fetal rat heart. While most of the studies involve the interaction of caffeine with adenosine receptors (Sutherland and Rall, 1958; Butcher and Sutherland, 1962; Snyder, 1984; Iglesia et al., 2006) caffeine also interacts with adrenergic, cholinergic, GABA, and serotonin receptors as well as calcium channels (Shi et al., 1993).

**Cardiovascular Effects**

Keller et al. (2007) provided an excellent review of cardiovascular development in which maternal exposure to hypoxic and bioactive chemicals, for example, caffeine, can rapidly impact embryonic/fetal cardiovascular function, growth, and outcome. No specific description of caffeine exposure in animals or humans was provided.

A study by Asadifar et al. (2005), while not relevant to toxicity produced as the result of in utero exposure of pregnant rats to caffeine, addresses the interaction of combined effects of caffeine and malnutrition on Cu content in the neonatal rat heart. The results of this study, in which neonates were administered a normal diet with 20% protein, 20% protein supplemented with caffeine (4 mg/100 g BW) or 6% protein diet (malnourished) or 6% protein supplemented with caffeine (4 mg/100 g BW) from birth to postnatal day 10 were surprising and not what was expected. The caffeine level was considered comparable to consumption of a heavy coffee drinker, defined as 4 cups of coffee containing an average of 100 mg of caffeine and an average body weight of 50 kg (400 mg/50 kg = 8 mg/kg). The results show that malnutrition did not impair mitochondria, and that although it was expected that caffeine exposure would aggravate their Cu status, the results were the opposite of the hypothesis. Caffeine exposure affected Cu status more in the normally nourished animals than in the malnourished animals, an apparent protective effect.

Moomo et al. (2008) further evaluated maternal and embryonic cardiovascular function in CD-1 mice administered 10 mg/kg/day caffeine subcutaneously on p.c.d.s. 9.5 to 18.5 of a 21-day pregnancy period (this information appears in error, because mice have an 18-day pregnancy). Blood levels were not reported, so it is not possible to extrapolate to human exposure, although the authors considered the exposure to be equivalent to modest daily maternal exposure. (It should be noted that the caffeine was administered by injection rather than by oral administration in the diet, so it is unlikely that this exposure was comparable to human caffeine exposures.) No maternal toxicity or increase in embryo resorption was observed. At p.c.d. 18.5, crown-rump length, forelimb length, and wet body weight of caffeine-treated embryos were smaller than the control.
embryos. The main findings of the study were reported as: (1) modest daily maternal caffeine exposure altered regional developing embryonic arterial blood flow and induced intraterine growth retardation without impacting maternal CV function or weight gain; (2) caffeine at peak maternal serum concentration transiently reduced embryonic carotid arterial flow to a greater extent than dorsal (and descending) aortic or umbilical arterial flow; (3) maternal adenosine A2A receptor blockade reproduced the embryonic hemodynamic effects of maternal caffeine exposure; and (4) adenosine A2A receptor gene expression in the uterus and developing embryo were downregulated by maternal caffeine exposure. The authors considered the 10 mg/kg dosage of caffeine to be a modest maternal caffeine dosage. They also stated that maternal caffeine effects in a mouse model may not reflect human effects, and concluded that modest daily maternal caffeine exposure may have a negative effect on embryonic CV function and overall embryonic growth, possibly mediated by adenosine A2A receptor blockade.

Another study in near-term fetal sheep (Tomimatsu et al., 2007) was performed to test the hypothesis that maternal caffeine administration does not significantly alter fetal cerebral oxygenation. The authors considered the dosage comparable to one that may be consumed by pregnant women in daily life. The pregnant ewes and their fetuses were instrumented at post conception day 125±3 (term ~145 days). A total of 800 mg of caffeine citrate (400 mg of caffeine, reported as approximately 8 mg/kg, that is, equivalent to 2–3 cups of coffee) into the maternal inferior vena cava over 30 min. Fetal arterial and sagittal sinus blood samples and maternal arterial samples were collected every 10 to 15 min and analyzed for blood gases, hemoglobin concentration, oxyhemoglobin saturation, and calculated O2 content. Maternal parameters were unaffected. Fetal arterial blood gas values at 5, 30, and 40 min after the 30-min maternal infusion of caffeine were also not significantly affected. Values at 5, 30, and 40 min after the 30-min maternal caffeine infusion, fetal LD-CBF decreased slightly (~4%). Fetal cortical PO2 decreased, and arterial content difference, cerebral fractional O2 extraction, and CMRO2 each increased 20 to 30% above baseline. Authors concluded that the results of their study showed findings that would suggest a small compromise in cerebral oxygenation occurred without affecting overall fetal systemic oxygenation. Further studies are needed to determine whether there are any related clinical findings.

**Encephalization**

There is some evidence that caffeine accelerates encephalization (development of the cerebral cortex). A publication by Sahir et al. (2000) describes a potential model for studying human holoprosencephaly. These investigators confirmed their previous in vitro work (Marret et al., 1997), describing effects of caffeine on early encephalization. In this work, they evaluated i.p. dosages of caffeine (12.5, 25, or 50 mg/kg) administered on p.c.d.s. 8, 9, and 10 and then scored the embryos for encephalization. Increased encephalization was noted on embryonic day 10 at all caffeine dosages, as compared with controls, and on embryonic day 9 at the 25 and 50 mg/kg/caffeine dosages. Normalization of brain anatomy and histology was noted within a few days after caffeine was discontinued, observations in agreement with the plasticity of the developing brain. The dosages tested were high and administered by an inappropriate route (12.5–50 mg/kg/day, i.p.) compared to human consumption (2.7–4 mg/kg/orally over a day). The results do not appear to represent a concern for humans, although the model may be useful for the evaluation of telencephalic vesicle formation. A later study by this group of investigators (Sahir et al., 2001) used similar methodology, i.p. injection once daily of mice on p.c.d.s. 8.5 to 10.5 with 25 mg/kg/day of caffeine or either of one of two inhibitors of cAMP dependent protein kinase (PKA). The dams were subsequently killed on p.c.d. 10.5, and the embryos were evaluated for histology and various tests for gene expression and sequencing. As cited previously, embryos treated with 25 mg/kg caffeine had significant acceleration of telencephalic vesicle formation, compared with control embryos. The authors concluded that the study showed involvement of PKA activity in caffeine-induced acceleration of encephalization, however, at relatively high exposures.

**Potential Model for the Production of Cataracts**

Two publications (Evereklioglu et al., 2003, 2004) reported results from the same set of rats. The Evereklioglu et al. (2003) study was designed to identify whether histopathology could reveal changes in the neonatal rat cornea resulting from caffeine exposure during pregnancy. The Evereklioglu et al. (2004) study focuses on the examination of the crystalline lenses in neonatal rats. Unfortunately, the study methodology was not well reported, and some tabular errors are evident, which preclude appropriate independent interpretation of the results.

Wistar pregnant rats and the i.p. route were used to treat a control and three dosage groups. As the result of the use of a route that is inappropriate for extrapolation to human exposure (i.p. dosages of 25, 50, and 100 mg/kg/day were administered between p.c.d.s. 9–21), exposure relevant to human exposure comparisons cannot be made. A fifth group was given caffeine via gavage at a toxic dosage of 50 mg/kg/day. Dams delivered normally (generally on p.c.d.s 20–21). Half of the newborn rats per litter were decapitated at postnatal day 1, and the eyes were examined. The remaining litters were raised with their biological mothers and sacrificed and decapitated at postnatal day 30 for eye evaluation. Pups were evaluated on postnatal days 1 or 30, and the eyes enucleated for corneal histopathology. Although the investigators refer to ‘‘pup’’ and ‘‘groups’’ and statistical analysis of these, it is somewhat unclear how this occurred because it appears that only one randomly selected eye (right eye) was evaluated. Thus, it appears that each litter and dosage group is represented by only one pup and one eye at each time interval.

No maternal toxicity was reported; however, 7 pups were reported as ‘‘miscarried’’ by 2 dams in 100 mg/kg/day caffeine Group 4 (high dosage), because rats do not generally abort but resorb their dead conceptuses. These
“late fetal deaths” were probably either a sequela of IP injection and/or apparent premature delivery associated with incorrect identification of the mating date. Pup body weights were slightly decreased in all groups in a dose-dependent pattern. It is unclear whether the number of litters evaluated included the aborted litter at birth, or whether these litters were included with those with pups evaluated on postnatal day 30. Table 1 in the Everekiloglu et al. (2003) publication appears to incorrectly report the number of pups per litter as the mean number of pups per litter at birth. The authors concluded dosages of 50 mg/kg/day and higher affected development of the cornea, particularly postnatal at 100 mg/kg/day. Interestingly, macroscopic changes were not observed in any corneas on postnatal day 30.

In the later publication regarding effects in the same rats (Everekiloglu et al., 2004), the ultimate objective was to establish a model for the study of cataract development, specifically, to investigate histologically the influence of maternal caffeine exposure during pregnancy on the development of the crystalline lenses in neonatal rats. In the control and 25 mg/kg/day dosage groups, both slit-lamp biomicroscopic and histopathologic examination of the crystalline lenses revealed normal findings. Histological examination of the 50 and 100 mg/kg/day IP groups and the 50 mg/kg/day PO group had findings suggesting cataractogenesis, including eosinophilic degeneration, lens fiber cell swelling and liquefaction, central lens fibers with retained nuclei, and prominent epithelial cells lining the posterior lens capsule behind the equator. Some lenses in the intraperitoneal 100 mg/kg/day group had immature cataract on slit-lamp biomicroscopic examination at postnatal day 30. The authors concluded that excessive maternal caffeine exposure during pregnancy had cataractogenic effects. As previously reported, no macroscopic ocular abnormalities were observed in control or experimental groups at birth, and the i.p. administration of high doses of caffeine prevents the ability to perform a valid risk assessment in humans.

EVALUATION, DISCUSSION, AND RISK ANALYSIS

The method of evaluation that has been utilized in past publications (Brent, 1978; Shepard, 1986, 1994; Brent 1986a,b, 1995a,b, 1997, 1999, 2003, 2005; Brent and Beckman, 1990; Christian and Brent, 2001) will be utilized in this publication and is described in Table 1. It consists of evaluating the (1) epidemiological studies, (2) determining whether secular trend analysis is an appropriate technique to utilize, (3) animal studies, (4) evaluating the available pharmacokinetic and toxicokinetic information, (5) Testing the biological plausibility of any reported findings or hypotheses based on (a) MOA (mechanism of action), (b) receptor agonistic and antagonistic effects, (c) enzymatic stimulation or suppression, and (d) basic reproductive and developmental teratology principles.

Epidemiological Studies

Epidemiological studies are the most important area of research for evaluating human risks from environmental exposures. It is most helpful if the epidemiology study results are in agreement (consistent). We know that cohort studies are the most likely to be accurate with regard to identifying causal associations; however, for rare events you need very large exposed populations to study. They are costly and difficult because the studies need large numbers of cases and controls. Case-control studies can be performed with smaller numbers of cases and controls and are more likely to find associations that are not causal. Consistent findings of increased risks or no increased risks strengthen the believability of the results. None of the new caffeine epidemiological studies of the 21st Century included complete pharmacokinetic data in their research protocol. The studies continued to measure exposure by cups per day, per week, or even per month of caffeine containing beverages. Some investigators hypothesized that slow metabolizers of caffeine may be at greater risk because the mother’s serum levels of caffeine and caffeine metabolites would be higher and protracted. The studies of CYPIA2 activity’s impact on the risk of SA were inconsistent and in some cases the results were the opposite of what was expected.

In this publication the epidemiological studies pertaining to SA, CMS, and fetal growth retardation were evaluated noting that appropriate animal studies can assist in the risk assessment analysis.

SA. Of all the reproductive and developmental events, SA is the most difficult to evaluate in epidemiological studies (Tables 2 and 3). The complexity of performing research involving the evaluation of whether a particular environmental agent is responsible for an increase in the prevalence of SA is discussed in detail in the SA section of this article. Furthermore, it will be obvious that many of the epidemiological studies failed to recognize the impact of the factors that alter the veracity of epidemiological studies dealing with SA, which are summarized in the earlier section. Seventeen caffeine epidemiological studies were reviewed that were concerned with SA. Almost all the studies reported no association in pregnant women consuming three or less cups of coffee per day. Eight of the studies were negative at all exposures that were studied. The epidemiology studies were not consistent in their conclusions. At high exposures it was difficult to eliminate confounding factors such as smoking, alcohol ingestion, decreased nutrition, the Susser effect, and many other confounding factors that may be associated with “excessive” caffeine ingestion. So the conclusion in this review was that caffeine ingestion at the usual or even very high exposures is an unlikely cause of SAs. Ten of the studies did not account for the impact of the “pregnancy signal”. Only one of the studies discussed the multiple etiologies of abortion and the complexity of performing SA research studies. Nor did these epidemiology studies cite the animal studies that examined embryonic and fetal resorptions that refuted the concept that caffeine is an abortifacient at the usual or even high exposures of caffeine in pregnant women.

Congenital malformations (CMs). Each of the 11 recent CM epidemiological studies evaluated only one particular isolated birth defect and none of them focused on a syndrome of abnormalities that is usually associated with a teratogenic effect. Most teratogens do not produce a single isolated defect. Teratogens produce an identifiable syndrome of effects that are caused by the teratogen.
growth retardation at exposures that are much higher (Pollard et al., 1987) did not result in rat or mouse fetal growth retardation, the studies many of the possible confounding factors that could be etiologically related to growth retardation (tobacco, alcohol, nutritional problems, maternal disease states, maternal behavioral, or psychiatric problems) were not evaluated (Smith, 1947). While large doses of caffeine administered parentally or by bolus to rats or mice can result in fetal growth retardation, the studies utilizing caffeine in drinking water or the food supply (Aeschbbacher et al., 1980; Nagasawa and Sakurai, 1986; Pollard et al., 1987) did not result in rat or mouse fetal growth retardation at exposures that are much higher than are likely to occur in the human. None of the epidemiological studies focused on the difference between reparable and nonreparable growth retardation. Many causes of growth retardation are permanent and the infant never recovers from the in utero growth retardation (chromosome abnormalities, in utero teratogenic and nonteratogenic infections, many teratogenic drugs and chemicals and some forms of nutritional deprivation). Reparable growth retardation following placental insufficiency or from pregnancies whose mothers smoked have much better prognosis than the fetuses that never recover completely from the in utero growth retardation. None of these studies determined whether the subjects in their studies recouped or recovered from their decreased growth in utero. Inconsistent findings of growth retardation and ignoring the importance of the pregnancy signal diminish the value of the conclusions of the epidemiology studies. Thus, fetal growth retardation is unlikely to be caused by the usual human exposures of caffeine.

Secular Trend Analysis

When a significant segment of the population is exposed to a drug or chemical, changes in population exposure may be associated with an increase or decrease in the incidence of reproductive or teratogenic effects. This can happen when a very popular drug is introduced or withdrawn from the market. Secular trend analysis cannot be utilized if only a very small segment of the population is exposed. Caffeine exposure is so universal and difficult to monitor that it would be impossible to attribute changes in reproductive or developmental effects to changes in population caffeine exposure.

Animal Developmental Toxicity Studies (Supplemental Table 1, and Tables 6–9)

When human epidemiological studies or a case series presumptively indicate that a cluster of malformations may be caused by a drug or chemical, an animal model may be developed that mimics the human developmental effect at clinically comparable exposures (Brent et al., 1986). There are over 50 proven human teratogens and for almost every teratogen scientists have been able to produce an animal model at exposures pharmacokinetically comparable to the human exposures (with the exception of infectious teratogens that primarily affect the human species) (Brent, 2004, 2008). Animal studies have demonstrated that the developmental NOEL in rodents is approximately 30 mg/kg/day, the teratogenic NOEL is 80 to 100 mg/kg/day, and the reproductive NOEL approximately 80 to 120 mg/kg/day (Knoche and Konig, 1964; Aeschbbacher et al., 1980; Nolen, 1981; Nagasawa and Sakurai, 1986; Pollard et al., 1987; Purves and Sullivan, 1993).

The animal studies that utilized pharmacokinetics estimated the teratogenic plasma NOEL at 60 μg/ml, a level that would rarely, if ever, be reached from caffeine nutritional exposures in pregnant women (Tables 8 and 9). The malformations described in the animal studies at very high doses fit the description of vascular disruptive types of malformations (Nishimura and Nakai, 1960). However, in the epidemiological studies reporting malformations in a caffeine-exposed population, the malformations that were selected for the study were not of the vascular disruptive type and no caffeine teratogenic syndrome has been described (Wilson and Brent, 1981; Brent, 1986, 1994, 1999, 2004, 2008; Christian and Brent, 2001). Fetal weight reduction. The 17 epidemiology studies dealing with the risk of fetal growth retardation from caffeine exposure during pregnancy did not consistently report that growth retardation was present in these studies. Four of the studies reported growth retardation with ingestions above 300 mg/day and eight of the studies did not. In eight of the studies the "pregnancy signal" was not included in the evaluation. The decrease in birth weight was very small and had minimal clinical significance. In some of the positive studies many of the possible confounding factors that could be etiologically related to growth retardation (tobacco, alcohol, nutritional problems, maternal disease states, maternal behavioral, or psychiatric problems) were not evaluated (Smith, 1947). While large doses of caffeine administered parentally or by bolus to rats or mice can result in fetal growth retardation, the studies utilizing caffeine in drinking water or the food supply (Aeschbbacher et al., 1980; Nagasawa and Sakurai, 1986; Pollard et al., 1987) did not result in rat or mouse fetal growth retardation at exposures that are much higher than are likely to occur in the human. When a significant segment of the population is exposed to a drug or chemical, changes in population exposure may be associated with an increase or decrease in the incidence of reproductive or teratogenic effects. This can happen when a very popular drug is introduced or withdrawn from the market. Secular trend analysis cannot be utilized if only a very small segment of the population is exposed. Caffeine exposure is so universal and difficult to monitor that it would be impossible to attribute changes in reproductive or developmental effects to changes in population caffeine exposure.
percentage of animal studies that utilized the administration of caffeine in the food or drinking water has yielded important information summarized in Table 10. It indicates that the NOEL for teratogenesis necessitates a plasma level of caffeine > 60 μg/ml. This is unattainable without pregnant women ingesting very large quantities of caffeine. For example, 10 cups of coffee over a period of 8–10 hr (1,000 mg of caffeine) would never be able to reach a plasma level of 60 μg/ml. This is true for growth retardation and pregnancy loss as well.

Pharmacokinetics

Some of the animal studies performed before 2000 have provided investigators with pharmacokinetic data that can be utilized for risk analysis (Knoche and Konig, 1964; Aeschbacher et al., 1980; Nolen, 1981; Nagasawa and Sakurai, 1986; Pollard et al., 1987; Perves and Sullivan, 1993) (Table 10). One of the recommendations of the Christian and Brent (2001) “Teratogen Update” was that any future caffeine epidemiological studies should measure caffeine and caffeine metabolites as an important component of the study. Extensive information regarding the metabolism of caffeine is presented in this publication. The information should be useful for future investigations in the caffeine field, so that any future caffeine toxicology studies will have a significant pharmacokinetic component. The inconsistencies of previous studies are partly the result of not knowing the actual exposures of the participants. In Tables 8 and 9 are the animal and human pharmacokinetic data that are available for human risk assessment. These tables are the most important tables in this publication because they demonstrate that it is unlikely that a pregnant woman could ingest enough caffeine via her diet to result in fetal growth retardation, pregnancy loss, or congenital malformations.

Biological Plausibility (Biological Common Sense)

Case reports and clusters. It is a common knowledge (a truism) that most teratogenes have been discovered by an alert physician or scientist from clusters of patients with a group of similar malformations (Brent et al., 1986; Carey et al., 2009). An historical example is Gregg’s observation of children in his ophthalmology practice with cataracts and associated malformations whose mothers had contracted Rubella during their pregnancy (Gregg, 1941). Case-control studies verified his observation as being correct. Another teratological truism is that a single case report of a drug exposure during pregnancy that resulted in a malformed child is rarely a causal relationship. Is a single case report ever useful? Bodineau et al. (2003) cited a case report of a newborn “intoxicated” by caffeine because the mother drank 24 cups of coffee/day during pregnancy.

The case report reads as follows (Khanna and Somani, 1984):

A male infant weighing 1,236 g was born to a 23-year-old Gravida 1, Para 0, white married woman at 27 weeks gestation. Amniotic fluid was leaking for 24 hr before delivery. The mother received 10% alcohol i.v. to attempt to stop the labor without success. She also received 16 mg of Dexamethasone i.v. 24 hr before delivery. The infant was spontaneously delivered vaginally. Apgar scores were 9 and 10. The infant developed respiratory distress and was administered 40% oxygen before being referred to a high-risk neonatal center. The gestational age was estimated to be 31 weeks at the neonatal center. He was diagnosed with transient tachypnea of the newborn. Cultures and electrolytes and the metabolic panel were all negative. Apnea of >20 sec was first noted at 4 days of age. On the 5th day, because of the apnea and the history of caffeine ingestion, a blood specimen was obtained for caffeine followed by caffeine administration (10 mg/kg), followed by 5 mg/kg every 12 hr. By the sixth day the apnea was no longer present. The serum caffeine concentration before the administration of caffeine was 40.3 μg/ml. The half-life of caffeine in a premature baby is estimated to be approximately 100 hr. It was estimated that at birth the infant had an estimated serum caffeine level of 80 μg/ml. On the 12th day postpartum, the serum caffeine concentration in the infant was 47.7 μg/ml.

No congenital malformations were detected and the infant’s birth weight was normal for gestational age. No further problems were encountered and the baby was discharged at 43 days of age. A serum sample was obtained from the infant at the time of discharge and was 0.7 mg/ml. At the postdischarge follow-up at 6 months later, the child was growing and developing normally.

The history of maternal caffeine intake is interesting. During the pregnancy she was taking as much as 24 cups of coffee per day. About 5 days before delivery, she reduced her coffee at work to 5 to 6 cups of coffee per day. After delivery she was drinking 5 to 6 cups of coffee per day. A maternal serum caffeine level on the 10th postpartum day was 18.4 μg/ml. Unfortunately, we do not have a caffeine level when she was taking 24 cups of coffee/day.

How much information can you obtain from one clinical report? It is apparent that this case report is extremely valuable. When a subject ingests 3 to 5 cups of coffee/day, a 60-kg subject is exposed to 5 to 8 mg/kg, which results in a serum concentration of 8 to 10 μg/ml. These are not absolute figures. For example, Stavric (1988) states that when a human consumes a cup of coffee delivering a 1 to 2 mg/kg dosage of caffeine it results in a blood concentration of 1 to 2 μg/ml, while a 3 to 5 mg/kg intake leads to a 5 μg/ml concentration. The serum measurements in this case-report indicate that the infant may have received a massive caffeine exposure as a fetus. If the infant was exposed to a very high level of caffeine why was the infant not growth retarded or malformed? Most likely, because the caffeine levels did not reach 60 μg/ml and lower levels do not produce congenital malformations or growth retardation.

The importance of the “mechanism of action” (MOA). The evidence that demonstrates that an environmental toxicant can produce reproductive or developmental effects in humans can be determined from the results of five areas of investigation (Table 1). Dose-response relationships in the reviewed epidemiology studies are primarily determined by estimates of exposures and there is meager data pertaining to the pharmacokinetics of caffeine and its metabolites. Since 2000, only four epidemiology studies reviewed in this article considered actual exposures. Even more
surprising is the fact that none of the epidemiological studies discussed the mechanism by which caffeine can produce SAs, congenital malformations, stillbirths, prematurity, fetal growth retardation, or fertility problems. The mechanisms by which reproductive toxicants produce their effects are listed in Table 10. Only one of the listed mechanisms in Table 10 have the possibility of providing a mechanism for reproductive toxicity of caffeine and that is agonistic or antagonistic effect on the adenosine, adrenergic, cholinergic GABA, or serotonin receptors. The pharmacokinetic levels of caffeine from low and high exposures are not cytotoxic or mutagenic. Nor is there definite data indicating that it can affect development or reproduction by any of the other mechanisms listed in Table 10.

**The importance of the “pregnancy signal”**. The “pregnancy signal phenomenon” has been discussed in many obstetrical and epidemiology publications (Weigel and Weigel, 1989; Lawson et al., 2004). In the Lawson et al. study, the authors reported that the vast majority of nonsmoking coffee drinkers decreased or quit drinking coffee during the first trimester. In fact 65% reported a decrease in coffee consumption between the 4th and 6th week of gestation. The authors were of the opinion that a decrease in coffee consumption may be a signal for a healthy pregnancy and therefore can act as a confounder. In many of the epidemiological studies published between 2000 and 2010, including the SA studies, the pregnancy signal was not considered. This omission could invalidate the results and conclusions of these studies.

**Fecundity and fertility studies**. Preconception exposure of sperm or ova (eggs) to mutagenic drugs and chemicals have theoretical risks of producing chromosome abnormalities or point mutations in the developing germ cells. Since caffeine is not a potent mutagen or carcinogen, an increase in the mutagenic risks would appear to be very unlikely (Table 10). There is extensive evidence supporting the conclusion that even potent mutagens at low exposures have a very low risk of having a significant effect on the developing surviving fetuses at term or a mutagenic effect (chromosomal abnormalities and point mutations). At high exposures, mutagenic agents can reduce ova survival and produce severe chromosomal abnormalities that result in very early embryonic death. This scenario is the classic dominant lethal test. However, caffeine is unlikely to increase the risk of birth defects by this mechanism because even potent chemical mutagens and ionizing radiation exposure to animals and humans before conception do not cause a significant increase in the incidence of genetic disease or birth defects in the live offspring (Mulvihill et al., 1987; Ames and Gold, 1990; Neel and Lewis, 1990; Nygaard et al., 1991a,b; Autrup, 1993; Brent, 1994, 1999, 2007; Byrne, 1999; Neel, 1999; Boice et al., 2003; Winther et al., 2004).

**CONCLUDING REMARKS**

After reviewing the 2000 to 2010 scientific epidemiology literature concerning the reproductive and developmental toxicology risks of caffeine, we conclude that major advances in the risk estimates have not been made and the confounding phenomena continue to be present in the present caffeine studies. An increase in pharmacokinetic studies has not occurred. We still do not know whether the increased risk estimates for some developmental and reproductive effects at higher exposures are due to caffeine or are due to other confounding factors. It appears that we should evaluate and continue to improve the animal studies to determine whether we can answer the many unanswered questions.

It may not be possible because of cost and invasiveness for epidemiological investigators to initiate pharmacokinetic studies to determine the actual caffeine exposure in the pregnant women exposed to caffeine that are being studied for reproductive and developmental effects. Further studies utilizing “cups” of tea, coffee, and colas will add little more to the understanding of caffeine “toxicity” from the plethora of studies that have been published.

The pharmacokinetics of caffeine and its metabolites was reviewed if only to demonstrate the complexity of evaluating caffeine’s toxic effects without knowing the basic science of caffeine metabolism. Caffeine’s main effect is on the central nervous system as a stimulant that interacts with the adenosine receptor and can also interact with adrenergic, cholinergic, GABA, or serotonin receptors, the implications of which are unknown.

1. In vivo animal caffeine studies should mimic human exposures, which is oral administration.
2. Second, every epidemiology study that is initiated should include recognition of the “pregnancy signal” as an important factor in determining the extent of reproductive and developmental risks in the population being studied.
3. Third, rarely has an investigator explained the MOA of caffeine. How does caffeine produce growth retardation, birth defects, SA, or premature births? Caffeine is not mutagenic, oncogenic, or cytotoxic at the usual human exposures. Agonism or antagonism of the adenosine receptor is unlikely to be related to developmental or reproductive toxic effects. It is interesting that scores of investigators are interested in the “toxic” effects of caffeine but not the mechanism to explain the toxic effects.
4. Planning and analyzing epidemiological studies by utilizing the principles of teratology would markedly improve the caffeine epidemiology studies (Table 5).

Our conclusion is that the dietary exposures of caffeine are not teratogenic or are directly responsible for an increased risk of SA or fetal growth retardation. Studies that involve very high exposures to caffeine are difficult to evaluate because of the many confounding factors that contribute to the risks that are not adequately evaluated; however, the animal studies indicate that even the highest human exposures in the epidemiological studies are unlikely to have reproductive and developmental effects (Table 10).

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Thomas Shepard, MD, Pediatrician, endocrinologist, embryologist, and teratologist, University of Washington.

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Richard Miller, Toxicologist, teratologists, and embryologists, University of Rochester.

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Robert Brent, Pediatrician, embryologist, and teratologist, Alfred I. duPont Hospital for Children.

Kenneth Lyon Jones, Pediatrician, clinical teratologist, and epidemiologist, U of Ca, SD.

REFERENCES


EVALUATION OF THE REPRODUCTIVE AND DEVELOPMENTAL RISKS OF CAFFEINE


Health Effects of Energy Drinks on Children, Adolescents, and Young Adults
Sara M. Seifert, Judith L. Schaechter, Eugene R. Hershorn and Steven E. Lipshultz

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The online version of this article, along with updated information and services, is
located on the World Wide Web at:
http://pediatrics.aappublications.org/content/127/3/511.full.html
Health Effects of Energy Drinks on Children, Adolescents, and Young Adults

**OBJECTIVE:** To review the effects, adverse consequences, and extent of energy drink consumption among children, adolescents, and young adults.

**METHODS:** We searched PubMed and Google using “energy drink,” “sports drink,” “guarana,” “caffeine,” “taurine,” “ADHD,” “diabetes,” “children,” “adolescents,” “insulin,” “eating disorders,” and “poison control center” to identify articles related to energy drinks. Manufacturer Web sites were reviewed for product information.

**RESULTS:** According to self-report surveys, energy drinks are consumed by 30% to 50% of adolescents and young adults. Frequently containing high and unregulated amounts of caffeine, these drinks have been reported in association with serious adverse effects, especially in children, adolescents, and young adults with seizures, diabetes, cardiac abnormalities, or mood and behavioral disorders or those who take certain medications. Of the 5448 US caffeine overdoses reported in 2007, 46% occurred in those younger than 19 years. Several countries and states have debated or restricted energy drink sales and advertising.

**CONCLUSIONS:** Energy drinks have no therapeutic benefit, and many ingredients are understudied and not regulated. The known and unknown pharmacology of agents included in such drinks, combined with reports of toxicity, raises concern for potentially serious adverse effects in association with energy drink use. In the short-term, pediatricians need to be aware of the possible effects of energy drinks in vulnerable populations and screen for consumption to educate families. Long-term research should aim to understand the effects in at-risk populations. Toxicity surveillance should be improved, and regulations of energy drink sales and consumption should be based on appropriate research. *Pediatrics* 2011;127:511–528
“Energy drinks” are beverages that contain caffeine, taurine, vitamins, herbal supplements, and sugar or sweeteners and are marketed to improve energy, weight loss, stamina, athletic performance, and concentration.1-3 Energy drinks are available in >140 countries and are the fastest growing US beverage market; in 2011, sales are expected to top $9 billion.4-10 Half of the energy drink market consists of children (<12 years old), adolescents (12-18 years old), and young adults (19-25 years old).7-10 Although healthy people can tolerate caffeine in moderation, heavy caffeine consumption, such as drinking energy drinks, has been associated with serious consequences such as seizures, mania, stroke, and sudden death.6,9,11-14 Numerous reports exist in the popular media, and there are a handful of case reports in the literature that associate such adverse events with energy drink consumption; it is prudent to investigate the validity of such claims (Appendix). Children, especially those with cardiovascular, renal, or liver disease, seizures, diabetes, mood and behavioral disorders, or hyperthyroidism or those who take certain medications, may be at higher risk for adverse events from energy drink consumption.6,9,14-24 Although the US Food and Drug Administration (FDA) limits caffeine content in soft drinks, which are categorized as food, there is no such regulation of energy drinks, which are classified as dietary supplements.1-3

Despite the large, unregulated market for energy drinks and reports in the literature and popular media of serious adverse events associated with their consumption, research into their use and effects has been sparse.25 However, schools, states, and countries increasingly are exploring content and sales regulations of these drinks.1,8,13,26-35 Given the rapidly growing market and popularity among youth, we reviewed the literature to (1) determine what energy drinks are, (2) compile consumption data of energy drinks by children, adolescents, and young adults, (3) compile caffeine and energy drink overdose data, (4) examine the physiologic effects of the ingredients in energy drinks, (5) identify potential problems of energy drinks among children and adolescents, (6) assess the marketing of energy drinks, (7) report current regulation of energy drinks, and (8) propose educational, research, and regulatory recommendations.

METHODS

We searched PubMed by using “energy drink,” “sports drink,” “guarana,” “caffeine,” “taurine,” “ADHD” (attention-deficit/hyperactivity disorder), “diabetes,” “children,” “adolescents,” “insulin,” “eating disorders,” and “poison control center” singly or in combination. We limited searches to English-language and foreign-language articles with English-language abstracts and selected articles by relevance to energy drink use in children and adolescents. We similarly searched Google for print and trade media. We reviewed articles and Internet sources by the above search through June 2010 and updated sections as new information became available through January 2011.

RESULTS

Two-thirds of the 121 references we found on energy drinks were in the scientific literature, although reports by government agencies and interest groups also contained much useful information (Table 1). Most information came from the United States, but European, Canadian, Australian, New Zealand, and Chinese sources are also represented.

DISCUSSION

What Are Energy Drinks?

Energy drinks may contain caffeine, taurine, sugars and sweeteners, herbal supplements, and other ingredients (Table 2) and are distinct from sports drinks and vitamin waters (Table 3).6,8 In 2008, the National Federation of State High School Associations, while recommending water and sports drinks for rehydration, specifically did not recommend energy drinks and cited potential risks, the absence of benefit, and drug interactions (Table 4).26,37 Caffeine is the main active ingredient in energy drinks; many of them contain 70 to 80 mg per 8-oz serving (~3 times the concentration in cola drinks) (Table 5).8,51 Caffeine content can be nearly 5 times greater than that in 8 oz of cola drinks when packaged as “energy shots” (0.8-3 oz) or as 16-oz drinks.5,29,38 Energy drinks often contain additional amounts of caffeine through additives, including guarana, kola nut, yerba mate, and cocoa.5,7,14,25 Guarana (Paulinia cupana) is a plant that contains caffeine, theobromine (a chronotrope), and theophylline (an inotrope).7,8,14,39 Each gram of guarana can contain 40 to 80 mg of caffeine, and it has a potentially longer half-life because of interactions with other plant compounds.7,14 Manufacturers are not required to list the caffeine content from these ingredients.7,14 Thus, the actual caffeine dose in a single serving may exceed that listed.9,29

Consumption of Energy Drinks by Children, Adolescents, and Young Adults

In the United States, adolescent caffeine intake averages 60 to 70 mg/day and ranges up to 800 mg/day.24,40 Most caffeine intake among youth comes from...
TABLE 1  Primary Literature and Media Sources Selected for Review, According to Relevance

<table>
<thead>
<tr>
<th>Source Description</th>
<th>No. of Results</th>
<th>Main Topics</th>
<th>Source Country</th>
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<td>Primary literature</td>
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<td>US, Poland, United Kingdom, Germany, China</td>
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<td>Systematic reviews</td>
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<td>—</td>
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<td>Energy drinks are a growing problem; safety issues with energy drinks;</td>
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<td>stimulant adverse effects; caffeine dependence; caffeine and fluid-electrolyte balance; caffeine and exercise performance; caffeine and mental performance; caffeine and apnea of prematurity; caffeine and bronchopulmonary dysplasia; caffeine and coronary heart disease; herb adverse effects; adolescents with ADHD and misuse of medication; ADHD and substance use; cardiovascular effects of antidepressants in children and adolescents; caffeine consumption and eating disorders; caffeine and bone gain in children and adolescents; incidence of pediatric cardiomyopathy; frequency of myocardial injury in children; epidemiology of hypertrophic cardiomyopathy</td>
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<td>Randomized controlled trials</td>
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<tr>
<td>Experimental studies</td>
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<td>Cognitive and physiological effects of energy drinks; caffeine and exercise performance, thermoregulation, and fluid-electrolyte balance; energy drink effects on hemodynamic and electrocardiographic parameters in young adults; caffeine effects in children; gender differences and caffeinated beverages; taurine and cardiac parameters; energy drinks and platelet and endothelial function; eye-tracking in adolescents viewing advertisements</td>
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<td>Caffeine and sleep patterns in children; caffeine exposure in children,</td>
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<td>adolescents, and young adults</td>
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<td>Surveys</td>
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<td>Government agency reports</td>
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<td>FDA overview of dietary supplements; energy drinks/high-caffeine beverages;</td>
<td>Australia, Canada, European Germany, New Zealand, United States</td>
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<td>caffeinated beverages and obesity; dietary supplements and military personnel;</td>
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<td>German assessment of energy drinks; Canadian assessment of energy drinks; health risks of energy shots; Poison Control Center data</td>
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<td>racial differences in caffeine metabolism; energy drink dangers for youth;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>restriction of drugs in youth; preparticipation screening and congenital heart defects; regulation of energy drinks; Hong Kong’s ban of Red Bull; questioning dietary supplements</td>
<td></td>
</tr>
<tr>
<td>Web sites</td>
<td>12</td>
<td>Energy drink sales; French ban on Red Bull; Germany questions energy drink</td>
<td>Holland, United Kingdom, United States</td>
</tr>
<tr>
<td></td>
<td></td>
<td>safety; a school’s ban on energy drinks; marketing of energy drinks; high caffeine levels in energy drinks in Australia; Ireland’s review of the safety of energy drinks; Dutch schools’ ban on Red Bull; preparticipation sports physicals</td>
<td></td>
</tr>
<tr>
<td>Books</td>
<td>1</td>
<td>History of energy drinks and health consequences</td>
<td>United States</td>
</tr>
<tr>
<td>Total</td>
<td>121</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
soda; however, energy drinks are becoming increasingly popular.7,24,41,42 Several self-report studies have examined energy drink consumption by children, adolescents, and young adults.7,24,41,42 One study found that 28% of 12- to 14-year-olds, 31% of 12- to 17-year-olds, and 34% of 18- to 24-year-olds reported regularly consuming energy drinks.5,45 Shortly after energy drinks were approved in Germany, a study of 1265 adolescents found that 94% were aware of energy drinks, 53% had tried them, 23% drank <1 can per week, and 3% drank 1 to 7 cans per week.44 Among 10- to 13-year-olds, 31% of girls and 50% of boys had tried energy drinks, and 5% of girls and 23% of boys reported drinking them regularly but at a rate of <1 can per week.44

### TABLE 2 Common Ingredients, Therapeutic Uses, and Adverse Effects of Energy Drink Ingredients8,14,25,30,49,53,82,83

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Description</th>
<th>Therapeutic Uses</th>
<th>Purported Effect From Energy Drinks</th>
<th>Adverse Effects (due to Idiosyncratic Reaction or Excessive Dosage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeine</td>
<td>An adenosine receptor antagonist: a central nervous system stimulant</td>
<td>As caffeine citrate, used to treat apnea and bronchopulmonary dysplasia in premature infants</td>
<td>Increases exercise endurance and improves cognition and mood when fatigued or sleep-deprived</td>
<td>Nervousness, irritability, anxiety, insomnia, tachycardia, palpitations, upset stomach, vomiting, abdominal pain, rigidity, hypokalemia, altered consciousness, paralysis, hallucinations, increased intracranial pressure, cerebral edema, seizures, rhabdomyolysis, supraventricular and ventricular tachyarrhythmias</td>
</tr>
<tr>
<td>Guarana</td>
<td>A South American plant that contains large amounts of caffeine, theobromine, and theophylline (a chronotrope and an inotrope) and tannins</td>
<td>None known</td>
<td>Stimulant, mainly through the effects of caffeine, and weight loss</td>
<td>Generally considered safe by the FDA Center for Food Safety and Applied Nutrition</td>
</tr>
<tr>
<td>Taurine</td>
<td>An abundant amino acid in the central nervous system; acts in neural growth and protection, cell metabolism, osmoregulation, antioxidation, and glycolysis; estimated daily intake is 400 mg/d</td>
<td>Infant formula has been supplemented with taurine since the 1980s because of evidence that it promotes healthy development; used to treat alcohol withdrawal, congestive heart failure, cystic fibrosis, palpitations/dysrhythmias, hypertension, diabetes, seizure disorders, hepatitis</td>
<td>Marketed to promote eye and biliary health and to prevent congestive heart failure by lowering blood pressure while improving cardiac contractility</td>
<td>Generally considered safe by the FDA</td>
</tr>
<tr>
<td>L-Carnitine</td>
<td>An amino acid involved in β-oxidation of fatty acids</td>
<td>Used as a therapeutic supplement in congenital and acquired deficiency states, end-stage renal disease, valproate toxicity, and dementia; increases attention and decreases hyperactivity in certain populations of children; nonstimulant l-acetyl-carnitine is used to treat ADHD in boys with fragile X syndrome and, in 1 study, children with typical ADHD; it may also protect against heart disease</td>
<td>Added to promote fat metabolism and increase endurance</td>
<td>In high doses, can cause nausea, vomiting, abdominal pain, and diarrhea; has been reported to cause seizures in patients with no known disease and to increase seizure frequency in patients with seizure disorder</td>
</tr>
<tr>
<td>Ginseng</td>
<td>An East Asian herb</td>
<td>Believed to improve memory, increase stamina, and stimulate immune function</td>
<td>Improve physical performance</td>
<td>Reported symptoms of ginseng toxicity include diarrhea, vaginal bleeding, headache, vertigo, mania, hypertension, rashes, insomnia, irritability, Stevens-Johnson syndrome, and agranulocytosis; some of these symptoms may be related to contaminants, such as phenylbutazone and aminopyrine, used in its processing</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>An alkaloid found in the plants <em>Paussinialalia yohimbe</em> and <em>Rauwolfa serpentina</em></td>
<td>An herbal supplement believed to be an aphrodisiac and to relieve chest pain, diabetic complications, depression, and erectile dysfunction</td>
<td>Increase energy, metabolism, and stamina; promotes well-being</td>
<td>Can cause hypertension at usual doses and hypotension at high doses; tachycardia, death</td>
</tr>
</tbody>
</table>
in the study consumed energy drinks in moderation, but a small group consumed extreme amounts. A survey of 496 college students found that 51% of those surveyed regularly consumed >1 energy drink per month; the majority of them habitually drank energy drinks several times per week. Insufficient sleep (67%) and the

| TABLE 3 | Characteristics of Sports Drinks, Vitamin Drinks, and Energy Drinks<sup>1,4,8,14,27,31,57,117</sup> |
| --- | --- | --- | --- |
| Common brand names | Gatorade; Powerade | Glacéau Vitamin Water; SoBe Lifewater; Dasani Plus | Red Bull, Full Throttle, Monster Energy, Rockstar |
| Advertised purpose | To rehydrate the body after intense exercise for ≥1 h | Broad claims that range from improving endurance to strengthening immune defense to relaxation | To rapidly increase energy, endurance, and performance |
| Ingredients | Glucose, electrolytes | Water, vitamins, nutrients, sometimes sweeteners; no stimulants | Caffeine, sugar, herbal supplements, other substances (eg, taurine, glucuronolactone) |
| Effects | Replaces lost electrolytes and carbohydrates during sustained strenuous exercise; prevents dehydration | Prevents dehydration, may replace some nutrients; other than the caloric sweeteners, the contents are unlikely to present health risks, and there is no published evidence of benefit | Temporarily increases heart and respiratory rates and blood pressure; not designed to hydrate the body |

| TABLE 4 | Potential Pharmaceutical Supplement Interactions With Energy Drink Ingredients<sup>30,32,33</sup> |
| --- | --- | --- |
| Energy Drink Ingredient | Interactions |
| 5-Hydroxy tryptophan | Should not be combined with monoamine oxidase inhibitors |
| Vinpocetine | Increases the risk for excessive bleeding and should not be combined with aspirin, clopidogrel, warfarin, pentoxyflline, vitamin E, garlic, and gingko |
| Yohimbine | Affects cardiac function and should not be combined with tricyclic antidepressants, bupropion, phenothiazines, clonidine, stimulants, decongestants, or other blood pressure-lowering medications |
| Ginseng | Prolongs bleeding time and should not be combined with warfarin; interacts with phenelzine sulfate in patients being treated for mania; may interfere with estrogens or corticosteroids; may impede digoxin metabolism; reduces blood glucose levels |

| TABLE 5 | Caffeine Content Reported by Manufacturers and Selected Additional Ingredients of Selected Products<sup>6,29,46</sup> |
| --- | --- | --- | --- | --- |
| Product | Selected Ingredients | Sugar Content per 8 oz, g | Average Caffeine Dose per 8 oz, mg | Amount per Serving, oz | Average Caffeine Dose per Container, mg |
| Comparison products |  |  |  |  |  |
| NoDoz | Caffeine | 0 | NA<sup>a</sup> | NA<sup>a</sup> | 200 mg per tablet |
| Excedrin | Acetaminophen, aspirin, caffeine | 0 | NA<sup>a</sup> | NA<sup>a</sup> | 65 mg per tablet (serving size: 2 tablets) |
| Hershey’s Kisses | Caffeine, sugar | 21 in 9 pieces | NA<sup>a</sup> | NA<sup>a</sup> | 9 mg in 9 pieces |
| Coca-Cola | Caffeine, sugar | 19–25 | 23 | 12 | 35 |
| Mountain Dew | Caffeine, sugar | 32 | 28 | 16 | 55 |
| Diet Pepsi Max | Ginseng | 0 | 47 | 12 | 69 |
| Tea, brewed | NA<sup>a</sup> | 0 | 54 | 8 | 54 |
| Coffee, generic brewed | NA<sup>a</sup> | 0 | 100 | 8 | 100 |
| Starbucks tall coffee | NA<sup>a</sup> | 0 | 174 | 12 | 260 |
| Energy drinks |  |  |  |  |  |
| Low calorie | B vitamins, dextrose, fumarate, gingko, ginseng, glucuronolactone, guarana, glucose, inositol, L-arginine, L-carnitine, milk thistle, n-acetyl-L-tyrosine, sucrose, taurine, yohimbine HCL, vinpocetine, 5-hydroxy tryptophane | 0 | 77–19 200 | 0.17–16.00 | 80–400 |
| Regular | B vitamins, dextrose, fumarate, gingko, ginseng, glucuronolactone, guarana, high-fructose corn syrup, glucose, inositol, L-arginine, L-carnitine, milk thistle, n-acetyl-L-tyrosine, sucrose, taurine, yohimbine HCL, vinpocetine, 5-hydroxy tryptophane | 17–30 | 71–286 | 8.3–16.0 | 75–300 |
| Super-caffeinated | B vitamins, dextrose, fumarate, gingko, ginseng, glucuronolactone, guarana, glucose, inositol, L-arginine, L-carnitine, milk thistle, n-acetyl-L-tyrosine, sucrose, taurine, yohimbine HCL, vinpocetine, 5-hydroxy tryptophane | 0 | 315–19 200 | 0.17–2.50 | 80–400 |

NA indicates not applicable.

<sup>a</sup>Product-specific information is from company Web sites; ingredients may vary according to country.
desire to increase energy (65%) were the most common reasons for use. In this study, 54% of the respondents reported mixing energy drinks with alcohol, and 49% drank ≥3 of them while partying. Another study of 795 college students found that 39% of the respondents had consumed an energy drink in the previous month and that, on average, men drank energy drinks 2.5 days/month, whereas women drank 1.2 days/month.

The estimated caffeine exposure of consuming energy drinks or energy shots was calculated for New Zealand children (5–12 years old), teenagers (13–19 years old), and young men (19–24 years old) (Figs 1–3). After consuming a single retail unit, 70% of the children and 40% of the teenagers who consumed caffeine were estimated to have exceeded the adverse-effect level of 3 mg/kg body weight per day beyond their baseline dietary exposure. An average child, teenager, or young man would all, on average, exceed the adverse-effect level after consuming a single retail unit of energy drink/energy shot above their baseline dietary caffeine exposure.

Caffeine and Energy Drink Overdoses

US poison control centers have not specifically tracked the prevalence of overdoses attributed to energy drinks, because exposures were coded as “caffeine” or “multisubstance exposures” and combined with other caffeine sources (Table 6) (American Association of Poison Control Centers Board of Directors, personal communication, 2010). Energy drinks were recently given unique reporting codes, so their toxicity can now be tracked (American Association of Poison Control Centers Board of Directors, personal communication, 2010). Germany has tracked energy drink–related incidents since 2002. Re-
reported outcomes include liver damage, kidney failure, respiratory disorders, agitation, seizures, psychotic conditions, rhabdomyolysis, tachycardia, cardiac dysrhythmias, hypertension, heart failure, and death. Ireland’s poison center reported 17 energy drink adverse events including confusion, tachycardia, and seizures and 2 deaths between 1999 and 2005. New Zealand’s poison center reported 20 energy drink/shot–related adverse events from 2005 to 2009; 12 cases were referred for treatment of vomiting, nausea, abdominal pain, jitteriness, racing heart, and agitation. The minimum and maximum symptomatic caffeine levels were 200 mg (4 mg/kg) in a 13-year-old with jitteriness and 1622 mg (35.5 mg/kg) in a 14-year-old. The maximum volume consumed was fifteen 250-mL cans (11.5 mg/kg caffeine) during 1 hour. One 23-year-old chronic energy drink consumer had a myocardial infarction.

**Physiologic Effects of the Ingredients in Energy Drinks**

**Caffeine Pharmacology and Physiology**

Caffeine, the most commonly used psychoactive drug worldwide, may be the only psychoactive drug legally available over-the-counter to children and sold among food and beverage products. Caffeine is an adenosine and benzodiazepine receptor antagonist, phosphodiesterase inhibitor, and central nervous system stimulant. In healthy adults, a caffeine intake of ≤400 mg/day is considered safe; acute clinical toxicity begins at 1 g, and 5 to 10 g can be lethal.

Physiologically, caffeine causes coronary and cerebral vasoconstriction, relaxes smooth muscle, stimulates skeletal muscle, has cardiac chronotropic and inotropic effects, reduces insulin sensitivity, and modulates gene expression in premature neo-
Large amounts of caffeine increase urine flow and sweat excretion and alter blood electrolyte levels.\textsuperscript{11,53} Although caffeine is a mild diuretic, consumption of \( \leq 500 \) mg/day does not cause dehydration or chronic water imbalance.\textsuperscript{54,55}

Caffeine is a ventilatory stimulant with anti-inflammatory and bronchoprotective effects.\textsuperscript{56} Caffeine has been linked to dyspnea on exertion from central and peripheral chemoreceptor stimulation.\textsuperscript{56} In addition, increased breathing work may divert blood flow away from locomotor muscles and negate any ergogenic advantage.\textsuperscript{56} Caffeine’s cardiovascular effects include decreased heart rate from stimulation of medullary vagal nuclei and increased blood pressure.\textsuperscript{24,57–61}

Adults who consume low-to-moderate amounts of caffeine (1–3 mg/kg or 12.5–100 mg/day) have improved exercise endurance, cognition, reaction time, and mood with sleep deprivation.\textsuperscript{9,24,56,62} However, these studies typically involve habitual caffeine consumers, and results reflect withdrawal-symptom reversal.\textsuperscript{58}

Consuming 4 to 12 mg/kg of caffeine has been associated with undesirable symptoms, including anxiety and jitteriness.\textsuperscript{63} Headache and fatigue, common withdrawal symptoms, can occur after short-term, high-dose use.\textsuperscript{64} Caffeine intoxication is a clinical syndrome of nervousness, irritability, anxiety, insomnia, tremor, tachycardia, palpitations, and upset stomach.\textsuperscript{6,7,9,14,26,65} Additional adverse effects include vomiting and abdominal pain, hypokalemia, hallucinations, increased intracranial pressure, cerebral edema, stroke, paralysis, rhabdomyolysis, altered consciousness, rigidity, seizures, arrhythmias, and death.\textsuperscript{1,2,8,29,48}

Caffeine intakes of \( > 300 \) mg/day have been associated with miscarriage and low birth weight.\textsuperscript{38,66,67} Long-term caffeine consumption relates to a lower
of caffeine in children increases.24 Whether the effects of caffeine consumed 50 mg of caffeine, or 1.1 mg/kg for a 70-kg male or 2.2 mg/kg for a 35-kg preteen.40 Whether the effects of caffeine in adults can be generalized to children remains unclear.63 In a study of 26 boys and 26 men, the same dose of caffeine affected blood pressure similarly, but heart rate was significantly lowered in boys, whereas there was no effect on heart rate in men.58 Boys also exhibited more increased motor activity and speech rates and decreased reaction time than did men.69 Caffeine can improve attention, but it also increases blood pressure and sleep disturbances in children.24,63,70,71 After cessation in children who habitually consume caffeine, attention decreases and reaction time increases transiently.24,38 Similarly, reaction time has been shown to decrease as the dose of caffeine in children increases.24 In a study of 9- to 11-year-olds with habitual (mean intake: 109 mg/day) and low (mean intake: 12 mg/day) caffeine consumption given 50 mg of caffeine after overnight abstinence, habitual caffeine users reported withdrawal-symptom (headache and dulled cognition) reversal. The children who did not habitually consume caffeine reported no marked changes in cognitive performance, alertness, or headache.63 Caffeine may affect future food and beverage preferences by acting on the developing child’s brain reward-and-addiction center; this effect may be gender specific.5 A study of 12- to 17-year-olds revealed that boys found caffeinated soda more reinforcing than did girls regardless of usual caffeine consumption.72

### Physiologic Effects of Other Ingredients in Energy Drinks and Potential Synergistic Effects

Popular media and case reports have associated adverse events with energy drink consumption (Appendix). Yet, few studies have examined the physiologic effects of individual ingredients or potential synergistic effects; furthermore, results of experimental studies have been inconclusive and occasionally contradictory.24,25,59,73 Some studies of adults revealed improved mental alertness, reaction times, and concentration with energy drinks; others revealed no improvement compared with caffeine or glucose alone.73 One study of 14 young adults compared a complete energy drink mixture to the glucose fraction, the caffeine fraction, and the herbal fraction.59,85 Although individual components did not enhance cognition, the combined ingredients did.59 Caffeine and taurine combined may synergistically decrease heart rate initially; one study found that 70 minutes after consumption, heart rate returned to normal and blood pressure increased.25,75 Taurine similarly produced a reflex bradycardia when injected into the rat cerebroventricular system.76 Another study of 15 healthy young adults in a 7-day trial in which they consumed 500 mL of an energy drink each day with 160 mg of caffeine and 2000 mg of taurine, reported an average increase in systolic blood pressure of 9 to 10 mm Hg and an average increased heart rate of 5 to 7 beats per minute 4 hours after consumption.25,38 Caffeine- and taurine-containing beverages increased left atrial contractility in 13 athletes, thereby increasing left ventricular end-diastolic volume and stroke volume.19 The caffeine-only group showed no changes in left ventricular function.76 Taurine may cause this increase in stroke volume by suppressing sympathetic nervous stimulation and influencing calcium stores in cardiac muscle.8 Results of human and animal studies have suggested that long-term taurine exposure may cause hypoglycemia25 but a decreased risk of coronary heart disease.77 In animal experiments, taurine also has shown anticonvulsive and epileptogenic properties.25 Among 50 young adults who drank one sugar-free energy drink, hematomatologic and vascular effects included increased platelet aggregation and

### Table 6: American Association of Poison Control Centers’ Data on Caffeine Toxicity, 2006–2008

| Year | Total calls to PCCs to Report Caffeine Toxicity, n | Calls That Reported Caffeine Toxicity in Children <6 y old, n (%) of Total Calls | Calls That Reported Caffeine Toxicity in 6- to 19-Year-Olds, n (%) of Total Calls | Calls That Reported Caffeine Toxicity in Adults (>19 y old), n (%) of Total Calls | Patients Subsequently Treated, n (%) of Total Calls | Patients With Moderately Severe Symptoms, n (%) of Total Calls | Patients With Life-Threatening Effects, n (%) of Total Calls | Deaths, n (%) of Total Calls |
|------|--------------------------------------------------|--------------------------------------------------------------------------------|--------------------------------------------------------------------------------|--------------------------------------------------------------------------------|-----------------------------------------------------------------|------------------------------------------------|------------------------------------------------|------------------------------------------------|------------------------|
| 2008 | 4852                                             | 1208 (24.9)                                                                     | 1170 (24.1)                                                                     | 1090 (22.5)                                                                     | 1281 (26.4)                                                     | 470 (9.7)                                                     | 11 (0.2)                                                      | 1 (0.02)                                                            |
| 2007 | 5448                                             | 1176 (21.6)                                                                     | 1328 (24.4)                                                                     | 1404 (25.8)                                                                     | 1561 (28.7)                                                     | 544 (10.0)                                                    | 16 (0.3)                                                      | 1 (0.02)                                                            |
| 2006 | 5686                                             | 1247 (21.9)                                                                     | 1427 (25.1)                                                                     | 1427 (25.1)                                                                     | 1799 (31.6)                                                     | 654 (11.5)                                                    | 18 (0.3)                                                      | 1 (0.02)                                                            |

PCC indicates poison control center.
Potential Problems of Energy Drinks Among Children and Adolescents

Cardiovascular Effects of Energy Drinks on Children and Adolescents

High doses of caffeine may exacerbate cardiac conditions for which stimulants are contraindicated. Of particular concern are ion channelopathies and hypertrophic cardiomyopathy, the most prevalent genetic cardiomyopathy in children and young adults, because of the risk of hypertension, syncope, arrhythmias, and sudden death.

Effects of Energy Drinks on Children and Adolescents With ADHD

ADHD occurs in 8% to 16% of US school-aged children and may be more prevalent in children with heart disease. Some 2.5 million US children take stimulants for ADHD, which may increase heart rate and blood pressure. Children with ADHD have higher rates of substance abuse, including the abuse of caffeine, which blocks the A2A adenosine receptors and thereby enhances the dopamine effect at the D2 dopamine receptor, similarly to the way guanfacine works for ADHD.

For the subpopulation with methylphenidate cardiotoxicity, energy drink use may increase cardiac events. As with the ADHD stimulants, the combined effects of energy drinks and antidepressants are unknown.

Energy Drink Use in Children and Adolescents With Eating Disorders

Children and adolescents with eating disorders, especially anorexia nervosa, may regularly consume high amounts of caffeine to counter caloric-restriction-associated fatigue, suppress appetite, and produce looser stools and some diuresis. Given that children and adolescents with eating disorders have a propensity for cardiac morbidity/mortality and electrolyte disorders, consumption of high-caffeine energy drinks may put them at further risk for cardiac dysrhythmias and intracardiac conduction abnormalities.

Effects on Caloric Intake and Diabetes

Because obesity is epidemic, caloric increases from energy drink consumption become important. Additional calories may increase blood pressure, blood glucose levels, BMI, calcium deficiency, dental problems, depression, and low self-esteem. Sugar and caffeine may also synergistically increase postprandial hyperglycemia, which is of concern for children with diabetes.

Effects on Bone Mineralization

Early adolescence is the time of maximal calcium deposition in bone, and caffeine interferes with intestinal calcium absorption. It remains controversial whether caffeine itself has the most marked effect on bone acquisition during adolescence or whether replacement of milk intake by caffeinated beverages is the leading contributor.

Marketing of Energy Drinks

Youth-targeted marketing strategies date to 1987 when Red Bull was introduced in Austria. When it took 5 years to get permission to export Red Bull to Germany, rumors about its legality and dangerous effects helped fuel its popularity, and it became known as “speed in a can,” “liquid cocaine,” and a “legal drug.”

Energy drink marketing strategies include sporting event and athlete sponsorships, alcohol-alternative promotion, and product placement in media (including Facebook and video games) oriented to children, adolescents, and young adults.

Newer alcoholic energy drinks, the cans of which resemble the nonalcoholic counterparts, target risk-taking youth.

Contrasting with voluntary fine-print warning label on some products, which state that they may not be safe for children, those who are sensitive to caffeine, or for pregnant or nursing women, voluntary fine-print warning label on some products, which state that they may not be safe for children, those who are sensitive to caffeine, or for pregnant or nursing women.

Regulation of Energy Drinks

The FDA imposes a limit of 71 mg of caffeine per 12 fl oz of soda. Energy drink manufacturers may circumvent this limit by claiming that their drinks are “natural dietary supplements.” Thus, safety determinations of energy drinks are made solely by the manufacturers, and there are no requirements for testing, warning labels, or restriction against sales or consumption by minors.

In contrast, over-the-counter dedicated caffeine stimulants (eg, No-Doz [Novartis Consumer Health, Parsippany, NJ]) must list the minimum age for purchase (12 years), adverse effects, cautionary notes, recommended dose, and the total daily recommended dose of caffeine. In November 2009, the FDA asked manufacturers of alcoholic energy drinks to prove their safety. The US Senate is considering a bill that would require supplement manufacturers to register annually with the FDA and allow FDA recalls of supplements suspected of being unsafe. Ingredients may also be restricted to those that have already been approved by the FDA.
<table>
<thead>
<tr>
<th>Country</th>
<th>Bans on Energy Drinks</th>
<th>Restrictions</th>
<th>Proposed or Attempted Regulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argentina</td>
<td>—</td>
<td>—</td>
<td>Senate has proposed banning energy drinks in nightclubs.</td>
</tr>
<tr>
<td>Australia</td>
<td>Recently banned 5 energy drinks on the basis of a caffeine content of &gt;320 mg/L.</td>
<td>—</td>
<td>Classifying energy drinks as pharmaceutical products, which are regulated, has been proposed.</td>
</tr>
<tr>
<td>Canada</td>
<td>—</td>
<td>Requires warning labels, recommends a maximum daily consumption amount, and advises against mixing energy drinks with alcohol until further research has been conducted.</td>
<td>—</td>
</tr>
<tr>
<td>Denmark</td>
<td>Prohibits energy drinks entirely.</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>European Food Safety Authority</td>
<td>—</td>
<td>Beverages that contain &gt;150 mg/L caffeine should be labeled “high caffeine content” and the exact amount present indicated on the label.</td>
<td>—</td>
</tr>
<tr>
<td>France</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Germany</td>
<td>11 of 16 German states banned Red Bull Cola because of trace amounts of cocaine.</td>
<td>—</td>
<td>Banned Red Bull but recently removed the ban after assessment by the European Food Safety Authority.</td>
</tr>
<tr>
<td>Ireland</td>
<td>—</td>
<td>—</td>
<td>Stricter regulations on warning labels have been requested by the government; the German Federal Institute for Risk Assessment recommends that energy shots be banned because of the high risk of overdose.</td>
</tr>
<tr>
<td>Netherlands</td>
<td>—</td>
<td>—</td>
<td>Ireland is reviewing energy drink safety; Ireland’s food-safety board has recommended that energy drinks be labeled as unsuitable for children &lt;16 y old and that a ban be placed on the promotion of Red Bull in sporting events and in combination with alcohol.</td>
</tr>
<tr>
<td>Norway</td>
<td>—</td>
<td>—</td>
<td>Reviewed energy drink safety and declared no risk.</td>
</tr>
<tr>
<td>Sweden</td>
<td>—</td>
<td>—</td>
<td>Drinks that contain &gt;150 mg/L of caffeine must be labeled “high caffeine content” and also must be labeled “not recommended for children, pregnant women, or people sensitive to caffeine”; energy drinks must also state the maximum amount to be used daily.</td>
</tr>
<tr>
<td>Finland</td>
<td>—</td>
<td>—</td>
<td>The UK’s Committee on Toxicity investigated Red Bull and determined that it was safe for the general public but that children &lt;16 y old or people sensitive to caffeine should avoid drinks with high caffeine content.</td>
</tr>
<tr>
<td>Turkey</td>
<td>Ban on all high-caffeine energy drinks.</td>
<td>—</td>
<td>In 2008, Kentucky, Maine, and Michigan introduced legislation that would ban the sale of highly caffeinated drinks to children &lt;16 y old, but the bills were defeated. California is considering a bill to require special labels on alcoholic beverages to avoid confusion with nonalcoholic beverages.</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Uruguay</td>
<td>Prohibits energy drinks entirely.</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>
| United States | —                     | The FDA has listed caffeine as generally recognized as safe in concentrations of <200 mg/kg; in November 2009, the FDA announced plans to examine the safety of caffeinated alcoholic beverages. | In 2008, Kentucky, Maine, and Michigan introduced legislation that would ban the sale of highly caffeinated drinks to children <16 y old, but the bills were defeated. California is considering a bill to require special labels on alcoholic beverages to avoid confusion with nonalcoholic beverages.
Regulatory controversies also extend internationally (Table 7). When France banned Red Bull, the manufacturers challenged the ban through the European Commission, which determined that the caffeine and taurine concentrations in energy drinks had not been proven to be health risks and ordered France to lift the ban; the European Food Safety Authority has encouraged international data-pooling to better assess risks in children, adolescents, and young adults. In 2008, authorities in Germany, Hong Kong, and Taiwan detected 0.13 μg per can of cocaine (average) in Red Bull Cola. Red Bull manufacturers insisted that active cocaine was removed from the coca leaf during processing and that the extract was used for flavoring. However, 11 of 16 German states banned the product.

CONCLUSIONS

On the basis of this review, we conclude that (1) energy drinks have no therapeutic benefit, and both the known and unknown pharmacology of various ingredients, combined with reports of toxicity, suggest that these drinks may put some children at risk for serious adverse health effects; (2) typically, energy drinks contain high levels of caffeine, taurine, and guarana, which have stimulant properties and cardiac and hematologic activity, but manufacturers claim that energy drinks are nutritional supplements, which shields them from the caffeine limits imposed on sodas and the safety testing and labeling required of pharmaceuticals; (3) other ingredients vary, are understudied, and are not regulated; (4) youth-aimed marketing and risk-taking adolescent developmental tendencies combine to increase overdose potential; (5) high consumption is suggested by self-report surveys but is underdocumented in children (deleterious associations with energy drink consumption have been reported globally in case reports and popular media); and (6) interactions between compounds, additive and dose-dependent effects, long-term consequences, and dangers associated with risky behavior in children remain to be determined.

EDUCATIONAL, RESEARCH, AND REGULATORY RECOMMENDATIONS

In the short-term, pediatric health care providers need to be aware of energy drink consumption by children, adolescents, and young adults and the potentially dangerous consequences of inappropriate use. Diet and substance-use histories should include screening for episodic/chronic energy drink consumption, both alone and with alcohol. Screening is especially important for athletes, children with high-risk behaviors, certain health conditions (eg, seizures, diabetes, hypertension, cardiac abnormalities), and children with behavioral changes, anxiety, poor nutrition, or sleep disturbances.

For most children, adolescents, and young adults, safe levels of consumption have not been established. Yet, heavy use may be harmful or interact with medications and cause untoward adverse effects. Health care providers should educate families and children at risk for the potential adverse effects of energy drinks.

Routine high school athletic physicals do not identify everyone at risk for sudden cardiac death. Children with cardiac conditions should be counseled regarding the risks of caffeine-containing products, including irregular heart rhythms, syncope, dysrhythmias, and sudden death. Community partners, including schools, athletic groups, and regulatory bodies, also need to promote risk awareness. The fourth edition of the “Preparticipation Physical Evaluation” monograph will feature a revamped health questionnaire focused on cardiac health problems that may be exacerbated by physical activity; thus, adding questions about stimulant use, including energy drink consumption, becomes important.

Long-term research objectives should aim to better define maximum safe doses, the effects of chronic use, and effects in at-risk populations (eg, those with preexisting medical conditions, those who consume energy drinks during and after exercise, or those who consume them in combination with alcohol), and better documentation and tracking of adverse health effects. Unless research establishes energy drink safety in children and adolescents, regulation, as with tobacco, alcohol, and prescription medications, is prudent. This approach is essential for reducing morbidity and mortality, encouraging research, and supporting families of children and young adults at risk for energy drink overdose, behavioral changes, and acute/chronic health consequences.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health grants HL072705, HL078522, HL053392, CA127642, CA068484, HD052104, AI50274, CA068484, HD052102, HL087708, HL079233, HL004537, HL087000, HL007188, HL094100, HL085127, and HD80002; Health Resources and Services Administration grant HCF0-C76HF15614; the Children’s Cardiomyopathy Foundation; and the Women’s Cancer Association.
REFERENCES


24. Temple JL. Caffeine use in children: what we know, what we have left to learn, and why we should worry. Neurosci Biobehav Rev. 2009;33(6):793–806


51. Dworzanski W, Opie1043–1048


77. Wójcik OP, Koenig KL, Zeleniuń-Jacquette A, Costa M, Chen Y. The potential protective


### Representative Sample of Adverse Events Reported in Association With Nonalcoholic Energy Drink Consumption

<table>
<thead>
<tr>
<th>Source of Information</th>
<th>No. and of Age Patients</th>
<th>Previous Health Conditions</th>
<th>Symptoms</th>
<th>Reported Association With Energy Drink</th>
<th>Ref No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Journal articles/ case reports</td>
<td>One 25-y-old woman</td>
<td>Mitral valve prolapse</td>
<td>Unknown</td>
<td>Drank a bottle of Race 2005 Energy Blast with guarana and ginseng on the day that she had a cardiac arrest; the autopsy and toxicological screen results were negative except for a caffeine concentration of 19 mg/L in aortic blood</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>One 25-y-old man</td>
<td>None reported</td>
<td>Generalized seizures on 2 occasions over 4 mo; the seizures did not recur within 6 mo after abstaining from energy drinks</td>
<td>Reportedly drank energy drinks on an empty stomach; he reported consuming two 24-oz energy drinks 30–60 min before the seizure</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>One 31-y-old man</td>
<td>None reported</td>
<td>Rhabdomyolysis and acute kidney failure with tubular necrosis</td>
<td>Active football referee drank 3 cans of Red Bull before a 3000-m competition; the authors stated that the taurine could have caused the rhabdomyolysis from hyperosmolarity because the distance was relatively short compared with his normal training</td>
<td>25 and 38</td>
</tr>
<tr>
<td></td>
<td>One 43-y-old man</td>
<td>Paranoid-type schizophrenia and alcohol dependence in full, sustained remission</td>
<td>6-wk history of worsening paranoia, delusions, and agitation resulting in hospitalization</td>
<td>Started drinking energy drinks 2 wk before becoming symptomatic; consumption increased to 8–10 cans per d; the cessation of caffeine reportedly improved symptoms</td>
<td>114</td>
</tr>
<tr>
<td></td>
<td>One 47-y-old man</td>
<td>None reported</td>
<td>Delusions and paranoia; the psychosis resolved within 7 wk after reducing caffeine consumption</td>
<td>High caffeine intake</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>2 patients</td>
<td>Migraine headaches</td>
<td>Seizures</td>
<td>In 1 case, the energy drink was consumed on an empty stomach; in the other case, caffeine tablets were also consumed with the energy drink</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>2 depressed patients and 1 patient with no psychiatric illness</td>
<td>Started on ginseng for several months</td>
<td>Unknown</td>
<td>Mania, which resolved after stopping taking ginseng</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>One young professional volleyball player</td>
<td>None reported</td>
<td>Developed orthostatic intolerance, postural tachycardia, and syncope, which resolved after energy drink consumption was stopped</td>
<td>4–5 cans of Red Bull per d</td>
<td>75</td>
</tr>
<tr>
<td>Newspaper articles</td>
<td>4 middle school students</td>
<td>None reported</td>
<td>All transported to the hospital with tachycardia, hypertension, paresthesias, diaphoresis, jitters/anxiety, hypokalemia and hyperglycemia were diagnosed in the emergency department</td>
<td>All 4 shared 1 can of Redline energy drink</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>7 high school students</td>
<td>None reported</td>
<td>Shortness of breath, heart palpitations, nausea; 2 students were treated in a hospital</td>
<td>SPIKE Shooter</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>One teenaged boy</td>
<td>None reported</td>
<td>Severe stomach pain for 2 mo; endoscopic findings included severe inflammation, bleeding, and ulcerations in the duodenum</td>
<td>Several Redline energy drinks (250 mg of caffeine per 8-oz serving) per d</td>
<td>32</td>
</tr>
<tr>
<td>Marin Institute</td>
<td>One 14-y-old girl</td>
<td>Diabetes</td>
<td>2 d after drinking 1 can of SPIKE Shooter; she was hospitalized for a seizure</td>
<td>SPIKE Shooter</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>One 18-y-old girl</td>
<td>None reported</td>
<td>Died after sharing 4 cans of Red Bull with friends and then playing basketball</td>
<td>Red Bull</td>
<td>43</td>
</tr>
<tr>
<td>Source of Information</td>
<td>No. and Age Patients</td>
<td>Previous Health Conditions</td>
<td>Symptoms</td>
<td>Reported Association With Energy Drink</td>
<td>Ref No.</td>
</tr>
<tr>
<td>----------------------</td>
<td>----------------------</td>
<td>-----------------------------</td>
<td>----------</td>
<td>----------------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Online news sources</td>
<td>One 14-y-old girl</td>
<td>Diabetes</td>
<td>Seizure</td>
<td>Reportedly drank 1 can of an energy drink before the seizure</td>
<td>25 and 38</td>
</tr>
<tr>
<td></td>
<td>One 17-y-old girl</td>
<td>None reported</td>
<td>Collapsed at the finish of a track race and was rushed to the emergency department after reporting chest pain and fatigue</td>
<td>Regularly skipped breakfast and drank 2 or 3 cans of Red Bull</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>One 29-y-old man</td>
<td>None reported</td>
<td>The man reportedly drank energy drinks and then engaged in the strenuous physical activity of motocross; he subsequently died from a cardiac arrest, presumably from coronary vasospasm caused by the energy drinks</td>
<td>Unknown energy drink; unspecified but large numbers of energy drinks that contained high levels of caffeine and taurine were consumed</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>One 47-y-old man</td>
<td>None reported</td>
<td>&quot;I thought I was having a heart attack. I thought I was going to die.&quot; The next day he reported feeling sore and exhausted from the experience</td>
<td>Two 8-oz cans of VPX Redline</td>
<td>119</td>
</tr>
</tbody>
</table>
An analysis of energy-drink toxicity in the National Poison Data System

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Division of Biostatistics, Department of Public Health Sciences, University of Miami Miller School of Medicine, Miami, FL, USA
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Context. Small studies have associated energy drinks—beverages that typically contain high concentrations of caffeine and other stimulants—with serious adverse health events. Objective. To assess the incidence and outcomes of toxic exposures to caffeine-containing energy drinks, including caffeinated alcoholic energy drinks, and to evaluate the effect of regulatory actions and educational initiatives on the rates of energy drink exposures. Methods. We analyzed all unique cases of energy drink exposures reported to the US National Poison Data System (NPDS) between October 1, 2010 and September 30, 2011. We analyzed only exposures to caffeine-containing energy drinks consumed as a single product ingestion and categorized them as caffeine-containing non-alcoholic, alcoholic, or “unknown” for those with unknown formulations. Non-alcoholic energy drinks were further classified as those containing caffeine from a single source and those containing multiple stimulant additives, such as guarana or yerba mate. The data were analyzed for the demographics and outcomes of exposures (unknown data were not included in the denominator for percentages). The rates of change of energy drink-related calls to poison centers were analyzed before and after major regulatory events. Results. Of 2.3 million calls to the NPDS, 4854 (0.2%) were energy drink-related. The 3192 (65.8%) cases involving energy drinks with unknown additives were excluded. Of 1480 non-alcoholic energy drink cases, 50.7% were children < 6 years old; 76.7% were unintentional; and 60.8% were males. The incidence of moderate to major adverse effects of energy drink-related toxicity was 15.2% and 39.3% for non-alcoholic and alcoholic energy drinks, respectively. Major adverse effects consisted of three cases of seizure, two of non-ventricular dysrhythmia, one ventricular dysrhythmia, and one tachypnea. Of the 182 caffeinated alcoholic energy drink cases, 68.2% were < 20 years old; 76.7% were referred to a health care facility. Educational and legislative initiatives to enhance understanding of the health consequences of energy drink consumption were significantly associated with a decreased rate of energy drink-related cases (p = 0.036). Conclusions. About half the cases of energy drink-related toxicity involved unintentional exposures by children < 6 years old. Educational campaigns and legal restrictions on the sale of energy drinks were associated with decreasing calls to poison centers for energy drink toxicity and are encouraged.

Keywords: energy drink; caffeine; poison control; overdose

Introduction

Energy drinks are beverages that typically contain high concentrations of caffeine, as well as vitamins, herbal supplements, and sweeteners. Most are marketed for their stimulant properties, likely linked to their caffeine content, with claims of increasing energy, weight loss, stamina, athletic performance, and concentration. Common additives, however, such as guarana, kola nut, yerba mate, and cocoa, also have stimulant, cardiac, and hematologic activity. These ingredients are often non-standardized in their caffeine content, sometimes considered as masked caffeine. Hence, the actual amount of caffeine content in energy drinks may be higher than that reported in their labels (Table 1).

The threshold of caffeine toxicity is 400 mg/day in healthy adults (≥ 19 years old), 100 mg/day in healthy adolescents (12–18 years old), and 2.5 mg/kg/day in healthy children (< 12 years old). One 8-ounce can of a popular energy
Energy drink toxicity

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Drink provides 77 mg of caffeine (or 1.1 mg/kg) for a 70-kg male and twice that, 2.2 mg/kg, for a 35-kg pre-teen. Caffeine intoxication is a clinical syndrome of nervousness, irritability, anxiety, insomnia, tremor, tachycardia, palpitations, and gastrointestinal upset. Additional adverse effects include vomiting and abdominal pain, hypokalemia, hallucinations, increased intracranial pressure, cerebral edema, stroke, paralysis, rhabdomyolysis, altered consciousness, rigidity, seizures, arrhythmias, and death. Some case reports, scientific studies, and surveys have associated many of these serious adverse effects with energy drink ingestion. The American Academy of Pediatrics holds that "Caffeine and other stimulants contained in energy drinks have no place in the diet of children and adolescents".

Prior to January 2011, pre-packaged alcoholic energy drinks were available for purchase. Due to concerns regarding their safety the Food and Drug Administration (FDA) removed these co-formulated beverages from the market. The use and effects of energy drinks are not well-understood, but several adverse effects have been linked with energy drink consumption. Emergency department visits for energy drink ingestion increased more than 10-fold between 2005 and 2009, from 1128 to 13,114, and continued to substantially increase since 2009 to 20,783 in 2011. In 2010, the US National Poison Data System (NPDS) added generic category codes (energy drinks with a single-source of caffeine, with caffeine from multiple sources, non-caffeinated, alcohol-containing, and unknown formulation) for caffeine toxicity so cases specifically related to energy drink calls can now be tracked. Here, we report the first year of data from these category codes and identify the demographic characteristics of individuals experiencing energy drink-related toxicities and the associated clinical effects and outcomes. We also evaluate the impact of regulatory actions and educational initiatives in the rate of energy drink exposures reported to NPDS.

**Methods**

The Human Subjects Research Office of the University of Miami Miller School of Medicine determined that this study does not constitute Human Subjects research. This was based on the fact that the data analyzed in this study do not contain any of the HIPAA identifiers. As such, it was not subject to Institutional Review Board review under 45 CFR 46.

We queried all closed, unique cases coded as energy drink exposures in the NPDS between October 1, 2010 and September 30, 2011. We analyzed only exposures to caffeine-containing energy drinks consumed as a single product ingestion. Energy drinks were categorized as caffeine-containing

<table>
<thead>
<tr>
<th>Top-selling energy drinks*</th>
<th>Ounces per bottle or can</th>
<th>Caffeine concentration, mg/oz</th>
<th>Total caffeine, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Bull</td>
<td>8.3</td>
<td>9.6</td>
<td>80</td>
</tr>
<tr>
<td>Monster</td>
<td>16.0</td>
<td>10.0</td>
<td>160</td>
</tr>
<tr>
<td>No Fear</td>
<td>16.0</td>
<td>10.9</td>
<td>174</td>
</tr>
<tr>
<td>Higher caffeine energy drinks†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wired X505</td>
<td>24.0</td>
<td>21.0</td>
<td>505</td>
</tr>
<tr>
<td>Wired X344</td>
<td>16.0</td>
<td>21.5</td>
<td>344</td>
</tr>
<tr>
<td>Jolt cola</td>
<td>23.5</td>
<td>11.9</td>
<td>280</td>
</tr>
<tr>
<td>Lower caffeine energy drinks†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bomba Energy</td>
<td>8.4</td>
<td>8.9</td>
<td>75</td>
</tr>
<tr>
<td>HiBall Energy</td>
<td>10.0</td>
<td>7.5</td>
<td>75</td>
</tr>
<tr>
<td>Vitamin Water (energy citrus)</td>
<td>20.0</td>
<td>2.5</td>
<td>50</td>
</tr>
<tr>
<td>High concentration energy drinks†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RedLine Power Rush</td>
<td>2.5</td>
<td>140.0</td>
<td>350</td>
</tr>
<tr>
<td>Powershot</td>
<td>1.0</td>
<td>100.0</td>
<td>100</td>
</tr>
<tr>
<td>Fuel Cell</td>
<td>2.0</td>
<td>90.0</td>
<td>180</td>
</tr>
<tr>
<td>Classic soft drinks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coca-Cola Classic</td>
<td>12.0</td>
<td>2.9</td>
<td>34.5</td>
</tr>
<tr>
<td>Dr Pepper</td>
<td>12.0</td>
<td>3.4</td>
<td>41</td>
</tr>
<tr>
<td>Mountain Dew</td>
<td>12.0</td>
<td>4.5</td>
<td>54</td>
</tr>
</tbody>
</table>

*Top selling energy drinks in the US in 2006, listed sequentially as a percentage of market share (packaged facts, 2007).
†Examples of energy drinks selected from the hundreds of energy drink products marketed in the US.

Adapted from Reissig et al. 2009

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nonalcoholic, alcoholic, or “unknown” for those with unknown formulations. Non-alcoholic energy drinks were further classified as those containing caffeine from a single source with no additional stimulant additives and those containing multiple stimulant additives, such as guarana or yerba mate. Because comparisons among substances in energy drinks could not be made when the formulation was unknown, energy drink exposures to substances with unknown ingredients were excluded from analysis. Energy drinks taken in conjunction with other substances (co-ingestants), such as other medications in overdose, illegal drugs or toxic chemicals, or those categorized as “unknown”, were also excluded from this analysis. The NPDS classifies the severity of adverse effects in five ordinal categories: no effect (no signs or symptoms resulting from the exposure); minor (“minimally bothersome signs and symptoms that resolved rapidly”); moderate (“more pronounced, prolonged, or systemic signs and symptoms requiring treatment but not life-threatening”); major (“life-threatening signs or symptoms or marked residual disability”); and death (as a direct result or complication of the poison exposure).

We also analyzed the demographics, clinical effects, therapies, outcomes of the included exposures, and the rates of change of NPDS reports of energy drink exposures in relation to the FDA warnings on alcoholic energy drinks reported on November 17, 2010, the release of the American Academy of Pediatrics' nutrition recommendations published on May 29, 2011, and increased media coverage surrounding February 2011 publications related to the health consequences of energy drinks.19,20,28–30

**Statistical analyses**

Data are reported as frequencies and percentages. Unknown data are reported but not included in the denominator for percentages. Chi-square tests were used for primary comparisons among age categories, sex, intentional or unintentional exposure, the presence or absence of alcohol in the drink involved, and the severity of adverse effects. The Spearman rank-sum test was used to analyze actual age differences. The effects of educational and federal legislative actions on reported exposure incidence trends in the NPDS over time were analyzed with a general linear model. The data conformed to the assumptions of the test used to analyze them. Alpha was set at 0.05, and all tests were two-tailed. The SAS 9.3 statistical software program was used in the analysis.

**Results**

Of the 2.4 million calls received during the study period, 4854 (0.2%) were for energy drink exposure cases. The 3192 (65.8%) cases involving energy drinks categorized as “unknown” or confounded with co-ingestants were excluded. Of the remaining energy drink-related cases, 1662 (34%)—1480 nonalcoholic and 182 alcoholic—were included in the analysis (Fig. 1). Overall, 46% of cases (734 of 1588 cases with reported age) were children less than 6 years old. The median (inner quartiles) ages for non-alcoholic and alcoholic energy drink cases were 5 (2–18) and 17 (15–21) years old.

**Fig. 1.** Sample selection and data availability of energy drink-related cases to the US National Poison Data System, October 1, 2010 through September 30, 2011.
Exposures to caffeinated non-alcoholic energy drinks

When non-alcoholic energy drink exposures are viewed as a whole, of 1480 cases, 717 (50.7% of 1,425 who reported age) were children less than 6 years old; 896 (60.8% of 1475 who reported gender) were males; 960 (76.7% of 1,251 who reported intentionality) were unintentional; and 120 (15.2% of 789 who had available referral data) were coded as “moderate” or “major” adverse effects (Table 2). This is a similar age distribution for other types of poisonings where in Table 2 we show that 49.6% (of 2,330,538 reporting) of all human exposure cases in the NPDS involve children less than 6 years old. Compared with the overall database, pre-adolescents and adolescents are more frequently represented in these exposures. Compared with all of the other age categories, children less than 6 years old had the highest proportion of unintentional exposures to non-alcoholic energy drinks, whereas teenagers had the highest proportions of intentional exposures in non-alcoholic energy drinks (Fig. 2a). When comparing only the teenagers with the ≥20 category, teenagers had a higher proportion of intentional exposures to non-alcoholic energy drinks (57.1% vs. 42.9%; Fig. 2b). Minor or moderate adverse effects were reported in 28% (103 of 372 reporting) of children less than 6 years old. No major effects were reported. Adolescents represented the largest proportion of cases of minor to moderate, with 1 major adverse effect (Fig. 3a). Other major adverse effects were reported among the 6–12 year old (n = 1) and the 20 years and older age group (n = 5). Overall, major effects reported consisted of three cases of seizures, two of non-ventricular dysrhythmias, one of ventricular dysrhythmia, and one of tachypnea.

Of the 1480 cases of non-alcoholic energy drinks, 946 were caffeine-only and 534 had additives. The two groups differed significantly in age, intentional or unintentional exposure, referrals to a health care facility, and severity of effects. Compared with energy drinks with additives, caffeine-only cases had a significantly greater proportion of cases less than 6 years old and unintentional exposures. A significantly greater proportion of cases involving additives was referred to a healthcare facility and experienced more minor, moderate, or major toxic effects (Table 2). Among those in the caffeine-only group who were referred to a healthcare facility, 34 cases resulted in hospital admissions (13 to a critical care unit, 18 to a non-critical care unit, and 3 to a psychiatric facility); the remaining 192 cases (84.9% of 233 reporting) were treated and released. There were no deaths.

Table 2. Characteristics of energy drink-related toxicity cases in the US National Poison Data System (NPDS). *

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All cases in the NPDS (n = 2,343,834)</th>
<th>Caffeine only (n = 946)</th>
<th>Caffeine + additives (n = 534)</th>
<th>p†</th>
<th>Non-alcoholic caffeinated (caffeine + additives) (n = 1,480)</th>
<th>p‡</th>
<th>Alcoholic caffeinated (n = 182)</th>
<th>p‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age categories, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–5 years</td>
<td>1,156,383 (49.6)</td>
<td>501 (55.6)</td>
<td>216 (42.0)</td>
<td>&lt;0.001</td>
<td>717 (50.7)</td>
<td>17 (9.8)</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>6–12 years</td>
<td>145,674 (6.3)</td>
<td>82 (9.1)</td>
<td>67 (13.0)</td>
<td></td>
<td>149 (10.5)</td>
<td>7 (4.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13–19 years</td>
<td>158,565 (6.8)</td>
<td>127 (14.1)</td>
<td>122 (23.8)</td>
<td></td>
<td>249 (17.6)</td>
<td>94 (54.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥20 years</td>
<td>869,916 (37.3)</td>
<td>191 (21.2)</td>
<td>109 (21.2)</td>
<td></td>
<td>300 (21.2)</td>
<td>55 (31.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>13,296</td>
<td>45</td>
<td>20</td>
<td></td>
<td>65</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1,136,598 (48.7)</td>
<td>568 (60.4)</td>
<td>328 (62.4)</td>
<td>0.688</td>
<td>896 (60.8)</td>
<td>91 (50.3)</td>
<td></td>
<td>0.007</td>
</tr>
<tr>
<td>Female</td>
<td>1,196,680 (51.3)</td>
<td>373 (39.6)</td>
<td>206 (38.6)</td>
<td></td>
<td>579 (39.2)</td>
<td>90 (49.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>10,556</td>
<td>5</td>
<td>0</td>
<td>0.022</td>
<td>5</td>
<td>1</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intentional exposure, n (%)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Unintentional</td>
<td>1,754,061 (83.3)</td>
<td>643 (78.8)</td>
<td>317 (72.9)</td>
<td>0.041</td>
<td>960 (76.7)</td>
<td>36 (21.6)</td>
<td></td>
<td>&lt;0.001</td>
</tr>
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<td>Intentional</td>
<td>350,883 (16.7)</td>
<td>173 (21.2)</td>
<td>118 (27.1)</td>
<td></td>
<td>291 (23.3)</td>
<td>131 (78.4)</td>
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<tr>
<td>Unknown</td>
<td>238,890</td>
<td>130</td>
<td>99</td>
<td></td>
<td>229</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Referrals, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>&lt;0.001</td>
</tr>
<tr>
<td>Non-healthcare facility</td>
<td>1,644,861 (71.5)</td>
<td>693 (75.4)</td>
<td>365 (70.5)</td>
<td>0.039</td>
<td>1058 (73.6)</td>
<td>41 (23.3)</td>
<td></td>
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</tr>
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<td>Healthcare facility</td>
<td>654,655 (28.5)</td>
<td>226 (24.6)</td>
<td>153 (29.5)</td>
<td></td>
<td>379 (26.4)</td>
<td>135 (76.7)</td>
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<tr>
<td>Unknown</td>
<td>44,318</td>
<td>27</td>
<td>16</td>
<td></td>
<td>43</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severity of toxicity, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No effect</td>
<td>450,605 (46.2)</td>
<td>232 (47.2)</td>
<td>127 (42.8)</td>
<td>0.039</td>
<td>359 (45.5)</td>
<td>13 (11.1)</td>
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<td></td>
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<tr>
<td>Minor effect</td>
<td>354,546 (36.4)</td>
<td>196 (39.8)</td>
<td>114 (38.4)</td>
<td></td>
<td>310 (39.3)</td>
<td>58 (49.6)</td>
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<td></td>
</tr>
<tr>
<td>Moderate effect</td>
<td>147,517 (15.1)</td>
<td>58 (11.8)</td>
<td>55 (18.5)</td>
<td></td>
<td>113 (14.3)</td>
<td>42 (35.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Major effect</td>
<td>22,049 (2.3)</td>
<td>6 (1.2)</td>
<td>1 (0.3)</td>
<td></td>
<td>7 (0.9)</td>
<td>4 (3.4)</td>
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<tr>
<td>Unknown</td>
<td>1,369,117</td>
<td>454</td>
<td>237</td>
<td></td>
<td>691</td>
<td>65</td>
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</tr>
</tbody>
</table>

* Numbers and percentages are based on the number of cases for which data are provided (see Fig. 1).
† p value for differences between cases of caffeine-only and caffeine + additives energy drinks.
‡ p value for differences between all non-alcoholic cases (caffeine-only plus caffeine + additives) and alcohol-containing energy drink cases.
§ “No effect” = no signs or symptoms resulting from the exposure; “Minor” = minimally bothersome signs and symptoms that resolved rapidly; “Moderate” = more pronounced, prolonged, or systemic signs and symptoms requiring treatment but not life-threatening; “Major” = life-threatening signs or symptoms or marked residual disability; “Death” = as a direct result or complication of the poison exposure.
Exposures to caffeinated alcoholic energy drinks

In the 182 cases (11% of 1662 reported single-substance energy drink cases) involving alcoholic energy drinks, males and females were equally involved, whereas most non-alcoholic cases involved males. Although more than half of these ingestions were among adolescents, 17 (9.8% of 173 reporting) were among children (Table 2). Overall, the proportion of intentional exposures was significantly higher in alcoholic than in non-alcoholic cases. However, 16 children less than 6 years old (44.1% of 36 reporting) had the highest proportion of unintentional exposures to alcoholic energy drinks, whereas 82 teenagers 13–19 years old (65.6% of 125 reporting) had the highest proportion of intentional exposures (Fig. 2c). Furthermore, the proportion of teenagers who intentionally consumed alcoholic energy drinks was twice that of those 20 years or older (68.3 vs. 31.7; Fig. 2b).

Overall, moderate or major effects were recorded in 46 cases (Table 2). Fifty (6 of 12 reporting) and 83% (5 of 6 reporting) of cases in children less than 6 years old and 6–12 year olds, respectively, reported only minor to moderately serious adverse effects, while 92% (60 of 65 reporting) of adolescents reported minor to moderately serious adverse effects, and 3 of 65 reporting (5%) reported major effects (Fig. 3b).

A greater proportion of alcoholic energy-drink cases, compared with non-alcoholic were advised to seek treatment in a health-care facility (Table 2). Of 23 reported admissions 9 were to a critical care unit, 12 to a non-critical care unit, and 2 to a psychiatric facility; 71 (75.5% of 94 reporting) were treated and released. The recommended care did not differ significantly from that for cases of non-alcoholic energy drinks.

Changes in the frequency of cases for energy drink-related toxicities

In the winter and spring of 2010–2011, consumer awareness efforts increased. Most notable were the FDA statement regarding the health risks of alcohol-containing energy drinks and their removal from the market; publications with extraordinarily large global media coverage in February 2011; the American Academy of Pediatrics’ statement that energy drinks have no nutritional value for children and adolescents; and extensive media coverage of these events, related research findings, and consumer concern about possible health risks. The number of cases involving energy drinks during that time, between
October 2010 and March 2011, increased at a significant rate (slope = $+17.7 \pm 4.33$; $p = 0.004$), and was then followed by a significant downward trend (slope = $-6.54$; $p = 0.036$; Fig. 4). Further, the trends were significantly different before and after regulatory and media effects ($R^2 = 0.83$; $p = 0.002$; Fig. 4). The trends in cases involving alcohol-containing energy drinks did not reach statistical significance, however, the intercepts before and after the FDA statement were each significant and were significantly different from each other.

**Discussion**

The true incidence of energy drink consumption is unknown but probably under-reported. Recent surveys in the US and elsewhere have produced findings similar to ours. A 2009 cross-sectional study of a convenience sample of 2158 respondents at least 18 years old from two San Diego emergency departments found that 1298 (60.1%) reported having consumed energy drinks, and most were male. Respondents 18–29 years old were most likely to use energy drinks, and more than 6% of all respondents used energy drinks with alcohol.  

In a retrospective study of 297 energy drink cases reported to an Australian poison information center between 2004 and 2010, the predominance of cases involving males increased annually (to 57%), and 21% involved accidental exposure of children (mean age, 38 months; range, 7 months–10 years). Of the recreational users, 50% simultaneously consumed alcohol, and 44% also consumed other caffeinated products. Forty-three percent (19% with no co-ingestants) required hospitalization, and 7% had adverse effects that included...
serious cardiac or neurological toxicity, including hallucinations, seizures, arrhythmias, or cardiac ischemia.\textsuperscript{34}

Children less than 6 years old were the largest proportion of the energy drink-related poison center calls (non-alcoholic and alcoholic combined). This is roughly equal to the proportion of exposures to other substances in this age group in NPDS, whereas the proportion of older children and adolescents exposed to energy drinks is much higher than to other substances in the database.

Given what is known about other unintentional childhood toxic exposures, these results suggest that energy drinks are easily within reach of children and that packaging is appealing and looks like other beverages children consume.\textsuperscript{35,36} If so, similar preventive measures should be used: childproof containers, unappealing packaging, and keeping products out of the reach of children. Adult consumers of energy drinks must be alerted to the potential adverse effects of energy drinks in children, which may occur in as many as 70% after only one serving.\textsuperscript{37}

Some energy drink containers are labeled with a warning that they may not be safe for children, those who are sensitive to caffeine, or for pregnant or nursing women. The labeling is voluntary and is contrary to marketing strategies that appear to be aimed at youth.\textsuperscript{38–40} Marketing campaigns often target extreme sporting events, sponsor athletes, associate products with illegal stimulant drugs, and advertise products in media (including social media websites and video games) oriented to children, adolescents, and young adults.\textsuperscript{26,41} Such strategies may explain the male and youth predominance of cases involving energy drink ingestion.\textsuperscript{39}

However, lawmakers question these marketing strategies, inquiring if any studies were done or underwritten by these marketers that have examined the effects of energy drink use on this younger population, given that the limited studies have taken place among adults.\textsuperscript{42}

Although the clinical effects of caffeine have been well studied, and caffeine is generally recognized as safe by the FDA,\textsuperscript{43} the effects of caffeine in energy drinks, where it may be combined with a number of other substances, is still relatively under-studied in vivo, and remain a concern, especially for children and people with certain medical conditions carrying increased risk for energy drink toxicity, such as cardiovascular, renal, or liver disease; seizures; diabetes; mood and behavioral disorders; hyperthyroidism; or those who take certain medications.\textsuperscript{1,6,7,8,14,15,44} Data on existing health conditions are routinely collected by regional poison centers, but these data are not included in the NPDS dataset and so were not analyzed here.

Nevertheless, the risk of energy drink toxicity to young children is apparent and likely unrecognized by caretakers. Poison prevention materials routinely alert parents to the dangers of household cleaning agents, medications, toxic plants, make-up, and art supplies,\textsuperscript{45–47} and the addition of the dangers of energy drinks may also have an impact on these exposures.

The NPDS has several limitations for determining the frequency and severity of product-related adverse effects. First, the NPDS is based on self-reported calls to poison control centers. Thus, NPDS exposures are only a portion of the total number of incidents that actually occur and likely underestimate the incidence of energy drink-related toxicity. There may be reporting bias, for example, in which parents of infants and children are more likely to call a poison center than for adolescents or for themselves. In fact,
the Institute of Medicine estimates that NPDS captures only about 50% of the actual poisonings that occur each year.\textsuperscript{48} Limitations in the data collection methods inherent in the database required excluding more than 3000 cases of energy drink ingestion for insufficient information. The NPDS database does not include detailed case information, association of managements or temporal relationships between clinical effects and treatments. The presence or absences of substances in the body are usually not confirmed by laboratory testing and, although we have attempted to limit the analysis to single-substance exposures, we cannot confirm exposures and co-ingestants that may have been present. Lastly, data are collected by telephone, so subtle clinical effects, such as electrocardiographic changes, could easily be missed or omitted during poison center follow-up, and in most cases, it is not possible to confirm exposure data through blood or other tests. If a portion of cases represents mistaken exposures, or if there is reporting bias in favor of exposures with greater toxicity, the true danger associated with energy drinks reported here would be overstated. On the other hand, under-reporting of clinical effects has been documented in some poison-control center data.\textsuperscript{49,50}

Conclusion

The NPDS provides the first comprehensive means to collect standardized information about potentially harmful exposures from energy drinks. Although it is limited to self-initiated calls, the database provides another method for tracking these types of exposures. We now have our first look into energy drink use and outcomes among children, adolescents, and adults across the US, which are consistent with those of poison centers in other countries.\textsuperscript{16,34,37,51} Most calls for alcoholic energy drink ingestion resulted in advice to seek medical care and resulted in critical care unit admissions for almost 10% of cases. Although some of the major effects could be secondary to the alcohol content and/or the interaction of alcohol with other energy drink constituents, nevertheless the findings here underscore the importance of the FDA ban on pre-mixed drinks.

The incidence of moderate to major adverse effects of energy drink-related toxicity was 15.2% and 39.3% for non-alcoholic and alcoholic energy drinks, respectively, although the incidence may be under-reported. About half the cases of energy drink-related toxicity involve unintentional exposures by children less than 6 years old. Educational campaigns and legal restrictions on the sale of energy drinks are associated with decreasing calls to poison centers for energy drink consumption. If a cause and effect relationship can be shown for regulatory and/or educational initiatives and a reduction in such exposures and for a correlation with the NPDS database, this national surveillance data may assist in guiding policy change decisions in an evidence-based way.\textsuperscript{52} The recent report from the American Medical Association stating their support to ban the marketing of energy drinks to children under 18\textsuperscript{53} together with statements from the American Academy of Pediatrics claiming energy drinks have no place in the diet of children and adolescents\textsuperscript{19} shows a growing support from physicians to protect children from the potentially adverse effects of caffeinated energy drinks by decreasing the likelihood of their exposure.

Declaration of interest

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

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References

RISK PROFILE:
CAFFEINE IN ENERGY DRINKS
AND ENERGY SHOTS

Prepared for New Zealand Food Safety Authority under project CFS/09/04 - Science Programme Reporting, as part of overall contract for scientific services

By

Dr Barbara Thomson
Sonja Schiess

April 2010
Maternal caffeine consumption during pregnancy and the risk of miscarriage: a prospective cohort study

Xiaoping Weng, PhD; Roxana Odouli, MSPH; De-Kun Li, MD, PhD

OBJECTIVE: The objective of the study was to examine whether the risk of miscarriage is associated with caffeine consumption during pregnancy after controlling for pregnancy-related symptoms.

STUDY DESIGN: This was a population-based prospective cohort study.

RESULTS: An increasing dose of daily caffeine intake during pregnancy was associated with an increased risk of miscarriage, compared with no caffeine intake, with an adjusted hazard ratio (aHR) of 1.42 (95% confidence interval 0.93 to 2.15) for caffeine intake of less than 200 mg/day, and aHR of 2.23 (1.34 to 3.69) for intake of 200 or more mg/day, respectively. Nausea or vomiting during pregnancy did not materially affect this observed association, nor did the change in intake pattern of caffeine during pregnancy. In addition, the magnitude of the association appeared to be stronger among women without a history of miscarriage (aHR 2.33, 1.48 to 3.67) than that among women with such a history (aHR 0.81, 0.34 to 1.94).

CONCLUSION: Our results demonstrated that high doses of caffeine intake during pregnancy increase the risk of miscarriage, independent of pregnancy-related symptoms.

Key words: abortion, caffeine, miscarriage, spontaneous

Caffeine, 1,3,7-trimethylxanthine, is among the most frequently ingested pharmacologically active substances in the world.1 Caffeine can readily cross the placental barrier to the fetus; its clearance is prolonged in pregnant women, and its metabolism rate is low in the fetus because of low levels of enzymes.2,3 It may also influence cell development through increasing cellular cyclic adenosine monophosphate concentrations4 and decrease intervillous placental blood flow via increasing circulating catecholamines.5 Therefore, caffeine could have an adverse effect on fetal development. Indeed, caffeine intake has been reported to increase the risk of miscarriage.6-9

Although numerous studies on maternal caffeine consumption and the risk of miscarriage have been published since the 1980s, the effect of caffeine intake on the risk of miscarriage remains controversial because of methodological limitations in past studies.10 Many studies have relied on retrospective information, which is subject to recall bias.6-8,11,12 Some had only a small number of participants, which limited their power to detect an effect.13 Some did not take into account potential confounding factors such as smoking, alcohol consumption, and most importantly, pregnancy-related symptoms including nausea and vomiting.7,14,15 Finally, some recruited women who sought prenatal care at their 13th to 28th weeks of gestation, therefore too late in pregnancy to study miscarriage.16,17 Such controversy has led to the uncertainty about the health effects of caffeine consumption during pregnancy among both clinicians and pregnant women alike.

In the United States, coffee, tea, and carbonated soft drinks are the main sources of caffeine intake. Mean daily caffeine consumption from these sources was estimated around 106-170 mg per day for adults and 58 mg per day for pregnant women, respectively.18 The objective of this population-based prospective study was to examine the effect of maternal caffeine intake during pregnancy on the risk of miscarriage, taking into account a number of potential confounders, especially the impact of nausea or vomiting during pregnancy.

MATERIALS AND METHODS

The study was conducted among pregnant members of the Kaiser Permanente Medical Care Program (KPMCP), a group model-integrated health care delivery system. During a 2 year period from October 1996 through October 1998, all KPMCP women who resided in the San Francisco and South San Francisco areas and had a positive pregnancy test in these facilities were identified as potentially eligible subjects. The KPMCP facilities require all women who suspect...
that they might be pregnant to undergo a pregnancy test at the KPMC laboratory regardless of whether they have already performed home pregnancy tests. Any woman who submitted a urine or blood sample for a pregnancy test was given a flyer explaining the purpose of the study and was informed of the possibility of being contacted for this study. A postage-paid and self-addressed return postcard was included with the flyer so that women who did not wish to be contacted for the study could inform us. Specially trained female interviewers contacted all women who did not return their refusal cards. Any woman who spoke English and intended to carry her pregnancy to term at the time of contact was considered eligible for the study. Women already included in the study for 1 pregnancy were not eligible to be included for subsequent pregnancies during the study period.

Of 2729 eligible women, 164 (6%) were contacted too far along in their pregnancy (more than 15 weeks) for interview; 317 (12%) initially agreed to participate but were unable to schedule an interview; 1185 (43%) refused to participate; and ultimately 1063 (39%) completed the interview. The main reasons for refusal were too busy, not interested, and too stressful to participate. A more detailed description of the study design and methods can be found elsewhere.19

Exposure assessment
Information on exposure to caffeine consumption during pregnancy was obtained during an in-person interview conducted soon after a woman’s pregnancy was confirmed (the median gestational age at interview was 71 days). Women were asked to report their intake of beverage including caffeine-containing beverages since their last menstrual period (LMP). They were asked about the types of their drinks; timing of initial drink; the frequency and amount of the intake; whether they changed consumption patterns since becoming pregnant; and, if so, the time, the frequency, and the amount of consumption after the change. Women might report their caffeine intake on either a daily or weekly basis and then average daily intake was calculated. Sources of caffeine included coffee (caffeinated or decaffeinated), tea (caffeinated or decaffeinated), decaffeinated soda (including 17 brands, such as Coca-Cola, Big Red, and Pepsi-Cola, etc), and hot chocolate. We used the following conversion factors to estimate the amount of caffeine intake: for every 150 mL of a beverage, we estimated 100 mg for caffeinated coffee, 2 mg for decaffeinated coffee, 39 mg for decaffeinated tea, 15 mg for caffeinated soda, and 2 mg for hot chocolate.8

Information on potential confounders, such as maternal age, race, education, household income, marital status, smoking, alcohol consumption, Jacuzzi use, exposure to magnetic fields (MF) during pregnancy, and symptoms related to pregnancy such as nausea and vomiting were also collected during the in-person interview.

Pregnancy outcome
Pregnancy outcomes up to 20 weeks of gestation were determined for all participants through the following 3 methods: (1) searching the KPMC inpatient or outpatient databases, (2) reviewing medical records, and (3) contacting participants whose outcomes could not be determined by using the previous 2 methods. Because, by definition, no miscarriage occurs after 20 weeks of gestation, pregnancy status was censored at 20 weeks of gestation for those pregnancies that continued beyond 20 weeks. We had information on pregnancy outcomes for all participants at 20 weeks of gestation. More than 95% of miscarriages in our study population occurred before 15 weeks of gestation. Because we recruited women at an early gestational age, a total of 102 subjects (59%) had already had a miscarriage at the time of initial contact for their participation. These subjects were interviewed soon after their miscarriage (median delay 19 days), and information on caffeine intake was ascertained only up to the end of pregnancy.

Statistical analysis
The Cox proportional hazards regression was used to take into account possible differing gestational ages at study entry between the exposed (caffeine intake) and unexposed.20,21 By using the Cox model with left truncation, we examined the association between caffeine consumption and the risk of miscarriage at any specific gestational age only for those women who had entered into the study and remained pregnant at the beginning of that specific gestational age. The interval between conception and study entry was truncated in this case (ie, treated as missing follow-up time). Using the Cox model also enabled us to easily assess whether the effect of caffeine consumption on the risk of miscarriage changed with gestational age.

Entry time was defined as gestational age at the positive pregnancy test because we started to follow up a woman’s pregnancy at her positive pregnancy test. The median gestational age at entry for the entire cohort was 40 days. The follow-up time was gestational age in days. Gestational age was determined by ultrasound (16.4%), an obstetrician (50.9%), or the self-reported last menstrual period (32.7%) if the determination by ultrasound or obstetricians was not available. All participants were followed up until miscarriage, termination of pregnancy because of other causes (eg, ectopic pregnancy), or 20 weeks of gestation.

The average daily caffeine intake during pregnancy was categorized as 0, less than 200 mg/day, or 200 or more mg/day in the overall analysis. Potential confounders, such as maternal age, race, education, household income, marital status, smoking, alcohol consumption, Jacuzzi use, MF exposure, and nausea and vomiting were included into the COX model for adjustment. A test for trend was performed with the categories of caffeine intake as an ordinal scale. All statistical analyses were performed using SAS 9.0 (SAS Institute, Cary, NC).

RESULTS
Overall 172 of women (16.18%) miscarried. Whereas 264 women (25%) reported no consumption of any caffeine-
TABLE 1
Characteristics of the study population by caffeine intake

<table>
<thead>
<tr>
<th></th>
<th>Total (n = 1063)</th>
<th>Caffeine intake</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 mg/day</td>
<td>0-200 mg/day</td>
<td>200 mg/day or greater</td>
</tr>
<tr>
<td></td>
<td>(n = 264)</td>
<td>%</td>
<td>(n = 635)</td>
<td>(n = 164)</td>
</tr>
<tr>
<td>Maternal age (y)</td>
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<td></td>
</tr>
<tr>
<td>24 or younger</td>
<td>153</td>
<td>45</td>
<td>(17.05)</td>
<td>97</td>
</tr>
<tr>
<td>25-29</td>
<td>294</td>
<td>77</td>
<td>(29.17)</td>
<td>181</td>
</tr>
<tr>
<td>30-34</td>
<td>358</td>
<td>83</td>
<td>(31.44)</td>
<td>219</td>
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<tr>
<td>35 or older</td>
<td>258</td>
<td>59</td>
<td>(22.35)</td>
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<tr>
<td>Race</td>
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<tr>
<td>White</td>
<td>405</td>
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<td>Black</td>
<td>77</td>
<td>25</td>
<td>(9.51)</td>
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<td>53</td>
<td>(20.15)</td>
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<td>Asian or Pacific Islander</td>
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<td>80</td>
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<td>176</td>
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<td>Other</td>
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<td>147</td>
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<td>College degree</td>
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<tr>
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<td>160</td>
<td>(63.49)</td>
<td>374</td>
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<td>$50,000 or more</td>
<td>389</td>
<td>92</td>
<td>(36.51)</td>
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<td>Marital status</td>
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<td>Married</td>
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<td>Living together or having a regular partner</td>
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<td>Other</td>
<td>54</td>
<td>5</td>
<td>(1.90)</td>
<td>16</td>
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<td>Previous miscarriage</td>
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<td>0</td>
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<td>1</td>
<td>164</td>
<td>35</td>
<td>(13.26)</td>
<td>92</td>
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<td>2 or more</td>
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<td>Vomiting since LMP</td>
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<td>109</td>
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<td>264</td>
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<td>221</td>
<td></td>
<td></td>
<td>(34.85)</td>
</tr>
<tr>
<td>No</td>
<td>413</td>
<td></td>
<td></td>
<td>(65.15)</td>
</tr>
<tr>
<td>Smoked since LMP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>107</td>
<td>8</td>
<td>(3.03)</td>
<td>63</td>
</tr>
<tr>
<td>No</td>
<td>956</td>
<td>256</td>
<td>(96.97)</td>
<td>572</td>
</tr>
</tbody>
</table>

Continued on page 279.e4.
containing beverages during pregnancy, 635 women (60%) reported 0-200 mg of caffeine intake per day, and 164 women (15%) had 200 mg or more of daily caffeine consumption. Table 1 compares the various characteristics of women who were at different levels of caffeine consumption. Caffeine intake was associated with a variety of risk factors for miscarriage, such as age of 35 years or older; having had a prior miscarriage; an absence of vomiting; and smoking, alcohol consumption, and use of Jacuzzi during pregnancy. Also, women with higher caffeine consumption were more likely to be white and to have a higher household income.

An increasing amount of caffeine intake was associated with an increased risk of miscarriage (Table 2). Compared with nonusers, women who consumed 0-200 mg caffeine daily had an increased risk of miscarriage (15% vs 12%), and the corresponding risk was much greater (25%) among women who consumed more than 200 mg caffeine daily. After adjustment for potential confounders including maternal age, race, education, household income, marital status, previous miscarriage, smoking, alcohol consumption, Jacuzzi use, MF exposure, and nausea and vomiting, the hazard ratio of miscarriage was 1.42 (95% confidence interval [CI], 0.93 to 2.15) and 2.23 (95% CI, 1.34 to 3.69) for daily caffeine consumption of 0-200 mg and 200 mg or more, respectively (P for trend < .01). Regarding the sources of caffeine, 63% of total caffeine consumed was from coffee. There were 152 women (19%) whose source of caffeine was solely from coffee, 293 (36.7%) from sources other than coffee, and the remaining 351 women (43.9%) from coffee and noncoffee sources (coffee, tea, soft drinks, etc.). We performed a stratified analysis according to the source of caffeine, and the association remained, regardless of the sources.

Table 3 shows the relationship between caffeine consumption and the risk of miscarriage separately for women whose pattern of caffeine consumption changed during pregnancy. A total of 631 women (79%) reduced their caffeine consumption since they became pregnant and 152 (19%) maintained the same consumption pattern, whereas 16 (2%) increased their consumption during the pregnancy. Caffeine intake of 200 mg or greater remained associated with an increased risk of miscarriage, regardless of whether a woman changed her pattern of caffeine intake after pregnancy, although the estimate in each stratum was no longer statistically significant because of reduced sample size from stratification. The number of women who increased their caffeine intake after pregnancy was too small to have a meaningful interpretation.

To examine whether the observed association was influenced by other risk factors, we conducted additional analy-

---

### Table 1

**Characteristics of the study population by caffeine intake**

Continued from page 279.e3.

<table>
<thead>
<tr>
<th>Caffeine intake</th>
<th>Total (n = 1063)</th>
<th>0 mg/day (n = 264)</th>
<th>%</th>
<th>0-200 mg/day (n = 635)</th>
<th>%</th>
<th>200 mg/day or greater (n = 164)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol use since LMP</td>
<td>Yes</td>
<td>439</td>
<td>77</td>
<td>(29.17)</td>
<td>272</td>
<td>(42.83)</td>
<td>90</td>
</tr>
<tr>
<td>No</td>
<td>624</td>
<td>187</td>
<td>(70.83)</td>
<td>363</td>
<td>(57.17)</td>
<td>74</td>
<td>(45.12)</td>
</tr>
<tr>
<td>Jacuzzi use</td>
<td>Yes</td>
<td>105</td>
<td>25</td>
<td>(9.51)</td>
<td>57</td>
<td>(9.03)</td>
<td>23</td>
</tr>
<tr>
<td>No</td>
<td>953</td>
<td>238</td>
<td>(90.49)</td>
<td>574</td>
<td>(91.97)</td>
<td>141</td>
<td>(85.98)</td>
</tr>
<tr>
<td>Drug use during pregnancy</td>
<td>Yes</td>
<td>60</td>
<td>14</td>
<td>(5.3)</td>
<td>36</td>
<td>(5.67)</td>
<td>10</td>
</tr>
<tr>
<td>No</td>
<td>1003</td>
<td>250</td>
<td>(94.7)</td>
<td>599</td>
<td>(94.33)</td>
<td>154</td>
<td>(93.90)</td>
</tr>
<tr>
<td>Exposure to MF, mG</td>
<td>16 or greater</td>
<td>780</td>
<td>195</td>
<td>(73.86)</td>
<td>458</td>
<td>(72.13)</td>
<td>127</td>
</tr>
<tr>
<td>Less than 16</td>
<td>283</td>
<td>69</td>
<td>(26.14)</td>
<td>177</td>
<td>(27.87)</td>
<td>37</td>
<td>(22.56)</td>
</tr>
<tr>
<td>Gestational age at entry, d</td>
<td>0-48</td>
<td>768</td>
<td>198</td>
<td>(75.00)</td>
<td>456</td>
<td>(71.81)</td>
<td>114</td>
</tr>
<tr>
<td>49-69</td>
<td>240</td>
<td>57</td>
<td>(21.59)</td>
<td>146</td>
<td>(22.99)</td>
<td>37</td>
<td>(22.56)</td>
</tr>
<tr>
<td>70-140</td>
<td>55</td>
<td>9</td>
<td>(3.41)</td>
<td>33</td>
<td>(5.20)</td>
<td>13</td>
<td>(7.93)</td>
</tr>
</tbody>
</table>

ses of the association stratified by presence or absence of nausea, smoking during pregnancy, and a history of miscarriage. To increase the stability of the estimates in these analyses, we categorized the caffeine consumption into less than 200 mg/day or 200 mg/day or more because the risk of miscarriage among women without any consumption of caffeine and those with consumption of caffeine less than 200 mg/day was quite similar.

The association existed among women both with and without the symptom of nausea during pregnancy, although the association was slightly stronger among women with the symptom (Table 4). A similar pattern of the association was observed for the symptom of vomiting during pregnancy.

The effect of caffeine consumption on miscarriage was higher in the nonsmoker group (adjusted hazard ratio [aHR] 2.04, 95% CI, 1.35 to 3.09) than the smoker group (aHR 1.49, 95% CI, 0.36 to 6.08) and was only statistically significant in the nonsmoker group. In addition, caffeine's effect on the risk of miscarriage remained strong among women without a history of miscarriage.

### Table 2

<table>
<thead>
<tr>
<th>Caffeine intake (mg/d)</th>
<th>Miscarriage</th>
<th>Caffeine intake (mg/d)</th>
<th>Miscarriage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes n (%)</td>
<td>No n (%)</td>
<td>cHR</td>
</tr>
<tr>
<td>Nonuser</td>
<td>33 (12.50)</td>
<td>231 (87.50)</td>
<td>1</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 200</td>
<td>97 (15.30)</td>
<td>538 (84.72)</td>
<td>1.23 (0.83 to 1.82)</td>
</tr>
<tr>
<td>200 or more</td>
<td>42 (25.45)</td>
<td>122 (74.39)</td>
<td>2.44 (1.54 to 3.85)</td>
</tr>
<tr>
<td>From coffee only</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 200</td>
<td>19 (16.81)</td>
<td>94 (83.19)</td>
<td>1.32 (0.76 to 2.33)</td>
</tr>
<tr>
<td>200 or more</td>
<td>12 (30.77)</td>
<td>27 (69.23)</td>
<td>2.82 (1.43 to 5.57)</td>
</tr>
<tr>
<td>From noncoffee only</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 200</td>
<td>54 (18.95)</td>
<td>231 (81.05)</td>
<td>1.61 (1.05 to 2.49)</td>
</tr>
<tr>
<td>200 or more</td>
<td>2 (25.00)</td>
<td>6 (75.00)</td>
<td>2.69 (0.65 to 11.22)</td>
</tr>
<tr>
<td>From both coffee and noncoffee</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 200</td>
<td>24 (10.17)</td>
<td>212 (89.83)</td>
<td>0.80 (0.47 to 1.36)</td>
</tr>
<tr>
<td>200 or more</td>
<td>28 (23.73)</td>
<td>90 (76.27)</td>
<td>2.23 (1.35 to 3.70)</td>
</tr>
</tbody>
</table>

cHR: crude hazard ratio.

* Hazard ratio adjusted for maternal age, race, education, family income, marital status, previous miscarriage, nausea and vomiting since LMP, smoking status, alcohol drinking, Jacuzzi use, and exposure to MFs.


### Table 3

<table>
<thead>
<tr>
<th>Caffeine intake (mg/d)</th>
<th>Miscarriage</th>
<th>Caffeine intake (mg/d)</th>
<th>Miscarriage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes n (%)</td>
<td>No n (%)</td>
<td>cHR</td>
</tr>
<tr>
<td>Nonuser</td>
<td>33 (12.50)</td>
<td>231 (87.50)</td>
<td>1</td>
</tr>
<tr>
<td>Reduction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 200</td>
<td>62 (12.06)</td>
<td>452 (87.94)</td>
<td>0.94 (0.62 to 1.43)</td>
</tr>
<tr>
<td>200 or more</td>
<td>20 (17.09)</td>
<td>97 (82.91)</td>
<td>1.50 (0.86 to 2.61)</td>
</tr>
<tr>
<td>No change</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 200</td>
<td>31 (28.44)</td>
<td>78 (71.56)</td>
<td>2.62 (1.60 to 4.27)</td>
</tr>
<tr>
<td>200 or more</td>
<td>20 (46.51)</td>
<td>23 (53.49)</td>
<td>5.61 (3.21 to 9.83)</td>
</tr>
</tbody>
</table>

cHR: crude hazard ratio.

* Hazard ratio adjusted for maternal age, race, education, family income, marital status, previous miscarriage, smoking status, alcohol drinking, Jacuzzi use, and exposure to MFs.

(aHR 2.33, 95% CI, 1.48 to 3.67), whereas the association no longer existed among women with such a history (aHR 0.81, 95% CI, 0.34 to 1.94) (Table 4). The test for the interaction was borderline significant ($P = .05$).

To determine whether the effect of caffeine on the risk of miscarriage varied by gestational age at miscarriage, we examined the effect separately for miscarriages that occurred before and after 8 weeks of gestation. A total of 57 miscarriages (33%) occurred before 8 gestational weeks, and 115 (67%) occurred on or after that. Higher caffeine consumption was associated with higher risk for both early and late miscarriage. However, the association appeared to be more pronounced for later rather than earlier miscarriage (Table 5).

**Comment**

In this prospective cohort study, we demonstrated an elevated risk of miscarriage associated with caffeine consumption during pregnancy and a dose-response relationship with most of the risk associated with caffeine consumption at 200 mg or greater per day. This observed effect was independent of many potential confounders including pregnancy-related symptoms such as nausea, vomiting, and aversion to caffeine consumption. Even among women who never changed caffeine consumption pattern during pregnancy, there was an almost 80% increased risk of miscarriage associated with caffeine consumption of 200 mg/day or greater, although it was not statistically significant because of reduced sample size by stratification. Finally, the increased risk of miscarriage appeared to be due to caffeine itself rather than other possible chemicals in coffee because caffeine intake from noncoffee sources showed the similarly increased risk of miscarriage (Table 2).

Although an increased risk of miscarriage associated with caffeine intake during pregnancy has been previously reported, a lack of adequate control of potential confounders, especially pregnancy-related symptoms such as nausea, vomiting, and aversion to caffeine, limited the validity of those findings. Some argued that the association was an artifact because of confounding by nausea and vomiting, which are generally associated with a low risk of miscarriage and possible reduction of the consumption of caffeine because of the symptoms. We ascertained detailed information on nausea and vomiting since the LMP and for the immediate 7 days before the interview. The association between caffeine intake and the risk of miscarriage remained after adjustment for nausea and vomiting, and the association also continued to exist among women both with and without nausea and vomiting during pregnancy.

To address this issue more thoroughly, we examined the association among women with and without actual change in caffeine consumption during pregnancy (a direct control of possible changes in caffeine consumption because of underlying risk of miscarriage that had been the critical point of the criticism of the association). We examined the association separately among those who reduced and who did not change their caffeine consumption during pregnancy. (The sample size was too small to evaluate this issue for those who increased their caffeine consumption during pregnancy.) The increased risk of miscarriage associated with caffeine consumption still existed after the stratification. These results did not support the argument that the observed association...
was due to confounding by the pregnancy-related symptoms that reduced both caffeine intake and the risk of miscarriage.

We also observed that the association appeared to be stronger among women without other risk factors for miscarriage, for example, women with no history of miscarriage, no smoking during pregnancy, and the presence of nausea and/or vomiting (Table 4). Although the underlying reason for this interaction is not known at this time, it could be that caffeine intake is a lesser risk factor in the presence of other risk factors of miscarriage as is the likely case among women with a history of repeated miscarriages. If our interpretation is correct, this observation is consistent with our other finding that the association was stronger among later miscarriage (Table 5), which, unlike early miscarriage, are not largely due to known strong risk factors such as chromosomal abnormalities.

One limitation of the study is the potential misclassification of caffeine intake. Caffeine content in a cup of tea/coffee varies by different brands and brewing methods; it is not practical to perform laboratory analysis on caffeine content from consumed coffee and tea in epidemiological studies. Even assays of biological specimens have limitations because they can measure only caffeine intakes in the very recent past. Therefore, most studies including ours used certain conversion factors to calculate caffeine amount given the sources of caffeine and amount of intake provided by the participants.8,13,15-17,22

Another concern is the potential recall bias because of some participants who were interviewed soon after their miscarriage. To assess the potential existence of recall bias, we conducted a stratified analysis based on whether the interview was conducted before or after their miscarriage. The results were essentially the same, providing no evidence of recall bias. Therefore, we combined the data in the final analyses. Because of low participation rates, selection bias could be a potential concern. Although we do not have information on caffeine intake for nonparticipants, we compared a few characteristics, including age and the rate of miscarriage between participants and nonparticipants. Both average age (30 vs 29 years) and the rate of miscarriage (16.4% vs 17.2%) for participants and nonparticipants were very similar, providing some assurance against participation bias.

The strengths of the present study included: (1) a cohort design, (2) a large study sample size, (3) recruitment of pregnant women at early gestational ages for identification of early miscarriages, (4) detailed information on caffeine intake including all sources, changing patterns of intakes, and timing and amount of intakes since LMP, and (5) ascertainment of detailed information on pregnancy-related symptoms including nausea, vomiting, and aversion to caffeine consumption during pregnancy. The available information on nausea, vomiting, and existence of aversion to caffeine consumption allowed us to examine whether these factors explained the observed association of caffeine intake during pregnancy with the risk of miscarriage.

In conclusion, the results from our prospective cohort study supported previous findings that high caffeine consumption during pregnancy may increase the risk of miscarriage. We provided new evidence that the observed association was not likely the result of confounding by the pregnancy-related symptoms of nausea, vomiting, and aversion to caffeine consumption. Therefore, it may be prudent to stop or reduce caffeine intake during pregnancy.

ACKNOWLEDGMENT

De-Kun Li conceived the concept, designed the study, obtained funding, oversaw the data collection and analyses, and was involved in the interpretation of results and preparation of the manuscript. Xiaoping Weng was responsible for data analysis, interpretation of the data, and preparation of the manuscript. Roxana Odooli was involved in the data collection and preparation of the manuscript. De-Kun Li is the guarantor of this paper, who took full responsibility for the conduct of the study, had access to the data, and controlled the decision to publish.

REFERENCES

RISK PROFILE:
CAFFEINE IN ENERGY DRINKS
AND ENERGY SHOTS

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Food Safety Programme Leader

Dr Barbara Thomson
Project Leader

Peter Cressey
Peer Reviewer
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Thanks to Peter Cressey, ESR, for undertaking the @Risk simulations.
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</table>

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EXECUTIVE SUMMARY

This Risk Profile addresses the risk from exposure to caffeine from energy drinks and energy shots available in New Zealand against a background dietary exposure from naturally occurring caffeine in foods and beverages and kola type soft drinks.

In the New Zealand diet, caffeine occurs naturally in coffee, tea, cocoa and foods containing these ingredients. Caffeine may also be added to a range of beverages including energy drinks, energy shots, kola type soft drinks and alcoholic beverages. A total of 28 energy drinks and 16 energy shots were identified as available on the New Zealand market at February 2010.

Caffeine has a range of adverse effects. Common acute adverse effects associated with stimulation of the central nervous system following caffeine ingestion include dizziness, rapid heartbeat, irritability, anxiety, tremors and insomnia. Irritation of the gastrointestinal tract can result in diarrhoea, nausea and/or vomiting. Single high doses of caffeine can affect the cardiovascular system causing rapid heart beat and high blood pressure. The risk of high blood pressure associated with coffee consumption may be higher in certain genotypes and in individuals with reduced liver function. Caffeine intake is associated with a slight deterioration in calcium balance, particularly if calcium intake is low. There is limited evidence for caffeine as a carcinogen but results are inconsistent and not conclusive. Caffeine consumption during pregnancy has been associated with an increased risk of foetal growth restriction. No studies have been reported for potential chronic effects of caffeine consumption by children. Tolerance to effects on blood pressure and heart rate, but not to sleep disturbance, develop quickly and virtually completely. Deaths attributed to caffeine consumption are rare.

Moderate daily caffeine intake by healthy adults with adequate nutrition, up to 400 mg/day (5.7 mg/kg bw/day for a 70kg adult) is unlikely to result in adverse effects.

Twenty calls relating to energy drinks and energy shots were recorded at the New Zealand National Poisons Centre in the five year period 2005 to 2009. Minimum and maximum self reported intakes that elicited symptoms of caffeine toxicity were 4.0 mg/kg and 35.5 mg/kg for persons 13 and 14 years of age respectively.

There is currently no recognised reference health standard established for caffeine exposure, such as an Acceptable Daily Intake (ADI). An upper exposure of 2.5 mg/kg bw/day has been suggested as a cautious toxicological limit on which to base risk assessments for children, based on limited evidence. An adverse effect level of 3 mg/kg bw/day for adults is a conservative reference level based on limited evidence of acute anxiety effects. A reference level of 200 mg/day for pregnant women is used in this report and is based on recent evidence of foetal growth restriction.

Baseline dietary exposure to caffeine (including coffee, tea, chocolate, kola type soft drinks and any foods containing these ingredients) was estimated for seven New Zealand population groups based on New Zealand caffeine concentration data for 52 foods and consumption information from the 1997 National Nutrition Survey and the 2002 Children’s Nutrition Survey. Most survey respondents consumed products containing caffeine on the surveyed day, with the proportion of caffeine consumers varying from 73 to 96 percent across the
seven population groups. Between two and 38 percent of New Zealand caffeine consumers were estimated to have a baseline dietary caffeine exposure above an adverse effect level of 3 mg/kg bw/day, depending on the population group to which they belonged.

Retail units of energy drinks ranged from 250 to 600 ml resulting in caffeine exposures of 75 to 240 mg caffeine per retail unit. Energy shots ranged from 30 to 120 ml resulting in exposures of 10 to 300 mg caffeine per retail unit consumed.

The estimated caffeine exposure following the consumption of energy drinks or energy shots was calculated for New Zealand children (5-12 yrs), teenagers (13-19 yrs) and young males (19-24 yrs). Scenarios were estimated for consumption of one, two, three or four retail units of any of the energy drinks or energy shots available in New Zealand. Our estimates indicate that approximately 70% of children and 40% of teenagers who consume caffeine are estimated to exceed an adverse effect level of 3 mg/kg bw/day after consumption of a single retail unit of an energy drink or energy shot in addition to a baseline dietary exposure.

Areas of uncertainty are: further products on the market not identified, out-dated consumption and concentration information, inappropriate mapping and caffeine concentration data, weak evidence of adverse effects on which to base safety levels, lack of market share weighting in the risk scenario modeling, inappropriate assumptions with respect to energy drink and energy shot choices and the contribution of caffeinated alcoholic beverages to caffeine exposure.

Risk management options include revision of regulations with respect to the composition and/or volume of energy drinks or energy shots and additional risk communication for children, teenagers, pregnant and caffeine sensitive individuals.

The most significant data gap in this assessment is the lack of current information on consumption of energy drinks and energy shots. There is no current information on how many New Zealanders are consuming which energy drinks or energy shots or how much they are consuming. Further data gaps are the lack of independently verified caffeine levels in energy drinks and energy shots and evidence of health effects of frequent high caffeine intakes for children and adults. For these reasons the present risk to New Zealanders from energy drinks and energy shots cannot be accurately quantified.
## GLOSSARY OF TERMS, ABBREVIATIONS AND ACRONYMS

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADI</td>
<td>Acceptable Daily Intake. The amount of a chemical that an individual may consume on a daily basis over a lifetime with no adverse effect to health</td>
</tr>
<tr>
<td>caffeinated beverage</td>
<td>A beverage to which caffeine has been added. May include energy drink, energy shot, kola type soft drink or caffeinated alcoholic beverage.</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System.</td>
</tr>
<tr>
<td>energy drink</td>
<td>A beverage containing added caffeine, vitamins and other bioactive substances marketed in retail units typically between 250ml and 600ml.</td>
</tr>
<tr>
<td>energy shot</td>
<td>A small volume liquid product containing added caffeine, vitamins and other bioactive substances.</td>
</tr>
<tr>
<td>FCT</td>
<td>New Zealand Food Composition Tables.</td>
</tr>
<tr>
<td>FSANZ</td>
<td>Food Standards Australia New Zealand.</td>
</tr>
<tr>
<td>IARC</td>
<td>International Agency for Research on Cancer</td>
</tr>
<tr>
<td>kola type soft drink</td>
<td>A flavoured carbonated beverage containing added caffeine. Caffeine can be added to a maximum level of 145mg/l (Standard 1.3.1 of the Australia New Zealand Food Standards Code)</td>
</tr>
<tr>
<td>mg/kg bw/day</td>
<td>A dosage unit of milligrams per kilogram body weight per day.</td>
</tr>
<tr>
<td>NIP</td>
<td>Nutrition Information Panel.</td>
</tr>
<tr>
<td>NZFSA</td>
<td>New Zealand Food Safety Authority.</td>
</tr>
<tr>
<td>QFFQ</td>
<td>Qualitative Food Frequency Questionnaire, a component of the 1997 NNS</td>
</tr>
<tr>
<td>RMF</td>
<td>Risk Management Framework.</td>
</tr>
</tbody>
</table>

1 definitions for the purposes of this report
1 STATEMENT OF PURPOSE

The purpose of a Risk Profile is to provide contextual and background information relevant to a food/hazard combination so that risk managers can make decisions and, if necessary, take further action. Risk Profiles are part of the Risk Management Framework (RMF, http://www.nzfsa.govt.nz/about-us/risk-management-framework/index.htm) approach taken by the New Zealand Food Safety Authority (NZFSA). The Framework consists of a four step process, as shown in Figure 1.

Figure 1: The four steps of the Risk Management Framework

This initial step in the RMF, Preliminary Risk Management Activities, includes a number of tasks:

- identification of food safety issues
- risk profiling
- establishing broad risk management goals
- deciding on the need for a risk assessment
- if needed, setting risk assessment policy and commissioning of the risk assessment
- considering the results of the risk assessment
- ranking and prioritisation of the food safety issue for risk management action.

Risk profiling may be used directly by risk managers to guide identification and selection of risk management options, for example where:

- rapid action is needed
- there is sufficient scientific information for action
- embarking on a risk assessment is impractical.

The sections in this Risk Profile are organised as much as possible as they would be for a conventional qualitative risk assessment, including hazard and food, evaluation of adverse health effects, evaluation of risk, and availability of control measures.
1.1 Hazard/Food Combination and Risk Management Questions

There is current public and regulatory concern regarding the exposure of children to caffeine from beverages to which caffeine has been added. There is now a wide range of energy drinks and energy shots available on the New Zealand market.

NZFSA has commissioned this Risk Profile in order to address the following specific risk management question:

Is any sub group of the New Zealand population at risk of adverse effects from the consumption of energy drinks or energy shots currently available in New Zealand?

This Risk Profile considers the potential risk from the consumption of energy drinks and energy shots in addition to a background exposure from the more traditional food sources of caffeine, namely, coffee, tea, chocolate and kola type soft drinks.
2 HAZARD AND FOOD

2.1 Caffeine

2.1.1 Caffeine structure and nomenclature

Caffeine (CAS 58-08-2) belongs to a class of compounds called methylxanthines. Its chemical structure is shown in Figure 2. Caffeine is found in many plant species and is believed to help protect the plant from insect pests (Nathanson, 1984).

Figure 2: Chemical structures of caffeine, theobromine and theophylline

2.2 Sources of Caffeine

Caffeine is produced commercially by both extraction from plant material and by synthesis (IARC, 1991).

Caffeine is found in more than 60 plant species worldwide, although there are only a few varieties that humans commonly eat (IARC, 1991; Steffen, 2000). Information on plant species and typical levels of caffeine found in them are summarised in Table 1. Caffeine content of plant materials may be affected by genetic and climatic factors, as well as fertiliser use (IARC, 1991). Caffeine content of tea leaves decreases with leaf maturity (Yao et al., 2006).

Guarana is used to refer both to the plant *Paullinia cupana* and the extract from its berries that may be used as an ingredient in energy drinks. The berries of the guarana plant contain large amounts of caffeine (4-8%), as well as significant amounts of the related methylxanthines, theobromine and theophylline (Figure 2) (Babu et al., 2008). Guarana is the richest known natural source of caffeine (Walker et al., 2000).
## Table 1: Caffeine content of various plant materials and products

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Part of plant</th>
<th>Processed form</th>
<th>Typical caffeine content of processed form (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coffee (Coffea arabica)</td>
<td>Berry/bean</td>
<td>Dried green beans</td>
<td>0.9-1.4</td>
<td>(IARC, 1991)</td>
</tr>
<tr>
<td>Coffee (Coffea canephora Var. robusta)</td>
<td>Berry/bean</td>
<td>Dried green beans</td>
<td>1.5-2.6</td>
<td>(IARC, 1991)</td>
</tr>
<tr>
<td>Tea (Camellia sinensis)</td>
<td>Leaf</td>
<td>Dried processed leaf</td>
<td>Mean = 3.5 (wide range)</td>
<td>(IARC, 1991)</td>
</tr>
<tr>
<td>Cacao (Theobroma cacao)</td>
<td>Bean</td>
<td></td>
<td>0.07-1.7</td>
<td>(IARC, 1991)</td>
</tr>
<tr>
<td>Mate (Ilex paraguariensis)</td>
<td>Leaf</td>
<td>Dried</td>
<td>0.5-2.2</td>
<td>(Cardozo Jr et al., 2007; IARC, 1991)</td>
</tr>
<tr>
<td>Guarana (Paullinia cupana)</td>
<td>Berry</td>
<td>Dried</td>
<td>2.0-4.5</td>
<td>(Bempong et al., 1993)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.5-7.0</td>
<td>(Walker et al., 2000)</td>
</tr>
<tr>
<td>Kola</td>
<td>Nut</td>
<td>Fresh</td>
<td>0.5-1.6</td>
<td>(Niemenak et al., 2008)</td>
</tr>
<tr>
<td>Cola acuminata</td>
<td></td>
<td></td>
<td>0.9-1.9</td>
<td></td>
</tr>
<tr>
<td>Cola nitida</td>
<td></td>
<td></td>
<td>0.4-1.2</td>
<td></td>
</tr>
<tr>
<td>Cola anomola</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 2.3 Caffeine in Food and Beverages

In the New Zealand diet, caffeine occurs naturally in coffee, tea, cocoa and foods containing these ingredients. The caffeine content of foods containing naturally occurring caffeine is not regulated.

Caffeine may also be added to a range of beverages including energy drinks, energy shots, kola type soft drinks and alcoholic beverages. Energy drinks and energy shots are beverages containing added caffeine, vitamins and other bioactive substances. For the purposes of this report, larger retail units of 250 ml or more, were considered as energy drinks and smaller retail units of 120ml or less, were considered as energy shots.

Caffeine added to food and beverages in New Zealand is regulated (see 5.1.1). A formulated caffeinated beverage must contain no less than 145 mg/L and no more than 320 mg/L total caffeine, regardless of the source of caffeine (Standard 2.6.4 of the Australia New Zealand Food Standards Code) (FSANZ, 2010). Prior to 31 March 2010 energy shots marketed as Dietary Supplements, had to include caffeine in the ingredient list but did not need to specify the amount of caffeine nor were advisory statements required (New Zealand Legislation, 2007). From 31 March 2010 food type dietary supplements such as energy shots are regulated by the New Zealand Food (Supplemented Food) Standard 2010. This new standard requires caffeine to be listed in the ingredient list and if the supplemented food contains...
added caffeine greater than 145mg/L the level of caffeine and the advisory statements that apply to formulated caffeinated beverages regulated under Standard 2.6.4 of the Australia New Zealand Food Standards Code must be included on the label (NZFSA, 2010).

Kola type soft drinks, which include Cola, Coke, and Pepsi products, contain added caffeine. The maximum permissible level of caffeine in these soft drinks is 145 mg/L (mg/kg) (Standard 1.3.1, Schedule 1) (FSANZ, 2010). Kola type soft drinks have been included in background exposure to caffeine within this Risk Profile.

The global energy drink market was 3.9 billion litres in 2008 and the market has shown 14% growth over the period 2003 to 2008. Consumption per person, globally, has risen from 0.4 litres in 2003 to 0.8 litres in 2008. North America is the leading region with a 37% share of global volume, followed by Asia Pacific with 30% share, and West Europe with 15% (Zenith, 2010).

2.4 Exposure Assessment

Exposure to caffeine from added caffeine in energy drinks and energy shots occurs against a background exposure from naturally occurring caffeine and kola type soft drinks.

2.4.1 Caffeine concentrations in New Zealand foods and beverages from natural sources and kola type soft drinks

Data on the caffeine content of non-alcoholic beverages, chocolate or cocoa flavoured foods and confectionery are reported in the New Zealand Food Composition Tables (Lesperance, 2009). Most of these data were sourced, or derived, from Australian analytical data (42/48) except for two foods (Milo made with water and self-saucing chocolate pudding) that were calculated from New Zealand data, and three foods (espresso brewed coffee, chocolate éclairs and McDonalds sundae) that were derived from a United States Department of Agriculture database (FOODfiles, 2006). Caffeine concentrations per 100 g (or 100 ml) and caffeine content per serving of food are shown in Appendix 1.

2.4.2 New Zealand data for dietary caffeine concentrations compared with overseas data

A compilation of caffeine levels reported in the open literature, for overseas countries, is shown in Appendix 2. Whilst the majority of these data are from publications in the peer reviewed scientific literature, some government website (NSW Food Authority, 2009) and industry supplied data on caffeine content of energy drinks is also included (Meltzer et al., 2008).

A comparison of the New Zealand and international concentration data for the caffeine content of foods and beverages shows reasonable consistency except for the concentrations of caffeine per serving of latte and espresso (Appendices 1 and 2). For these beverages, the New Zealand levels per serve appear unrealistically high. The New Zealand values for caffeine per serve are extrapolated from a concentration per 100g to a serve of 272 ml (i.e. the caffeine concentration per 100g is multiplied by 272/100). It is likely a serve of latte or espresso is equivalent to a coffee shot, typically of 50-100 mg per shot, rather than a...
multiplication factor based on volume. This may account for the apparent difference between New Zealand and overseas data for these two beverages.

The caffeine content of coffee and tea beverages varies greatly depending on the preparation of the beverage. McCusker et al. (2003, 2006) reported variabilities expressed as coefficient of variation of 81% and 34% for the caffeine content of the same coffee purchased from the same outlet on different occasions. A comprehensive study of caffeine levels in 400 samples of teas, instant and ground coffee beverages as consumed, was undertaken by the UK Food Standards Agency in 2004. These samples were collected from family homes, workplaces, cafes and restaurants from 10 areas over the UK and measured by validated methodology. This data set provides robust data on the variability of caffeine concentrations, as likely to be encountered by the consumer (FSA, 2004).

2.4.3 New Zealand caffeinated beverages

Information on caffeinated beverages available in New Zealand, and their claimed caffeine content was retrieved by browsing retail outlets, representing both the major supermarket companies, namely Progressive and Foodstuffs, two independent outlets (dairies), two petrol service station outlets and four liquor outlets in Christchurch, augmented with information from NZFSA (Ursula Egan, NZFSA, personal communication, October 2009). Further information was sourced from the Manufactured Food Database (Alannah Steeper, Manufactured Food Database, personal communication, October 2009) from the Energy Fiend website (Energy Fiend, 2009) and other New Zealand websites relating to specific products. The availability of drinks was also confirmed against those analysed by the NSW Food Authority (Ursula Egan, NZFSA, personal communication, October 2009).

As independent analytical data were not available, information on the claimed caffeine content of energy drinks and energy shots are summarised in Table 2. Information on the claimed caffeine content of caffeinated alcoholic beverages and kola type soft drinks are shown in Table 3.

A total of 64 products containing added caffeine were identified as available on the New Zealand market at the present time. These comprised energy drinks (28), energy shots (16), caffeinated alcoholic beverages (5) and kola type soft drinks (15) including multiple flavours and sugar free options when available. In addition, and excluded from product summaries, some products are available in more than one retail unit volume. The caffeinated beverage market is dynamic with online purchasing available. It is possible that additional products are currently being sold in New Zealand that were not captured in this report.

Retail units of energy drinks ranged from 250 to 600 ml resulting in caffeine exposures of 75 to 240 mg caffeine per retail unit consumed. Energy shots ranged from 30 to 120 ml resulting in exposures of 10 to 300 mg caffeine per retail unit consumed. The caffeine dose per retail unit consumed was similar for energy drinks and energy shots.

Caffeinated alcoholic beverages ranged in volume from 250 to 300 ml. These products claimed to contain between 7 and 32 mg/100ml of caffeine. Consumption of any one of these five products would provide between 21 and 96 mg caffeine per retail unit consumed.
### Table 2: Claimed caffeine content of energy drinks and energy shots available in New Zealand

<table>
<thead>
<tr>
<th>Product</th>
<th>Retail Unit Size (ml)</th>
<th>Caffeine concentration (mg/100 ml)</th>
<th>Caffeine per retail unit consumed (mg)</th>
<th>Guarana concentration (mg/100ml)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy Drinks</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angel Energy Drink</td>
<td>300</td>
<td>32</td>
<td>96</td>
<td>NA</td>
<td>1,2,3,6</td>
</tr>
<tr>
<td>Big Cock</td>
<td>440</td>
<td>30</td>
<td>132</td>
<td>NA</td>
<td>1,2,6</td>
</tr>
<tr>
<td>Charlie’s Vitamin Water, Energy</td>
<td>500</td>
<td>15</td>
<td>75</td>
<td>Ingredient</td>
<td>2</td>
</tr>
<tr>
<td>Cocaine</td>
<td>250</td>
<td>32</td>
<td>80</td>
<td>Nil</td>
<td>2</td>
</tr>
<tr>
<td>Demon Energy Drink</td>
<td>250 and 500</td>
<td>32</td>
<td>80/160</td>
<td>NA</td>
<td>1,2,3,6</td>
</tr>
<tr>
<td>Demon Killa Troppo Energy Drink</td>
<td>500</td>
<td>32</td>
<td>160</td>
<td>NA</td>
<td>1,3,6</td>
</tr>
<tr>
<td>Hemp Huge</td>
<td>440</td>
<td>32</td>
<td>141</td>
<td>45</td>
<td>2,6</td>
</tr>
<tr>
<td>Ink</td>
<td>500</td>
<td>32</td>
<td>160</td>
<td>120</td>
<td>1,2,4</td>
</tr>
<tr>
<td>Monster Original</td>
<td>500</td>
<td>32</td>
<td>160</td>
<td>Ingredient</td>
<td>1,2,3</td>
</tr>
<tr>
<td>Monster Ripper</td>
<td>500</td>
<td>32</td>
<td>160</td>
<td>NA</td>
<td>1,2,3</td>
</tr>
<tr>
<td>Mother</td>
<td>500</td>
<td>32</td>
<td>160</td>
<td>Nil</td>
<td>1,2,3</td>
</tr>
<tr>
<td>Mother Inferno</td>
<td>500</td>
<td>32</td>
<td>160</td>
<td>Nil</td>
<td>1,2,3</td>
</tr>
<tr>
<td>NOS Liquid Energy</td>
<td>500</td>
<td>48</td>
<td>240</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>Nutrient Water - Passionfruit Citrus</td>
<td>575</td>
<td>14</td>
<td>81</td>
<td>25</td>
<td>2,3</td>
</tr>
<tr>
<td>Rasta Blasta</td>
<td>500</td>
<td>32</td>
<td>160</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>Red Bull</td>
<td>473</td>
<td>17</td>
<td>80</td>
<td>Nil</td>
<td>1,2,3</td>
</tr>
<tr>
<td>Red Bull Sugar Free</td>
<td>473</td>
<td>17</td>
<td>80</td>
<td>Nil</td>
<td>1,2,3</td>
</tr>
<tr>
<td>Rockstar Juice</td>
<td>473</td>
<td>32</td>
<td>151</td>
<td>Nil</td>
<td>1,2,3</td>
</tr>
<tr>
<td>Rockstar Original</td>
<td>473</td>
<td>32</td>
<td>151</td>
<td>Nil</td>
<td>1,2,3</td>
</tr>
<tr>
<td>Rockstar Punched</td>
<td>500</td>
<td>32</td>
<td>151</td>
<td>NA</td>
<td>1,3,6</td>
</tr>
<tr>
<td>Supplement Water - Energise</td>
<td>600</td>
<td>17</td>
<td>102</td>
<td>Nil</td>
<td>2,4</td>
</tr>
<tr>
<td>Supplement Water - Revive</td>
<td>600</td>
<td>15</td>
<td>90</td>
<td>Nil</td>
<td>2</td>
</tr>
<tr>
<td>V Berry</td>
<td>250</td>
<td>31</td>
<td>77</td>
<td>120</td>
<td>2,3</td>
</tr>
<tr>
<td>V Black</td>
<td>250 and 500</td>
<td>31</td>
<td>77/155</td>
<td>120</td>
<td>1,2,3,4</td>
</tr>
<tr>
<td>V Green</td>
<td>250 and 500</td>
<td>31</td>
<td>77/155</td>
<td>120</td>
<td>2,3,4</td>
</tr>
<tr>
<td>V Lemon</td>
<td>250</td>
<td>31</td>
<td>77</td>
<td>500</td>
<td>2,3,4</td>
</tr>
<tr>
<td>V Sugarfree</td>
<td>250</td>
<td>31</td>
<td>77</td>
<td>120</td>
<td>2,3,4</td>
</tr>
<tr>
<td>Vitaminwater Energy</td>
<td>600</td>
<td>16</td>
<td>96</td>
<td>Ingredient</td>
<td>2,4</td>
</tr>
<tr>
<td><strong>Energy shots</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacchus D</td>
<td>100</td>
<td>30</td>
<td>30</td>
<td>Nil</td>
<td>2,3</td>
</tr>
<tr>
<td>6 Hour Power</td>
<td>60</td>
<td>208</td>
<td>125</td>
<td>Nil</td>
<td>1,6</td>
</tr>
<tr>
<td>Clear Shot</td>
<td>60</td>
<td>208</td>
<td>125</td>
<td>Nil</td>
<td>5</td>
</tr>
</tbody>
</table>

*Risk Profile: Caffeine in energy drinks and energy shots*
<table>
<thead>
<tr>
<th>Product</th>
<th>Retail Unit Size (ml)</th>
<th>Caffeine concentration (mg/100 ml)</th>
<th>Caffeine per retail unit consumed (mg)</th>
<th>Guarana concentration (mg/100ml)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demon Citrus Blast</td>
<td>60</td>
<td>333</td>
<td>200</td>
<td>12</td>
<td>2,3</td>
</tr>
<tr>
<td>Demon Tropical Punch’d</td>
<td>60</td>
<td>333</td>
<td>200</td>
<td>12</td>
<td>2,3</td>
</tr>
<tr>
<td>Monster Hitman</td>
<td>89</td>
<td>Ingredient</td>
<td>NA</td>
<td>Ingredient</td>
<td>2</td>
</tr>
<tr>
<td>NOS Energy Shot</td>
<td>60</td>
<td>416</td>
<td>250</td>
<td>NA</td>
<td>1,6</td>
</tr>
<tr>
<td>NOS High Octane</td>
<td>60</td>
<td>333</td>
<td>200</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>NOS Supercharged</td>
<td>60</td>
<td>333</td>
<td>200</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Octane</td>
<td>30</td>
<td>33</td>
<td>10</td>
<td>Nil</td>
<td>1,2</td>
</tr>
<tr>
<td>Octane Citrus</td>
<td>30</td>
<td>50</td>
<td>15</td>
<td>Nil</td>
<td>2</td>
</tr>
<tr>
<td>Reckless Energy Shot</td>
<td>70</td>
<td>178</td>
<td>125</td>
<td>NA</td>
<td>1,6</td>
</tr>
<tr>
<td>Red Bull Energy Shot</td>
<td>60</td>
<td>133</td>
<td>80</td>
<td>Nil</td>
<td>1,2,3</td>
</tr>
<tr>
<td>Top Flight</td>
<td>60</td>
<td>Ingredient</td>
<td>NA</td>
<td>Nil</td>
<td>5</td>
</tr>
<tr>
<td>V Pocket Rocket Energy Shot</td>
<td>60</td>
<td>267</td>
<td>160</td>
<td>Nil</td>
<td>1,2</td>
</tr>
<tr>
<td>X Shot</td>
<td>120</td>
<td>250</td>
<td>300</td>
<td>20</td>
<td>5</td>
</tr>
</tbody>
</table>

NA = not available, Nil = none present, Ingredient = present, but amount not specified
1=Energy Fiend, 2009, A website run by caffeine enthusiasts James Foster and Ted Kallmyer, owned by Exis Holdings Limited, a New Zealand corporation that is neither a beverage manufacturer nor lobby group. Caffeine amounts reported on this website were obtained from brand websites, nutrition panels or directly from beverage manufacturers. The site includes links to a number of scientific papers, consumer reports, and governmental sources or reports.
2=Christchurch retail outlets
3=Included in NSW study, October 2009
4=Alannah Steeper, Manufactured Foods Database, personal communication, identifies caffeine and guarana as ingredients but not amounts
5=Ursula Egan, NZFSA, personal communication, 9 November, 2009.
6=New Zealand company websites
### Table 3: Claimed caffeine content of caffeinated alcoholic beverages and kola type soft drinks available in New Zealand

<table>
<thead>
<tr>
<th>Product</th>
<th>Retail Unit Size(s) (ml or g)</th>
<th>Caffeine concentration (mg/100 ml or mg/100 g)</th>
<th>Caffeine per retail unit consumed (mg)</th>
<th>Guarana concentration (mg/100ml)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Caffeinated Alcoholic Beverages</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulse Red</td>
<td>300</td>
<td>32</td>
<td>96</td>
<td>100</td>
<td>1,2</td>
</tr>
<tr>
<td>Pulse Blue</td>
<td>300</td>
<td>7</td>
<td>21</td>
<td>150</td>
<td>2</td>
</tr>
<tr>
<td>Pulse Green</td>
<td>300</td>
<td>32</td>
<td>96</td>
<td>100</td>
<td>2</td>
</tr>
<tr>
<td>Pulse Black</td>
<td>300</td>
<td>9</td>
<td>27</td>
<td>150</td>
<td>2</td>
</tr>
<tr>
<td>Vudu</td>
<td>250</td>
<td>31</td>
<td>77</td>
<td>150</td>
<td>2</td>
</tr>
<tr>
<td><strong>Kola type soft drinks</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coca-Cola</td>
<td>250,420,440, 600,1500 2250</td>
<td>10</td>
<td>25/250ml</td>
<td>Nil</td>
<td>1,2,3</td>
</tr>
<tr>
<td>Coke Zero</td>
<td>250,420,440, 600,1500 2250</td>
<td>10</td>
<td>25/250ml</td>
<td>Nil</td>
<td>1,2,3</td>
</tr>
<tr>
<td>Vanilla Coke</td>
<td>NA</td>
<td>Ingredient</td>
<td>NA</td>
<td>Nil</td>
<td>3</td>
</tr>
<tr>
<td>Diet Coke</td>
<td>200,250,355 420,440,600 1500, 2250</td>
<td>10</td>
<td>25/250ml</td>
<td>Nil</td>
<td>1,2,3</td>
</tr>
<tr>
<td>Diet Coke Vanilla, Lime, Raspberry</td>
<td>NA</td>
<td>Ingredient</td>
<td>NA</td>
<td>Nil</td>
<td>3</td>
</tr>
<tr>
<td>Diet Pepsi</td>
<td>355,1500</td>
<td>Ingredient</td>
<td>NA</td>
<td>Nil</td>
<td>2,3</td>
</tr>
<tr>
<td>Frozen Coke</td>
<td>355,1500</td>
<td>Ingredient</td>
<td>NA</td>
<td>Nil</td>
<td>2,3</td>
</tr>
<tr>
<td>Dr Pepper Cherry</td>
<td>355</td>
<td>39</td>
<td>138</td>
<td>Nil</td>
<td>2</td>
</tr>
<tr>
<td>Illigit</td>
<td>1500</td>
<td>Ingredient</td>
<td>NA</td>
<td>Nil</td>
<td>2</td>
</tr>
<tr>
<td>Lift Plus</td>
<td>249</td>
<td>15</td>
<td>37</td>
<td>Nil</td>
<td>1,2</td>
</tr>
<tr>
<td>Lift Plus Sugar Free</td>
<td>355</td>
<td>14</td>
<td>50</td>
<td>Nil</td>
<td>2</td>
</tr>
<tr>
<td>Mountain Dew</td>
<td>1500</td>
<td>14</td>
<td>35/250ml</td>
<td>Nil</td>
<td>2,3</td>
</tr>
<tr>
<td>Pepsi- Max</td>
<td>1500</td>
<td>12</td>
<td>30/250ml</td>
<td>Nil</td>
<td>1,2,3</td>
</tr>
<tr>
<td>Pepsi Cola</td>
<td>1500</td>
<td>11</td>
<td>27/250ml</td>
<td>Nil</td>
<td>2,3</td>
</tr>
<tr>
<td>Royal Crown Draft Premium Cola</td>
<td>340</td>
<td>Ingredient</td>
<td>NA</td>
<td>Nil</td>
<td>2</td>
</tr>
</tbody>
</table>

1=Energy Fiend, 2009,  
2=Christchurch retail outlets,  
3=Alannah Steeper, Manufactured Foods Database, personal communication, identifies caffeine and guarana as ingredients but not amounts.  
NA = not available
New Zealand data for concentrations of caffeine in caffeinated beverages compared with overseas data

Caffeine levels for ten energy drinks available in Ireland ranged from 50 to 80 mg per retail unit (250ml) consumed (Stimulant Drinks Committee 2002).

The caffeine content of energy drinks in Nordic countries, as supplied by the brewery industry, was 15 or 32 mg/100g (equivalent to mg/100ml), equating to 37.5 or 80 mg/250ml serving (Meltzer et al., 2008).

The caffeine content of energy drinks and energy shots in New Zealand, per retail unit consumed, cover a wider range than those from Ireland and the Nordic countries. This could be because many of the energy drinks and energy shots now available in New Zealand did not exist at the time of the work undertaken in Ireland in 2002.

Caffeinated beverage consumption information

It should be noted that the market for caffeinated beverages is relatively recent but rapidly developing. For this reason existing sources of information on the consumption of these products may not be representative of the current situation.

Frequency of consumption of caffeinated beverages in New Zealand

The Qualitative Food Frequency Questionnaire (QFFQ) administered as part of the 1997 National Nutrition Survey (1997 NNS; covering adults 15 years and older) (Russell et al., 1999) did not ask respondents for information concerning their consumption of caffeinated beverages or foods. Examination of the 24-hour dietary recall records from the 1997 NNS, revealed six instances of consumption of Red Bull, with five of these by the same person, and one instance of consumption of V. This would equate to 3/4636 of respondents (0.06%) consuming caffeinated beverages on any day.

The 2002 National Children’s Nutrition Survey (2002 CNS; children 5-15 years) (Ministry of Health, 2003) asked questions related to consumption of ‘New Age Drinks’. While this food description contains both caffeinated (V, Red Bull) and non-caffeinated (E2, Lift) beverages it can be treated as useful indicative information on potential frequency of consumption of caffeinated beverages. Overall, 7.6% of respondents reported consuming ‘New Age Drinks’ at least once per week. However, only 0.6% reported consuming these foods daily. Approximately 81% of respondents reported never consuming these beverages or consuming them less frequently than once per month. Of the ‘New Age Drinks’ listed the most frequently consumed were E2 (39%), V (23%) and Lift (16%). It should be noted that of these three brands only V is definitely caffeinated. Examination of records from the 24-hour dietary recall study gave consistent results with only 0.3% of respondents reporting consumption of caffeinated beverages in the previous 24-hour period. Non-caffeinated ‘New Age Drinks’ were far more frequently consumed. One respondent reported consuming three servings of a beverage (Burn, no longer on the market) in a 24-hour period. Given that most of the products listed in Table 2 were not reported as consumed, this consumption information is clearly dated and represents a data gap.
Consumption of caffeinated beverages overseas

A 1994 German study of 1265 children aged between 10 and 19 years, indicated that 40% of children aged 10-13 years had tasted stimulant drinks (equivalent to an energy drink or energy shot in this report), with 23% drinking on average one 250 ml can of a stimulant drink per week (Stimulant Drinks Committee, 2002). An Austrian survey in 1999 reported that 45% of respondents 15-50 years had consumed one or more cans of Red Bull during the previous week (Stimulant Drinks Committee, 2002). In an Australian study of 381 children, 27% of males and 12% of females aged between 8 and 13 years old reported having tasted stimulant drinks (O’Dea and Rawstorne, 2000). A study of 1260 individuals, 11-35 year olds, undertaken in Ireland showed that 10% of respondents were regular consumers, with the highest prevalence among those aged 19-24 years (Stimulant Drinks Committee, 2002). The weekly consumption for this group was approximately three cans among consumers, rising to about eight cans among the highest consumers. Similar quantities were consumed in single sessions, suggesting that weekly consumption took place in a single session. This latter finding is important when modelling various consumption scenarios. For the Irish study group, stimulant drinks were frequently consumed with alcohol.

2.4.6 New Zealand baseline dietary exposure estimate for caffeine

New Zealand dietary exposures were estimated for seven population groups using published techniques (Thomson, 2009). Concentration data of foods and beverages containing caffeine (including tea, coffee, chocolate and kola type soft drinks) (Appendix 1) were combined with 24 hour diet recall information from the 1997 NNS and the 2002 CNS using Microsoft Foxpro software. Details of the exposure assessment are provided in Appendix 3.

Most respondents consumed caffeine containing products (Table 4).

Table 4: Respondent numbers and proportion (%) of caffeine consumers for exposure estimates

<table>
<thead>
<tr>
<th></th>
<th>Children 5-12 yrs</th>
<th>Teenagers 13-19 yrs</th>
<th>Young males 19-24 yrs</th>
<th>Adults 20-64 yrs *</th>
<th>Older people 65+ yrs</th>
<th>Females 16-44 yrs *</th>
<th>Females 16-44 yrs pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td># respondents</td>
<td>2579</td>
<td>803</td>
<td>141</td>
<td>3282</td>
<td>774</td>
<td>1388</td>
<td>64</td>
</tr>
<tr>
<td>% respondents consuming caffeine</td>
<td>73</td>
<td>79</td>
<td>78</td>
<td>95</td>
<td>96</td>
<td>92</td>
<td>86</td>
</tr>
</tbody>
</table>

* excluding pregnant women

Exposure estimates for those respondents who consumed caffeine within seven population groups are shown in Table 5.
Table 5: Dietary baseline caffeine exposure estimates for various New Zealand population groups (mg/day).

<table>
<thead>
<tr>
<th></th>
<th>Children 5-12 yrs</th>
<th>Teenagers 13-19 yrs</th>
<th>Young males 19-24 yrs</th>
<th>Adults 20-64 yrs*</th>
<th>Older people 65+ yrs</th>
<th>Females 16-44 yrs*</th>
<th>Females 16-44 yrs pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td>20</td>
<td>82</td>
<td>277</td>
<td>236</td>
<td>156</td>
<td>226</td>
<td>125</td>
</tr>
<tr>
<td><strong>Median</strong></td>
<td>7</td>
<td>41</td>
<td>148</td>
<td>180</td>
<td>140</td>
<td>149</td>
<td>57</td>
</tr>
<tr>
<td><strong>Min</strong></td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td><strong>Max</strong></td>
<td>644</td>
<td>2664</td>
<td>2220</td>
<td>3785</td>
<td>1998</td>
<td>3256</td>
<td>795</td>
</tr>
<tr>
<td><strong>P5</strong></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>22</td>
<td>33</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td><strong>P95</strong></td>
<td>74</td>
<td>294</td>
<td>1080</td>
<td>666</td>
<td>354</td>
<td>623</td>
<td>479</td>
</tr>
</tbody>
</table>

P5 = 5th percentile and P95 = 95th percentiles, representing low and high consumers respectively.
* excluding pregnant women

Since the adverse effect level is expressed in mg/kg bw/day, except for pregnant women, exposure was also calculated in mg/kg bw/day by dividing the exposure (mg/day) by the body weight for each individual (Table 6).

Table 6: Dietary baseline caffeine exposure estimates for caffeine consumers for various New Zealand population groups (mg/kg bw/day)

<table>
<thead>
<tr>
<th></th>
<th>Children 5-12 yrs</th>
<th>Teenagers 13-19 yrs</th>
<th>Young males 19-24 yrs</th>
<th>Adults 20-64 yrs*</th>
<th>Older people 65+ yrs</th>
<th>Females 16-44 yrs*</th>
<th>Females 16-44 yrs pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td>0.6</td>
<td>1.2</td>
<td>3.5</td>
<td>3.5</td>
<td>2.3</td>
<td>3.4</td>
<td>NA</td>
</tr>
<tr>
<td><strong>P95</strong></td>
<td>2.0</td>
<td>4.5</td>
<td>14.4</td>
<td>9.1</td>
<td>5.3</td>
<td>9.6</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA = not available because of changing weight during pregnancy.
P95 = 95th percentile and represents a high consumer.
* excluding pregnant women

The distribution of exposure estimates for each population groups was skewed, with more people having exposures lower than the mean value. This is shown graphically for three population groups (Figures 3-5) with the 95th percentile exposure shown as a reference point.
Figure 3: Distribution of dietary baseline caffeine exposure estimates for children (5-12 yrs)

Figure 4: Distribution of dietary baseline caffeine exposure estimates for teenagers (13-19 yrs)
2.4.7 Overseas estimates of caffeine exposure

Details of estimated caffeine exposures reported in the scientific literature are given in Appendix 4. Average caffeine consumption in the United States and Canada varied from 0.4 to 1 mg/kg bw/day for children, and 1.8 to 3 mg/kg bw/day for adults (Babu et al., 2008; Frary et al., 2005). Average intakes in Argentina were around 1 mg/kg bw/day for children, 3 mg/kg bw/day for teenagers and up to 5 mg/kg bw/day for adults with high consumers estimated to consume up to 13 mg/kg bw/day (Olmos et al., 2009). The average consumption of caffeine in Brazil, across all population groups from 10 to over 60 years was 2.7 mg/kg bw/day (Rojo Carmargo, 1999). In Denmark, average consumption for children and older teenagers (15-19 yrs) was 0.6 and 2.1 mg/kg bw/day respectively. Danish adults consumed, on average about 8 mg/kg bw/day of caffeine with high consumers up to 18 mg/kg bw/day (Barone and Roberts, 1996). Estimates from the UK (1988) were 2 mg/kg bw/day for children and teenagers and about 4.5 mg/kg bw/day for adults, with high consumers exposed to around 8 mg/kg bw/day.

2.4.8 Comparison of New Zealand dietary caffeine exposures with overseas estimates

The New Zealand estimates for average caffeine intake by children are similar to those from the USA (Frary et al., 2005) and Denmark (Barone and Roberts, 1996) but lower than those for Argentina (Olmos et al., 2009) and the UK where mate (a traditional South American drink made from infused dried mate leaves), coffee and tea account for major contributions to
caffeine exposure. For adults, the New Zealand estimates are higher than the more recent assessments from the USA (Frary et al., 2005), similar to those from the UK (Barone and Roberts, 1996), and lower than those for European and South American countries where a coffee culture is more traditional (Barone and Roberts, 1996, Olmos et al., 2009, Rojo Camargo, 1999). The estimate of average caffeine exposure for New Zealand pregnant women (125 mg/day) is in near agreement with the more recent estimate from the UK (159 mg/day) (CARE Study Group, 2008).

2.4.9 Major contributing foods

The major contributing foods for each population group were calculated by summing the caffeine exposure from each food across all consumers in each population group (mg/day) and expressing that sum as a percentage of total caffeine exposure for that population group. The individual foods were grouped into nine broader groups. The relative contributions of these food groups are shown in Table 7.

The 13-15 year and 15-19 year teenagers are presented as two different groups as their consumption information came from two different surveys.

For children (5-12 yrs) and younger teenagers (13-15 yrs), caffeine exposure was mostly from tea and kola type soft drinks with a lesser contribution from coffee. For older teenagers (15-19 yrs) interviewed in the 1997 NNS, and all adults, coffee was the major contributor to caffeine exposure followed by tea. Older people had a greater contribution to caffeine exposure from tea than for other adult population groups but coffee was still the highest contributing food for older people.

Table 7: Percentage contributions of caffeine containing foods to caffeine dietary exposure for different population groups

<table>
<thead>
<tr>
<th>Food</th>
<th>Children 5-12 yrs</th>
<th>Teenagers 13-15 yrs</th>
<th>Teenagers 15-19 yrs</th>
<th>Young males 19-24 yrs</th>
<th>Adults 20-64 yrs</th>
<th>Older people 65+ yrs</th>
<th>Females 16-44 yrs</th>
<th>Females 16-44 yrs pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biscuits, cakes, pastries</td>
<td>11</td>
<td>6</td>
<td>1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Cereal</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Choc desserts</td>
<td>1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Chocolate confectionery</td>
<td>6</td>
<td>4</td>
<td>1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>1</td>
</tr>
<tr>
<td>Cocoa &amp; choc drinks</td>
<td>7</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>&lt;1</td>
<td></td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Coffee</td>
<td>10</td>
<td>23</td>
<td>73</td>
<td>83</td>
<td>75</td>
<td>61</td>
<td>76</td>
<td>57</td>
</tr>
<tr>
<td>Energy drink</td>
<td>2</td>
<td>3</td>
<td>&lt;1</td>
<td>1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Soft drinks</td>
<td>30</td>
<td>32</td>
<td>13</td>
<td>10</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Tea</td>
<td>32</td>
<td>29</td>
<td>10</td>
<td>5</td>
<td>20</td>
<td>38</td>
<td>19</td>
<td>39</td>
</tr>
</tbody>
</table>

Those food groups contributing 10 or more percent to total caffeine exposure are bolded.

* excluding pregnant women
2.4.10 Potential caffeine exposures from ingestion of energy drinks and energy shots, additional to a baseline dietary exposure for children, teenagers and young males

The exposure to additional caffeine from the consumption of energy drinks and energy shots was calculated for children (5-12 yrs), teenagers (13-19 yrs) and young males (19-24 yrs). Scenarios were estimated for one, two, three or four retail units of any of the energy drinks and energy shots identified in Table 2 for which caffeine concentration data were available. Details of the exposure methodology are provided in Appendix 3. Caffeinated alcoholic beverages were not included since these are not legally available for purchase by young people aged less than 18 years.

The distributions of exposures are illustrated in Figures 6-8, for scenarios of one, two, three or four energy drinks or energy shots in addition to baseline dietary caffeine exposure. An adverse effect level of 3 mg/kg bw/day was used as a basis for risk evaluation (see 4.1.1 for details). The area under each curve to the right of the adverse effect line represents the proportion of consumers potentially at risk from adverse effects of caffeine or the probability of a random consumer exceeding the adverse effect level. As expected, there is a much wider range of exposures when four retail units are consumed than for consumption of a single retail unit.

An alternative representation of consumers that are potentially at risk is illustrated by cumulative probability curves (Appendix 5).

![Figure 6: Estimated distribution of exposure for children (5-12 yrs) following the consumption of 1-4 retail units of energy drinks or energy shots.](image-url)
Figure 7: Estimated distribution of exposure for teenagers (13-19 yrs) following the consumption of 1-4 retail units of energy drinks or energy shots.

Figure 8: Estimated distribution of exposure for young males (19-24 yrs) following the consumption of 1-4 retail units of energy drinks or energy shots.
The impact of consumption of energy drinks and/or energy shots on mean and 95th percentile caffeine exposures is tabulated in Table 8.

Table 8: Mean and 95th percentile (P95) daily baseline caffeine exposures (mg/kg bw/day) of New Zealand children, teenagers and young males and potential additional exposures if 1–4 retail units of energy drinks or energy shots are consumed

<table>
<thead>
<tr>
<th></th>
<th>Children 5-12 yrs</th>
<th>Teenagers 13-19 yrs</th>
<th>Young males 19-24 yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>P95</td>
<td>Mean</td>
</tr>
<tr>
<td>Baseline</td>
<td>0.6</td>
<td>2.0</td>
<td>1.2</td>
</tr>
<tr>
<td>Baseline + 1 retail units</td>
<td>4.4</td>
<td>9.1</td>
<td>3.2</td>
</tr>
<tr>
<td>Baseline + 2 retail units</td>
<td>8.3</td>
<td>17.2</td>
<td>5.2</td>
</tr>
<tr>
<td>Baseline + 3 retail units</td>
<td>12.2</td>
<td>25.3</td>
<td>7.2</td>
</tr>
<tr>
<td>Baseline + 4 retail units</td>
<td>16.0</td>
<td>33.6</td>
<td>9.2</td>
</tr>
</tbody>
</table>

The mean baseline dietary exposure of children and teenagers to caffeine is low compared with the contribution from the consumption of energy drinks or energy shots. An individual child (5-12 years), teenager (13-19 years) and young male (19-24 years) would all, on average, exceed the adverse effect level (3 mg/kg bw/day) from a single retail unit of energy drink or energy shot consumed over and above a baseline dietary caffeine exposure.
3 EVALUATION OF ADVERSE HEALTH EFFECTS

3.1 Absorption, Distribution and Pharmacokinetics

Following ingestion, caffeine is rapidly absorbed from the gastrointestinal tract with 99% of ingested caffeine absorbed in humans within 45 minutes after ingestion. Absorption is less complete when caffeine is consumed as coffee (Fredholm et al., 1999). Ingestion of a single cup of coffee provides a dose of 0.4 to 2.5 mg/kg and peak plasma caffeine concentration is reached within 1-1.5 hours of ingestion. Absorbed caffeine is readily distributed throughout the entire body. It passes across the blood-brain barrier, through the placenta into amniotic fluid and the foetus, and into breast milk. Caffeine has also been detected in semen (Nawrot et al., 2003). Saliva concentrations of caffeine reach 65 to 85% of plasma concentrations (Fredholm et al., 1999).

Caffeine is metabolised in the liver. In adults, virtually all caffeine is metabolized to 1-methylxanthine and 1-methyluric acid from a paraxanthine intermediate. Some metabolites, including paraxanthine are pharmacologically active (Nawrot et al., 2003). Caffeine half-lives range from 2.5 to 4.5 hours in humans with no differences in caffeine half-life with age except for newborns, due to their lower cytochrome P-450 activity and the immaturity of some metabolic pathways. In adult males, caffeine half-life is reduced by 30 to 50% (disappears more quickly) in smokers compared with non smokers, whereas it is approximately doubled in women taking oral contraceptives (Fredholm et al., 1999).

3.2 Adverse Effects

Caffeine has a range of pharmacological and psychological effects both beneficial (increased energy, alertness, motivation and concentration) and potentially harmful. The prevalence of caffeine consumption has stimulated both public and scientific interest in potential adverse effects of caffeine and a number of extensive reviews have been published (Nawrot et al., 2003; Smith et al., 2000; Stimulant Drinks Committee, 2002). This section draws on the findings of these reviews with reference to more recent publications where appropriate.

The most important mechanism of action of caffeine is the competitive binding to adenosine receptors, resulting in the release of norepinephrine, dopamine and serotonin in the brain and the increase of circulating catecholamines (Nawrot et al., 2003).

3.2.1 General adverse effects

Ingested caffeine may also irritate the gastrointestinal tract resulting in diarrhoea, nausea and vomiting (Durrant, 2002; Nawrot et al., 2003). Caffeine may reduce bladder control for women (Nawrot et al., 2003).

3.2.2 Central nervous system effects

The most significant effect of caffeine is its role as a potent stimulant of the central nervous system (CNS) although its effects are generally milder and of shorter duration than those of amphetamines (Durrant, 2002). Common adverse effects associated with excessive CNS stimulation from caffeine ingestion include dizziness, rapid heartbeat, irritability, anxiety, tremors and insomnia (Durrant, 2002; Nawrot et al., 2003).
3.2.3 **Cardiovascular effects**

A single high dose of caffeine (4-6 mg/kg/day, equating to 300-400 mg for an average male) can cause tachycardia (abnormally rapid heartbeat) and increased blood pressure (Stimulant Drinks Committee, 2002). The evidence for an association between habitual caffeine intake and cardiovascular disease is less clear (Stimulant Drinks Committee, 2002). Nawrot et al. (2003) concluded that moderate caffeine intake (\(\leq 400\) mg/day) does not adversely affect cardiovascular health but that there was insufficient evidence to draw conclusions about the risk of cardiovascular effects associated with high caffeine consumption (\(\geq 1000\)mg/day). In a recent review of experimental and epidemiological studies, Riksen et al., (2009) postulated that coffee drinking (as a major source of caffeine) may have an acute effect in triggering coronary events, rather than a chronic effect of promoting the development of atherosclerosis in the general population. The risk of high blood pressure associated with coffee consumption varies according to genotype. Individuals with a slow CYP1A2 allele (the key enzyme in caffeine metabolism) are at increased risk of hypertension from coffee whereas individuals with a fast CYP1A2 allele are not (Palatini et al., 2009). Whilst the causal link between genotype and hypertension in this study was clinically based, the association with coffee or caffeine was based on a dietary questionnaire and warrants confirmation. Limited evidence of the prevalence of slow and fast CYP1A2 alleles in the general population was found. In a single study of 229 healthy Chinese, 5% of subjects were poor CYP1A2 metabolizers (Ou-Yang et al., 2000). Assuming “poor” equates to “slow” and a similar prevalence applies to the New Zealand population, 5% of individuals may be at increased risk of acute coronary effects from coffee consumption.

3.2.4 **Mutagenicity, carcinogenicity**

Although caffeine was reported to induce mutations and inhibit DNA repair in a number of microorganisms and cell lines, it is considered unlikely that at normal levels of exposure, caffeine would result in mutagenic effects in humans (Nawrot et al., 2003).

In 1991, the International Agency for Research on Cancer concluded that caffeine was not classifiable as to carcinogenicity of caffeine to humans (Group3) (IARC, 1991).

Caffeine exposure, as measured by coffee consumption, has been associated with cancer development at some, but not all sites. Overall, the evidence indicates that caffeine, as present in coffee, does not cause bowel cancer. The evidence for caffeine as a carcinogen for bladder and pancreatic cancer is inconsistent and not conclusive. At other sites (ovary, stomach, liver) data are insufficient to conclude that caffeine consumption is related to carcinogenesis (Nawrot et al., 2003). More recently Tang et al. (2009) concluded a possible influence of high coffee consumption or increased coffee consumption, on the risk of breast cancer, although the meta analysis is not compelling. Overall, Nawrot et al. (2003) concluded that caffeine is not likely to be a human carcinogen when consumed at \(<500\)mg/day.

3.2.5 **Pregnancy outcomes and reproductive effects**

Caffeine consumption above 200 mg/day was associated with an increased risk of fetal growth restriction (generally measured as low birth weight) with a significant trend for greater reduction in birth weight with higher caffeine intake. This finding held after accounting for maternal age, weight, height, ethnicity, parity, duration of gestation, sex, maternal smoking status and alcohol consumption (CARE Study Group, 2008). Based on the
CARE and other studies, the Committee on Toxicity (COT) concluded that caffeine intake during pregnancy is associated with an increased risk of fetal growth restriction. COT concluded that although there is some uncertainty, it was prudent to assume this relationship was causal. Furthermore, COT concluded that based on current evidence, it is not possible to identify a threshold level of caffeine intake below which there is no elevation of risk, although it seems likely that risk is increased in association with intakes in the order of 200 mg/day and perhaps lower (COT, 2008).

Nawrot et al. (2003) concluded, from epidemiological studies, that consumption of caffeine at doses >300 mg/day may reduce women’s fertility and increase the risk of miscarriage. The COT review also concluded a positive association of caffeine intake with miscarriage, but there are uncertainties relating to possible recall bias and confounding factors (COT, 2008). In addition, COT concluded that data on caffeine consumption during pregnancy and associations with other adverse effects such as pre-term birth and congenital malformations are inconclusive (COT, 2008).

Based on limited data, caffeine consumption >400 mg/day may decrease sperm motility and/or increase the percentage of dead spermatozoa (in heavy smokers) but is unlikely to adversely affect male fertility in general (Nawrot et al., 2003).

3.2.6 Effects on bone and calcium balance

Caffeine intake is associated with a slight deterioration in calcium balance (Nawrot et al., 2003). Caffeine may be a risk factor for bone fracture but results are inconsistent. The association between caffeine exposure and bone metabolism is complicated by other risk factors for osteoporosis (calcium intake, age, smoking and alcohol consumption). Overall, current evidence suggests that caffeine exposures of <400 mg/day do not have significant effects on bone status or calcium balance in individuals ingesting at least 800 mg calcium per day. Since more than 50% of New Zealand women do not achieve an adequate calcium intake (Russell et al., 1999), those women with a caffeine exposure >400 mg/day may be at risk of adverse calcium balance and impaired bone health.

3.2.7 Effects on children

Only a few studies have considered the adverse effects of caffeine on children and these have mostly included only a small number of subjects. Results are variable. Caffeine has been reported to cause nervousness, jitters, stomach aches and nausea in children (Nawrot et al., 2003; Smith et al., 2000; Stimulant Drinks Committee, 2002). More recently, Luebbe and Bell (2009) reported depression, but not increased anxiety in both children (10-12 years) and teenagers (15-17 years) who consumed caffeine (Luebbe and Bell, 2009). No studies have been reported for potential chronic effects of caffeine consumption by children. Given that the human nervous system (including the brain) continues to develop and mature throughout childhood, it is possible that children may be more sensitive to any adverse effects of caffeine than other population groups (Nawrot et al., 2003).

3.2.8 Death

Death due to excessive caffeine ingestion is not common but cases of death due to caffeine intoxication have been reported (Garriott et al., 1985; Kerrigan and Lindsey, 2005), including cases linked to consumption of caffeinated beverages (Cannon et al., 2001). Pre-existing medical conditions (Cannon et al., 2001), consumption after exercise (Kapner, 2008) or
consumption in combination with alcohol (Kapner, 2008) have been reported as contributory factors in caffeine-related fatalities.

3.2.9 Tolerance, dependence and sensitivity

Tolerance, dependence and sensitivity to caffeine is widely assumed to occur but is poorly documented.

Tolerance develops to some caffeine effects but not to others (Fredholm et al., 1999; Meltzer et al., 2008). Tolerance to effects on blood pressure and heart rate develop quickly and virtually completely. The CNS stimulant effects show partial tolerance while there is little tolerance to the effects on sleep. Although Meltzer et al. (2008) cite much variation in tolerances between individuals, it is not clear if this variability relates to the degree, or the time taken, to develop tolerance.

Caffeine meets the criteria for substance dependence defined in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) with some people compelled to continue caffeine use despite efforts to the contrary (Meltzer et al., 2008). However, dependence is not universally accepted (Satel, 2006). The mechanisms of caffeine dependence are not fully understood.

A variety of withdrawal symptoms are described by most regular caffeine consumers who abruptly halt caffeine consumption, with the most frequent symptom being headaches. Other symptoms include drowsiness, depression, anxiety, fatigue, irritability and lack of concentration (Meltzer et al., 2008). The frequency of withdrawal symptoms is highly variable. Between 11 and 100% of caffeine consumers have claimed to experience withdrawal symptoms on stopping caffeine (Dews et al., 2002). Based on limited evidence, Meltzer et al. (2008) reported muscle ache as the predominant withdrawal symptom for 10-12 years olds and headaches to be more common in adolescents. Children consuming high amounts of caffeine were reported to be more angry and unfocused when deprived of caffeine compared with low consumers, implying a dose-response effect for withdrawal symptoms. Withdrawal effects in children can be induced at exposures of 50 mg caffeine (half a cup of coffee) (Dews et al., 2002).

Individuals with compromised liver function, and some individuals who consume caffeine in combination with medications that inhibit caffeine metabolism, are sensitive to caffeine exposure (Smith et al., 2000). No other substantiated sensitivities were identified.

3.3 Dose Response

Further to the description of adverse effects covered in the previous section, the doses at which effects from caffeine are observed are summarized as follows:

Fatalities are usually associated with ingestion of caffeine in excess of 5 g, although recovery after ingestion of 30 g has been reported (Nawrot et al., 2003; Kerrigan and Lindsey, 2005). Caffeinism, or caffeine intoxication, may occur with doses greater than 250 mg and can result in symptoms of anxiety (restlessness, nervousness, facial flushing) and diuresis (increased urine production). In a comprehensive review Nawrot et al. (2003) concluded that for the healthy adult population, moderate daily caffeine intake at a dose up to 400 mg/day was not associated with adverse effects such as general toxicity, cardiovascular effects, effects on bone status and calcium balance (so long as enough calcium is consumed) behavioural changes, cancer and male fertility. The implication is that habitual daily use of more than
500-600 mg (four to seven cups of coffee or seven to nine cups of tea) is a health risk for healthy adults. However, clinical data concerning health effects of persistent, high caffeine intakes is lacking.

Increased anxiety levels in children (8-12 years) at doses of 2.5 mg/kg bw/day and at 3 mg/kg bw/day in 70kg adults have been reported in a limited number of studies (Bernstein et al. 1994; Nickell and Uhde, 1994).

Positive mood effects, such as feelings of increased energy, imagination, efficiency, self-confidence, alertness, motivation and concentration were associated with low doses of caffeine (20-200 mg/day) (Smith et al., 2000; Stimulant Drinks Committee, 2002).

A reduced ability to sleep, for some people, at doses of 100mg (1.4 mg kg bw/day in 70kg adults) at bedtime has been reported (Smith et al., 2000).

A recent longitudinal study of 2635 pregnant women, reported a statistically significant increased risk of foetal growth retardation with caffeine exposures of 200-299 mg/day and above (Care Study Group, 2008). This finding confirmed an earlier conclusion of Nawrot et al. (2003).

3.4 Establishment of Safe Limits

There is currently no recognised reference health standard for caffeine exposure, such as an Acceptable Daily Intake (ADI). A number of assessments have been made overseas and exposure limits adopted for different population groups.

3.4.1 General population

In their review, Nawrot et al. (2003) concluded that for the general population of healthy adults, moderate caffeine exposure of 400 mg/day (5.7 mg/kg bw/day for a 70 kg adult) is not associated with adverse effects such as general toxicity, cardiovascular effects, changes in adult behaviour, increased incidence of cancer and effects on male fertility.

This level of exposure is higher than an adverse effect level of 3 mg/kg bw/day, (210 mg/day for a 70kg adult) based on observations of increased anxiety, suggested by Smith et al. (2000).

3.4.2 Children

Based on limited evidence for altered behaviour, including anxiety (Bernstein et al., 1994), the fact that the nervous system in children is continually developing, and the lack of information on long term effects of caffeine, an upper exposure of 2.5 mg/kg bw/day has been suggested as a cautious toxicological limit on which to base risk assessments for children (Nawrot et al., 2003). Based on these findings, Health Canada recommends a maximum daily caffeine intake of no more than 2.5 mg/kg bw/day for children aged 12 and under (Health Canada, 2007).

Smith et al. (2000) proposed a comparable effect level for children based on the same study as Nawrot et al. (2003), namely that by Bernstein et al. (1994), but rounded this number to 3 mg/kg bw/day.
In a risk assessment of caffeine among children and adolescents in Nordic countries (Meltzer et al., 2008), a no observed adverse effect level (NOAEL) of 0.3 mg/kg bw/day for caffeine tolerance development, and lowest adverse effect levels (LOAEL)s of 1.0-1.25 mg/kg bw/day, 2.5 mg/kg bw/day and 1.4 mg/kg bw/day were identified for tolerance development, anxiety and sleep disturbance respectively.

3.4.3 Reproductive aged women

Reproductive-aged women are a group “at risk” of possible adverse reproductive effects (Nawrot et al., 2003). Health Canada recommends a maximum caffeine exposure of 300 mg, or a little more than two cups of coffee per day (Health Canada, 2010). In November 2008 the UK Food Standards Agency issued new advice on caffeine consumption during pregnancy, advising pregnant women to limit their daily caffeine intake to 200 mg/day (FSA, 2008).

The New Zealand Ministry of Health in its “Food and Nutrition Guidelines for Healthy Pregnant and Breastfeeding Women” advises pregnant and breastfeeding women to limit caffeine consumption to 300mg per day which is roughly equivalent to one large long black, or three cappuccinos, or four cups of plunger coffee, or six cups of instant coffee, or six cups of tea, or 400g of plain chocolate. Breastfeeding women should consider their caffeine intake if the infant is irritable or wakeful. (Ministry of Health, 2006).

3.5 New Zealand reports of Caffeine Poisoning

3.5.1 National Poisons Centre

In the five year period from February 2005 to December 2009, the New Zealand National Poisons Centre received 82 calls relating to caffeine containing substances (Lucy Shieffelbien, National Poisons Centre, University of Otago, personal communication, January 2010). Approximately a quarter (20/82) involved the consumption of energy drinks or energy shots. The remainder related to capsules or tablets, most notably No-Doz which accounted for 44/82 calls. The following summary of caffeine calls is based on self reported information.

Twenty calls related to the consumption of energy drinks (18 calls) or energy shots (2 calls). Of these, the most prevalent product was the energy drink V. Twelve of the individuals were referred for medical treatment on the basis of these calls. Symptoms presented included vomiting, nausea, abdominal pain, jitteriness, racing heart, and agitation.

Four calls related to instant coffee, three of these by children less than two years old.

Caffeine levels are not available for all 20 energy drink or energy shot calls. The minimum caffeine level available that elicited symptoms was 200 mg, or 4.0 mg/kg of caffeine consumed by a 13 year old patient who presented with jitteriness 15 minutes after consuming one Demon Energy Shot. The maximum volume consumed by any patient was 15 x 250 ml cans, equivalent to 11.5 mg/kg of caffeine for this patient, over a one-hour period. This patient reportedly did not sleep, had a steady slow (rather than the more common quickened) heart rate, minor abdominal discomfort and no chest palpitations. A maximum total intake of caffeine of 1622 mg or 35.5 mg/kg was estimated for a 14 year old (40 kg) who reportedly consumed 14 No-Doz, capsules (1400 mg) plus 600 ml of V energy drink (222 mg).
3.5.2 Additional report

A 23-year-old Auckland mother claims to have lost 45 kg in eight months by drinking only Red Bull, 10-14 cans per day, with a handful of dry cereal. The woman who gained weight during pregnancy found Red Bull was an appetite suppressant and as she was losing weight, continued to drink it. The energy drink habit became an addiction and led to a minor heart attack and two weeks in hospital. The woman suffered severe withdrawal symptoms such as sweating, nausea and shaking and has been left with a residual heart murmur, severe pain and cramping in her stomach and bowel, and anxiety attacks (Medical-News, 2009). This was an extreme diet and the symptoms may not have been related to the energy drink per se.
4 EVALUATION OF RISK

4.1 Estimate of Risk for New Zealand

4.1.1 Adverse effect levels applied to New Zealand exposures

In the absence of a recognised reference health standard for caffeine, the estimated exposures from caffeine-containing foods were compared with an adverse effect level of 3 mg/kg bw/day for all population groups except pregnant women for whom an adverse effect level of 200 mg/day was applied. An adverse effect level of 3 mg/kg bw/day for children is consistent with international reviews (Nawrot et al., 2003; Smith et al., 2000). An adverse effect level of 3 mg/kg bw/day for adults is a conservative reference dose based on acute anxiety effects (Nickell and Uhde, 1994) and adopted by Smith et al., (2000) but is less than recommendations by Health Canada (see 5.2.1). A reference dose of 200 mg/day for pregnant women is based on recent evidence of an association between caffeine exposure and foetal growth restriction (Care Study Group, 2008) and is a little more conservative than the earlier conclusions drawn by Nawrot et al., (2003).

4.1.2 Comparison of baseline dietary exposure to caffeine with adverse effect level

The exposure of each individual assessed in the exposure assessment, as described in Section 2.4.6 and summarised in Tables 5 and 6, was ranked. The proportion of consumers exceeding an adverse effect level of 3 mg/kg bw/day, or 200 mg/day for pregnant women, from dietary sources excluding energy drinks or energy shots, was calculated as a percentage of all caffeine consumers and as a percentage of all respondents (including those who did not consume caffeine) (Table 9).

Table 9: Proportion (%) of caffeine consumers and respondents with estimated baseline dietary exposure to caffeine greater than caffeine adverse effect levels for different population groups

<table>
<thead>
<tr>
<th></th>
<th>Children 5-12 yrs</th>
<th>Teenagers 13-19 yrs</th>
<th>Young males 19-24 yrs</th>
<th>Adults 20-64 yrs</th>
<th>Older people 65+ yrs</th>
<th>Females 16-44 yrs</th>
<th>Females 16-44 yrs pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>% consumers &gt;adverse effect level **</td>
<td>2</td>
<td>11</td>
<td>35</td>
<td>37</td>
<td>27</td>
<td>38</td>
<td>25</td>
</tr>
<tr>
<td>% respondents &gt;adverse effect level **</td>
<td>2</td>
<td>8</td>
<td>28</td>
<td>35</td>
<td>26</td>
<td>35</td>
<td>22</td>
</tr>
</tbody>
</table>

* excluding pregnant women
** An adverse effect level of 3.0 mg/kg/day was applied (Smith et al., 2000) for all population groups except for pregnant females where an adverse effect level of 200 mg/day was applied.

4.1.3 Impact of caffeinated beverages on caffeine exposure relative to adverse effect level

Further to Section 2.4.10, the proportion of children (5-12 yrs), teenagers (13-19 yrs) and young males (19-24 yrs) estimated to exceed an adverse effect level of 3 mg/kg bw/day, hence potentially be at risk from adverse caffeine effects, following the consumption of one, two, three or four retail units of either an energy drink or an energy shot is given in Table 10.
Table 10: Proportion (%) of New Zealand children, teenagers and young males with potential caffeine intakes above an adverse effect level of 3mg/kg bw/day if one - four retail units of energy drinks or energy shots are consumed

<table>
<thead>
<tr>
<th></th>
<th>Children 5-12 yrs</th>
<th>Teenagers 13-19 yrs</th>
<th>Young males 19-24 yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>2</td>
<td>11</td>
<td>35</td>
</tr>
<tr>
<td>Baseline + 1 retail unit</td>
<td>68</td>
<td>42</td>
<td>62</td>
</tr>
<tr>
<td>Baseline + 2 retail units</td>
<td>89</td>
<td>77</td>
<td>84</td>
</tr>
<tr>
<td>Baseline + 3 retail units</td>
<td>94</td>
<td>89</td>
<td>92</td>
</tr>
<tr>
<td>Baseline + 4 retail units</td>
<td>95</td>
<td>93</td>
<td>95</td>
</tr>
</tbody>
</table>

At a population level, approximately 70% of children and 40% of teenagers who consume caffeine are estimated to exceed an adverse effect level of 3 mg/kg bw/day after consumption of a single retail unit of an energy drink or energy shot in addition to a baseline dietary exposure.

4.2 Uncertainties and Data Gaps

4.2.1 Uncertainties

There are uncertainties and limitations in the baseline exposure estimates relating to mapping and variability in concentration data that are common to any exposure assessment utilizing mean concentration data and 24 hr diet records (see Appendix 3.1.2). Of particular note, it is now 13 years since the 1997 NNS was undertaken. Consumption choices, amounts and available products are likely to have changed over this period, introducing a level of uncertainty in the baseline dietary exposure assessments.

With respect to food concentration data used in this exposure assessment it was assumed that food concentrations of Australian foods purchased in 2002 are the same as for New Zealand foods. This is probably reasonable but the range of products now available will be different. Given that tea, coffee, kola type soft drinks and chocolate flavoured bakery goods are major contributing foods to baseline caffeine exposure, the concentration values used for these foods is particularly important.

The energy drink and energy shot market is dynamic with online purchasing available. It is possible that additional products are currently available in New Zealand that were not captured in this report.

Information on the relative market share for different energy drinks and energy shots was not factored into the exposure scenarios presented. However the assumption used in the scenarios that they are equally likely to be chosen for consumption may not be correct.

The absence of information on current consumption practices necessitated assumptions of no product bias if multiple retail units were consumed, that a full retail unit was always
consumed and that baseline caffeine preferences did not influence consumption of energy drinks or energy shots. There is an element of uncertainty in these assumptions.

Caffeine from alcoholic beverages and Ready to Drink (TD) products containing kola type soft drinks were not included in the exposure scenarios, since alcohol sales are illegal for persons under 18 and alcohol consumption is not encouraged in persons under 18. Neither is the frequency and prevalence of consumption of these products known. Thus the caffeine estimates for individuals consuming either caffeinated alcoholic drinks or RTDs containing kola type soft drinks are underestimated. Since three of the caffeinated alcoholic beverages claimed to have caffeine levels similar to energy drinks, these products are likely to be a significant source of caffeine to those who drink them.

4.2.2 Data Gaps

The priority area of uncertainty in assessing the proportion of the New Zealand population at risk of adverse effects from the consumption of caffeinated beverages currently available in New Zealand is due to the lack of information on current consumption patterns of energy drinks and energy shots in New Zealand. Information on the frequency of consumption, the number of products that may be consumed at any one drinking event and whether energy drinks or energy shots are consumed with alcohol or not, is all lacking. Therefore it is not possible to determine with precision whether any sub-groups of the New Zealand population are at risk currently of adverse effects from caffeine. The scenario simulations indicate that the consumption of one energy drink or energy shot may exceed the adverse effect level of 3 mg/kg bw/day. Limited overseas evidence suggests that perhaps 20% of people under the age of 19 may consume one energy drink or energy shot per week and high consumers may drink up to 8 retail units in a session (Stimulant Drinks Committee, 2002). Limited, and uncorroborated, New Zealand reports indicate some individuals consume up to 15 retail units per session (see sections 3.5.1 and 3.5.2 above).

The contribution of energy drinks and energy shots to caffeine exposure is based on claimed concentrations. There is no independent laboratory data on actual caffeine levels in these products and this represents a data gap.

Current adverse effect levels for children are based on single studies of acute anxiety effects. There is a lack of data concerning health effects of frequent, high caffeine intakes. Further evidence of adverse effects, both acute and chronic, for children and adults would strengthen the evidence and confirm the appropriateness of the adverse effect levels.
5 AVAILABILITY OF CONTROL MEASURES

5.1 Existing Control Measures in New Zealand

5.1.1 Regulatory

Levels of naturally occurring caffeine in coffee, tea and chocolate products are not regulated, and the level of caffeine from these sources is not required on nutrition information panels (NIPs).

Caffeine added to food and beverages is regulated in Standard 1.3.1 ‘Food Additives’ and Standard 2.6.4 ‘Formulated Caffeinated Beverages’ of the Australia New Zealand Food Standards Code. The maximum amount of caffeine permitted in kola type soft drinks is 145 mg/L (mg/kg) (Standard 1.3.1, Schedule 1 of the Australia New Zealand Food Standards Code). A formulated caffeinated beverage must contain no less than 145 mg/L and no more than 320 mg/L total caffeine, regardless of the source of caffeine (Standard 2.6.4 of the Australia New Zealand Food Standards Code). Standard 2.6.4 also prescribes that the quantity of caffeine and advisory statements to the effect that the food contains caffeine and is not recommended for children, pregnant or lactating women, and individuals sensitive to caffeine be included on the label.

Prior to 31 March 2010, energy shots marketed as Dietary Supplements had to include caffeine in the ingredient list, but did not need to specify the amount of caffeine, nor were advisory statements required (New Zealand Legislation, 2007). From 31 March 2010, food type dietary supplements such as energy shots are regulated by the New Zealand Food (Supplemented Food) Standard 2010 (NZFSA, 2010). This new standard requires caffeine to be listed in the ingredient list. If the supplemented food contains added caffeine greater than 145mg/L, the level of caffeine, and the advisory statements that apply to formulated caffeinated beverages regulated under Standard 2.6.4 of the Australia New Zealand Food Standards Code, must be included on the label.

If *Paullinia cupana* or guarana is added as a food ingredient in New Zealand foods the label must include an advisory statement to the effect that the product contains caffeine (Standard 1.2.3 of the Australia New Zealand Food Standards Code). The label on the package of a supplemented food containing guarana must include an advisory statement to the effect that the supplemented food contains caffeine (NZFSA, 2010).

Whilst the concentration of caffeine in kola type soft drinks and formulated caffeinated beverages is regulated, there are no regulations on the volume (pack size) of retail units (hence dose of caffeine consumed per retail unit).

5.1.2 Advisory

The following advisory statement to pregnant women is published on the NZFSA website: “Drinks containing caffeine, including coffee, teas and colas, should be limited during pregnancy. You may want to consider giving up caffeine altogether. Have no more than six cups of tea or instant coffee, or three espresso-style coffees daily. Energy drinks and ‘smart drinks’ are not recommended as they may contain high levels of caffeine and other ingredients not recommended for pregnant and breastfeeding women” (NZFSA, 2009).
The following advisory to pregnant women is issued by the New Zealand Ministry of Health to pregnant women:
“Limit drinks containing caffeine, such as coffee, tea and cola drinks. Have no more than six cups of tea or instant coffee (or three ‘single’ espresso-type coffees or one ‘double’ espresso-type coffee) each day” (Ministry of Health, 2008).

5.2 Existing Control Measures Overseas

5.2.1 Canada

Health Canada recommends:
“For children age 12 and under, Health Canada recommends a maximum daily caffeine intake of no more than 2.5 milligrams per kilogram of body weight. Based on average body weights of children, this means a daily caffeine intake of no more than:
45 mg for children aged 4 - 6;
62.5 mg for children aged 7 - 9; and
85 mg for children aged 10 - 12.
Those recommended maximums are equivalent to about one to two 12-oz (355 ml) cans of cola a day.

Health Canada has not developed definitive advice for adolescents 13 and older because of insufficient data. Nonetheless, Health Canada suggests that daily caffeine intake for this age group is no more than 2.5 mg/kg body weight.”

“For women of childbearing age, the recommendation is a maximum daily caffeine intake of no more than 300 mg, or a little over two 8-oz (237 ml) cups of coffee.

For the rest of the general population of healthy adults, Health Canada advises a daily intake of no more than 400 mg” (Health Canada, 2010).

5.2.2 United Kingdom

In November 2008, the UK FSA issued new advice to pregnant women to limit caffeine consumption to 200 mg/day. The Agency had previously recommended a maximum daily intake of 300 mg but reduced this limit on the basis of new evidence, including, but not only, the CARE Study (Care Study Group, 2008; FSA, 2008).

5.2.3 USA

In a review of caffeine undertaken in 1978, the US Select Committee on GRAS Substances (SCOGS) concluded that “While no evidence in the available information on caffeine demonstrates a hazard to the public when it is used in cola type beverages at levels that are now current and in the manner now practiced, uncertainties exist requiring that additional studies be conducted. It is inappropriate to include caffeine among the substances generally recognized as safe (GRAS)” (FDA, 1978). Thus they conclude caffeine is GRAS for cola type beverages but not generally.

The US Food and Drug Administration (FDA) has listed caffeine as GRAS as an ingredient in cola-type beverages in concentrations of no greater than 200 parts per million (mg/kg or 20 mg/100ml). In November, 2009, the FDA announced plans to look into the safety of caffeinated alcoholic beverages (FDA, 2009).
5.2.4 European Food Safety Authority (EFSA)

The EU Directive 2002/67/EC on the labelling of foodstuffs containing quinine, and of foodstuffs containing caffeine, requires that beverages, other than those based on coffee or tea, containing more than 150 mg/l (15 mg/100ml) caffeine should be labelled “high caffeine content” and the exact amount present indicated on the label. It also required that where caffeine is used as flavouring in a foodstuff it must be listed in the ingredient list. (EC, 2002).

Caffeine was evaluated by the European Commission Scientific Committee for Food in relation to its presence in “energy” drinks in 1999 and 2003. The 1999 opinion was upheld in 2003, “For caffeine, it was concluded that the contribution of “energy” drinks to overall caffeine intake was not a matter of concern for non-pregnant adults. For children who do not normally consume much tea or coffee, and who might substitute “energy” drinks for cola or other soft drinks, consumption of “energy” drinks might represent an increase in daily caffeine exposure compared with their previous intake. The Committee considered that this could result in transient behavioural changes, such as increased arousal, irritability, nervousness or anxiety. For pregnant adults, the Committee concluded that while intakes of caffeine up to 300 mg/day appeared to be safe, the question of possible effects on pregnancy and the offspring at regular intakes above 300 mg/day remained open. This suggested that moderation of caffeine intake, from whatever source, was advisable during pregnancy” (SCF, 2003).

5.2.5 Finnish Food Safety Authority (Evira)

The Finnish Food Safety Authority, Evira, considers the moderate consumption of caffeine is unlikely to present any health risks for most people but that the stimulatory effect of caffeine is very individual lasting from a few to many hours depending on the dose and the person’s metabolism.

Evira considers that labelling according to Directive 2002/67/EC, that a drink that contains more than 150 mg/l (15 mg/100ml) of caffeine from any source must be labelled “high caffeine content”, is not sufficient to protect the most sensitive consumers. Evira requires that drinks, confectionery, chewing gums, chocolate bars and other similar products and food supplements containing added caffeine must also be labeled “Not recommended for children, pregnant women or people sensitive to caffeine”. The drinks and confectionery must also precisely declare the maximum amount to be used daily, for example, “a maximum of two cans per day” (Evira, 2010).

5.3 Control Options

Caffeine exposure could be controlled further by regulation and/or risk communication.

Options could include regulating the permissible volume of a caffeinated beverage, thus limiting the dose per retail unit consumed. No international precedent for this option was noted.

The maximum permissible concentration of caffeine in caffeinated beverages could be lowered.
Advisory information with respect to an amount of caffeine or amounts of caffeine containing foods, for different population groups, represents an option for risk communication. Aside from product labelling requirements current advisory statements in New Zealand are limited to pregnant women.
6 REFERENCES


APPENDIX 1: Concentration of Caffeine In New Zealand Foods (NZ Food Composition, 8th Edition, 2009)
<table>
<thead>
<tr>
<th>Food</th>
<th>Caffeine concentration (mg/100g)</th>
<th>Serve/ unit (g)</th>
<th>Caffeine concentration mg/serve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bakery products</td>
<td></td>
<td></td>
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<tr>
<td>Biscuit, `Afghan'</td>
<td>4</td>
<td>1 biscuit/ 17</td>
<td>1</td>
</tr>
<tr>
<td>Biscuit, chocolate base, 'Digestive/Wheat'</td>
<td>8</td>
<td>1 biscuit/ 10.5</td>
<td>1</td>
</tr>
<tr>
<td>Biscuit, chocolate coated</td>
<td>8</td>
<td>1 biscuit/ 10.5</td>
<td>1</td>
</tr>
<tr>
<td>Biscuit, chocolate coated, 'Mallowpuff'</td>
<td>8</td>
<td>1 biscuit/ 21.5</td>
<td>2</td>
</tr>
<tr>
<td>Biscuit, chocolate coated, 'Toffee Pop'</td>
<td>8</td>
<td>1 biscuit/ 17</td>
<td>1</td>
</tr>
<tr>
<td>Biscuit, cookie, chocolate chip</td>
<td>8</td>
<td>1 biscuit/ 13</td>
<td>1</td>
</tr>
<tr>
<td>Biscuit, peanut brownie, commercial</td>
<td>4</td>
<td>1 biscuit/ 12</td>
<td>1</td>
</tr>
<tr>
<td>Cake, chocolate, standard</td>
<td>3</td>
<td>1/8 cake/ 79</td>
<td>3</td>
</tr>
<tr>
<td>Cake, chocolate, butter icing</td>
<td>3</td>
<td>1/8 cake/ 79</td>
<td>3</td>
</tr>
<tr>
<td>Cookies, `Cookie Time', original</td>
<td>12</td>
<td>1 biscuit/ 92</td>
<td>11</td>
</tr>
<tr>
<td>Lamington, chocolate</td>
<td>3</td>
<td>140</td>
<td>5</td>
</tr>
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<td>Muffin, chocolate</td>
<td>3</td>
<td>60</td>
<td>2</td>
</tr>
<tr>
<td>Eclairs w/ choc. icing &amp; cream filling</td>
<td>2</td>
<td>1 éclair/ 42</td>
<td>1</td>
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<td>Beverages, non alcoholic</td>
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<tr>
<td>Lucozade</td>
<td>0</td>
<td>1 cup/ 264</td>
<td>0</td>
</tr>
<tr>
<td>Milo, made with trim milk</td>
<td>T</td>
<td>1 cup/ 280</td>
<td>1</td>
</tr>
<tr>
<td>Milo, made with water</td>
<td>T</td>
<td>1 cup/ 255</td>
<td>1</td>
</tr>
<tr>
<td>Milo, powder</td>
<td>10</td>
<td>1 tsp/ 2.5</td>
<td>T</td>
</tr>
<tr>
<td>Nesquik, powder</td>
<td>10</td>
<td>1 tsp/ 2.5</td>
<td>T</td>
</tr>
<tr>
<td>Chocolate, drinking, powder</td>
<td>51</td>
<td>1 tsp/ 2.5</td>
<td>1</td>
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<td>Cocoa, powder</td>
<td>227</td>
<td>1 tsp</td>
<td>4</td>
</tr>
<tr>
<td>Coffee, cafe latte, caffeinated</td>
<td>99</td>
<td>1 cup/ 272</td>
<td>269</td>
</tr>
<tr>
<td>Coffee, espresso brewed</td>
<td>212</td>
<td>1 cup/ 272</td>
<td>577</td>
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<tr>
<td>Coffee, decaffeinated</td>
<td>160</td>
<td>1 tsp/ 1.8</td>
<td>3</td>
</tr>
<tr>
<td>Coffee, instant</td>
<td>3700</td>
<td>1 tsp/ 1.5</td>
<td>56</td>
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<tr>
<td>Fruit Drink, 'V Drink'</td>
<td>28</td>
<td>1 can/ 250</td>
<td>72</td>
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<td>Soft drink, 'Cola-Diet'</td>
<td>14</td>
<td>1 can/ 350</td>
<td>49</td>
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<tr>
<td>Soft drink, 'Coca-Cola'</td>
<td>9</td>
<td>1 can/ 355</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 bottle/ 500</td>
<td>46</td>
</tr>
<tr>
<td>Tea, Indian, infused</td>
<td>22</td>
<td>1 cup/ 251</td>
<td>55</td>
</tr>
<tr>
<td>Chocolate confectionery</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Bounty bar, Cadbury</td>
<td>16</td>
<td>1 bar</td>
<td>8</td>
</tr>
<tr>
<td>Crunchie bar, Cadbury</td>
<td>16</td>
<td>1 bar</td>
<td>8</td>
</tr>
<tr>
<td>Kit Kat, Nestle</td>
<td>16</td>
<td>1 finger/ 12</td>
<td>2</td>
</tr>
<tr>
<td>Mars bar</td>
<td>16</td>
<td>1 bar/ 60</td>
<td>10</td>
</tr>
<tr>
<td>Pinky bar, Cadbury</td>
<td>16</td>
<td>1 bar/ 45</td>
<td>7</td>
</tr>
<tr>
<td>Chocolate, Cadbury, 'Moro',bar</td>
<td>16</td>
<td>1 bar/ 70</td>
<td>11</td>
</tr>
<tr>
<td>Chocolate, candy coated, 'Pebbles' etc</td>
<td>16</td>
<td>10 pieces/ 9</td>
<td>1</td>
</tr>
<tr>
<td>Chocolate, dark</td>
<td>59</td>
<td>1 cup, grated/ 88</td>
<td>52</td>
</tr>
<tr>
<td>Chocolate, white</td>
<td>5</td>
<td>1 cup, grated/ 88</td>
<td>4</td>
</tr>
<tr>
<td>Chocolate bar, milk</td>
<td>20</td>
<td>1 large bar/ 120</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 small bar/ 50</td>
<td>10</td>
</tr>
<tr>
<td>Chocolate bar, with peanuts</td>
<td>16</td>
<td>1 slab/ 52</td>
<td>8</td>
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<tr>
<td>Food</td>
<td>Caffeine concentration (mg/100g)</td>
<td>Serve/ unit (g)</td>
<td>Caffeine concentration mg/serve</td>
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<tr>
<td>-------------------------------------</td>
<td>----------------------------------</td>
<td>-----------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>Chocolates, fancy and filled</td>
<td>16</td>
<td>1 chocolate/ 5</td>
<td>1</td>
</tr>
<tr>
<td><strong>Chocolate flavoured products</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chocolate coated Ice cream, 'Jelly Tip’</td>
<td>2</td>
<td>1 cup/ 200</td>
<td>4</td>
</tr>
<tr>
<td>Dessert, dairy food, chocolate flavour</td>
<td>2</td>
<td>1 cup/ 246</td>
<td>5</td>
</tr>
<tr>
<td>Muesli bar, chocolate chip</td>
<td>2</td>
<td>1 bar/ 32</td>
<td>1</td>
</tr>
<tr>
<td>Muesli bar, chocolate coated</td>
<td>2</td>
<td>1 bar/ 33</td>
<td>1</td>
</tr>
<tr>
<td>Nutella</td>
<td>11</td>
<td>1 tbsp/ 14</td>
<td>2</td>
</tr>
<tr>
<td>Pudding, self-saucing, chocolate, baked</td>
<td>6</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td>Sauce, chocolate</td>
<td>10</td>
<td>1 tbsp/ 15</td>
<td>1</td>
</tr>
<tr>
<td>Sundae, chocolate, McDonald's</td>
<td>1</td>
<td>1 sundae/ 198</td>
<td>2</td>
</tr>
<tr>
<td>Coco Pops, Kellogg's</td>
<td>5</td>
<td>1 cup/ 40</td>
<td>2</td>
</tr>
</tbody>
</table>

T = trace
## APPENDIX 2: Overseas concentration of caffeine in coffee, tea and chocolate and food products containing coffee or chocolate

<table>
<thead>
<tr>
<th>Product</th>
<th>Average (range) caffeine concentration (mg/100ml or mg/100g)</th>
<th>Country</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Coffee</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Espresso</td>
<td>74.3 (58.1-92.5) mg/shot 78 (46-159) mg/100ml 106 (25-214) 62 per serving</td>
<td>USA</td>
<td>(McCusker <em>et al.</em>, 2003)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Argentina</td>
<td>(Olmos <em>et al.</em>, 2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Australia</td>
<td>(Desbrow <em>et al.</em>, 2007)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Various</td>
<td>(Mandel, 2002)</td>
</tr>
<tr>
<td>Brewed/Perhted</td>
<td>57 (43-83) 39.7 (31.2-54.8) 74-112 per serving 36 49 (7-118)</td>
<td>Various</td>
<td>(Barone and Roberts, 1996)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Brazil</td>
<td>(Rojo Camargo, 1999)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>USA</td>
<td>(McCusker <em>et al.</em>, 2003)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Various</td>
<td>(Mandel, 2002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>USA/Canada</td>
<td>(Knight <em>et al.</em>, 2006)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>UK</td>
<td>(FSA, 2004)</td>
</tr>
<tr>
<td>Instant</td>
<td>40 (62-82) 41 (21-44) 60-71 per serving 32 23 (9-51)</td>
<td>Various</td>
<td>(Barone and Roberts, 1996)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Brazil</td>
<td>(Rojo Camargo, 1999)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Argentina</td>
<td>(Olmos <em>et al.</em>, 2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Various</td>
<td>(Mandel, 2002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>USA/Canada</td>
<td>(Knight <em>et al.</em>, 2006)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>UK</td>
<td>(FSA, 2004)</td>
</tr>
<tr>
<td>Decaffeinated</td>
<td>2 (0-2.9) 1-4 per serving 2 0.5-5</td>
<td>Various</td>
<td>(Barone and Roberts, 1996)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>USA</td>
<td>(McCusker <em>et al.</em>, 2006)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Various</td>
<td>(Mandel, 2002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>USA/Canada</td>
<td>(Knight <em>et al.</em>, 2006)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>UK</td>
<td>(FSA, 2004)</td>
</tr>
<tr>
<td><strong>Tea</strong></td>
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<td></td>
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</tr>
<tr>
<td>Black (teabag, or not specified)</td>
<td>23 (21-24) 47.3 ± 0.1 27-40 per serving 5-28 12 (8-17) 20 13 17 (0.4-39) 8-30 per serving 27(22-33)</td>
<td>Brazil</td>
<td>(Rojo Camargo, 1999)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Taiwan</td>
<td>(Mandel, 2002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Various</td>
<td>(Pena <em>et al.</em>, 2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Portugal</td>
<td>(Olmos <em>et al.</em>, 2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Argentina</td>
<td>(Knight <em>et al.</em>, 2006)</td>
</tr>
<tr>
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<td></td>
<td>USA/Canada</td>
<td>(Knight <em>et al.</em>, 2006)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>UK</td>
<td>(FSA, 2004)</td>
</tr>
<tr>
<td>Green Tea (leaves)</td>
<td>1</td>
<td>Various</td>
<td>(Mandel, 2002)</td>
</tr>
<tr>
<td>Decaffeinated</td>
<td></td>
<td>Argentina</td>
<td>(Olmos <em>et al.</em>, 2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>UK</td>
<td>(FSA, 2004)</td>
</tr>
<tr>
<td><strong>Chocolate</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Dark chocolate</td>
<td>125 43 (33-73)</td>
<td>Various</td>
<td>(Mandel, 2002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Brazil</td>
<td>(Rojo Camargo, 1999)</td>
</tr>
<tr>
<td>Milk chocolate</td>
<td>21</td>
<td>Various</td>
<td>(Mandel, 2002)</td>
</tr>
<tr>
<td>White chocolate</td>
<td>23 (13-40)</td>
<td>Brazil</td>
<td>(Rojo Camargo, 1999)</td>
</tr>
<tr>
<td>Product</td>
<td>Average (range) caffeine concentration (mg/100ml or mg/100g)</td>
<td>Country</td>
<td>Reference</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>-------------------------------------------------------------</td>
<td>--------------------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>Cocoa, hot chocolate</td>
<td>31, 9 (4-15) 2.1</td>
<td>Various</td>
<td>(Barone and Roberts, 1996)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Brazil</td>
<td>(Rojo Camargo, 1999)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>USA/Canada</td>
<td>(Knight et al., 2006)</td>
</tr>
<tr>
<td>Chocolate milk</td>
<td>3 3.4 (3.0-4.2)</td>
<td>Various</td>
<td>(Barone and Roberts, 1996)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Argentina</td>
<td>(Olmos et al., 2009)</td>
</tr>
<tr>
<td>Chocolate candy</td>
<td>3-13</td>
<td>Various</td>
<td>(Barone and Roberts, 1996)</td>
</tr>
<tr>
<td><strong>Caffeinated beverages</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Energy drinks</td>
<td>14.5-&gt;42 32 26 (13-31) 42 (2-217) 21.5 (11-32) 28 (17-36) 15 32 15 32</td>
<td>Australia</td>
<td>(NSW Food Authority, 2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Various</td>
<td>(Mandel, 2002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>USA</td>
<td>(McCusker et al., 2006)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Portugal</td>
<td>(Pena et al., 2005)</td>
</tr>
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<td></td>
<td>Saudi Arabia</td>
<td>(Abourashed and Mossa, 2004)</td>
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<td></td>
<td></td>
<td>Argentina</td>
<td>(Olmos et al., 2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Denmark</td>
<td>(Meltzer et al., 2008)</td>
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<td>Finland</td>
<td>(Meltzer et al., 2008)</td>
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<td></td>
<td></td>
<td>Iceland</td>
<td>(Meltzer et al., 2008)</td>
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<td>Norway</td>
<td>(Meltzer et al., 2008)</td>
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<tr>
<td></td>
<td></td>
<td>Sweden</td>
<td>(Meltzer et al., 2008)</td>
</tr>
<tr>
<td>Carbonated sodas</td>
<td>11.6 (10.7-12.9) 9 (5-13) 8-17 10.4 (1.4-20.8) 11 (9-12) 11.5-15.3</td>
<td>Various</td>
<td>(Mandel, 2002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>USA</td>
<td>(McCusker et al., 2006)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Portugal</td>
<td>(Pena et al., 2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>USA</td>
<td>(Chou and Bell, 2007)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Argentina</td>
<td>(Olmos et al., 2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>USA/Canada</td>
<td>(Knight et al., 2006)</td>
</tr>
<tr>
<td>Water (cafeinated)</td>
<td>120</td>
<td>USA/Canada</td>
<td>(Knight et al., 2006)</td>
</tr>
<tr>
<td><strong>Other</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Cocoa Puffs</td>
<td>7.7</td>
<td>USA</td>
<td>(Caudle et al., 2001)</td>
</tr>
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<td>Chocolate fudge poptarts</td>
<td>5.4</td>
<td>USA</td>
<td>(Caudle et al., 2001)</td>
</tr>
<tr>
<td>Chocolate instant pudding</td>
<td>12.1</td>
<td>USA</td>
<td>(Caudle et al., 2001)</td>
</tr>
<tr>
<td>Chocolate biscuits</td>
<td>12</td>
<td>USA</td>
<td>(Caudle et al., 2001)</td>
</tr>
</tbody>
</table>

1 Standard values based on data 1975-1993
2 concentration in chocolate drink,
3 per 13g serving of powder
APPENDIX 3: New Zealand Exposure Estimate to Caffeine

A3.1 Baseline Exposure from Caffeine Containing Foods and Beverages

A3.1.1 Methodology

Concentration data of foods containing naturally occurring caffeine (Appendix 1) were combined with 24 hour diet recall information from the 1997 NNS and the 2002 CNS using Microsoft FoxPro software.

Concentration values for caffeine in 49 New Zealand foods were used (Appendix 1) with three changes to concentration values for coffee to better map to products as itemised in the 1997 NNS and 2002 CNS with reference to more recent overseas data (Appendix 2). Thus, coffee was mapped to:

- coffee, cafe latte, caffeinated (99 mg/100ml), (Appendix 1)
- coffee, instant (3700 mg/100g of dry powder) (Appendix 1) and coffee, decaffeinated (2mg/100ml) (Appendix 2).
- coffee, brewed (53 mg/100ml) (Appendix 2),
- coffee, espresso (80 mg/100ml), (Appendix 2)

Chocolate milk was included at a concentration of 3 mg/100ml based on overseas data and a concentration value of 17 mg/100ml was included for Red Bull (Table 2). Where a trace was noted in Appendix 1 (milo products) this was assigned a concentration value of 0.5 mg/100ml to represent a low, but non zero level of caffeine.

Exposures were estimated for seven population groups, namely, children (5-12 yrs), teenagers (13-19 yrs), young males (19-24 yrs), adults (20 to 64 yrs), older people (over 65 years old), non pregnant females of childbearing age (16 to 44 yrs) and pregnant females (16-44 yrs). The number of respondents for these population groups ranged from 64 to 3282 individuals (Table 4). Children (5-12 yrs) were assessed because of concern over the effects of caffeine on children and availability of consumption information. Teenagers (13-19 yrs) were assessed as the target group for energy drinks and energy shots. Young males (19-24 yrs) were included as a group representing high consumers and for consistency with the New Zealand Total Diet Survey population groups. An exposure assessment of women of childbearing age, both those who identified themselves as pregnant and not pregnant, was included because of concerns of caffeine exposure on pregnancy outcomes. Adults and older people were also included as separate groups due to a higher perceived level of consumption of coffee and tea by these population groups.

Each food descriptor identified by each consumer in the previous 24 hour period (approximately 6000 in total) was mapped to one of 52 foods for which a caffeine concentration was available. All other foods were mapped to “other” and assigned a caffeine concentration of 0 mg/100g. The modelling software multiplies the specified concentration of caffeine by the amount of food that an individual consumed in any one serving in order to estimate the exposure from each food item. Once completed for all foods, the total amount of caffeine consumed from all foods is summed for each individual. All individual’s exposures were then ranked and population statistics (mean, median, high and low percentiles) were derived.
The exposure estimates were for individual respondents and were not statistically weighted to the New Zealand population. Exposure estimates were based on food consumption on a single day, given that effects are based on acute and not habitual exposures.

A3.1.2 Limitations of the exposure assessment

Whilst the methodology described provides a realistic estimate of the actual caffeine exposure for the selected sub populations, there are recognized limitations. Not all caffeine containing foods will have been included in the assessment. Whilst every effort was made to include the likely major contributors, for which caffeine concentration data are available, it was not practical to include the complete array of foods that exists and therefore exposures will be underestimated for some consumers. The 52 foods included in the study were mapped to a very much wider range of foods described in the 1997 NNS/2002 CNS (approximately 6000) requiring assumptions that mapped foods have similar caffeine concentrations to the analysed foods. There is a measure of uncertainty around these assumptions. The variability of caffeine in coffee and tea is problematic for an exposure and risk assessment as clearly, the method of preparation will influence the level of exposure. The 24-hour dietary recall records from the 1997 NNS and 2002 CNS are a snapshot of consumption on any one day and will not necessarily represent the typical diet for the individual respondent. The 1997 NNS data is now dated and will likely differ from current consumption practices. These arguments also apply to comparative studies from overseas.

A3.2 Estimated exposure from Additional Caffeine from the Consumption of Energy Drinks or Energy Shots

Given that energy drinks and energy shots are targeted to young people, and based on overseas consumption intelligence, exposure to additional caffeine from the consumption of these products was calculated for children (5-12 yrs), teenagers (13-19 yrs) and young males (19-24 yrs). Scenarios were defined for one, two, three or four servings of any of the 42 energy drinks or energy shots identified in Table 2 for which caffeine concentration values were available. Two products that listed caffeine as an ingredient but did not state a quantity were not included. Caffeinated alcoholic beverages were not included since these are not legally available for purchase by young people aged less than 18 years.

A3.2.1 Methodology

Exposure estimates were simulated using @Risk software and a Monte Carlo approach for the selected population groups. Each iteration of the simulation randomly selected a base caffeine exposure (mg/day), the body weight of the respondent with that exposure (kg), and a retail unit of any one caffeinated beverage (mg) and used this combination to calculate an overall caffeine exposure adjusted for body weight (mg/kg bw/day). Simulations were run for 20,000 iterations for each scenario (one, two, three or four caffeinated drinks). An advantage of a Monte Carlo approach over point estimates is that it allows inclusion of all respondents and all food products, reducing bias towards any particular product.

In the absence of detail regarding market share and current consumption practices of caffeinated energy drinks or energy shots in New Zealand, the following assumptions were made:

- Only consumers with a background caffeine exposure were included since consumers who do not currently consume caffeine are assumed to be unlikely to seek caffeine from caffeinated beverages.
• There is no selection bias to particular products and all 42 products (energy drinks and energy shots) were equally weighted.
• Energy drinks and energy shots were assumed to be alternatives. That is, a consumer would choose either an energy drink, or an energy shot.
• If a person consumed more than one serving of caffeinated beverage, all servings were assumed to be of the same product. There is no data to inform this assumption, but is plausible if it is assumed that consumers are likely to display some brand loyalty.
• All individuals are equally likely to consume these products irrespective of base caffeine intake (i.e. those with high or low base caffeine exposures are equally likely to consume caffeinated beverages).
• A serving equalled a full retail unit.
### APPENDIX 4: Overseas Estimates Of Caffeine Exposure

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Population group</th>
<th>Caffeine exposure (mg/kg/day)</th>
<th>Major Contributors (In descending order of importance)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argentina</td>
<td>2005</td>
<td>2-10 years</td>
<td>Mean (95th percentile)</td>
<td>Soft drinks, chocolate milk</td>
<td>(Olmos et al., 2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11-15 years</td>
<td>1.3 (3.2)</td>
<td>Mate, coffee, soft drinks</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>16-20 years</td>
<td>2.3 (6.6)</td>
<td>Mate, coffee, soft drinks</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>21-30 years</td>
<td>4.1 (12.7)</td>
<td>Mate, coffee, soft drinks</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>31-40 years</td>
<td>3.8 (7.7)</td>
<td>Mate, coffee, soft drinks</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>41-50 years</td>
<td>4.8 (10.5)</td>
<td>Mate, coffee</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>51-60 years</td>
<td>5.1 (9.6)</td>
<td>Mate, coffee</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>60+ years</td>
<td>4.2 (9.6)</td>
<td>Mate, coffee</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean (90th percentile)</td>
<td>4.2 (9.6)</td>
<td>Mate, coffee</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Overall</td>
<td>3.7 (8.4)</td>
<td>Coffee, mate</td>
<td></td>
</tr>
</tbody>
</table>

| Brazil | 1993     | 10-19 years      | Mean mg/day                  | Coffee, tea, soft drinks                             | (Rojo Camargo, 1999)       |
|        |          | 20-29 years      | 150                          | Coffee, tea                                          |                            |
|        |          | 30-39 years      | 189                          | Coffee, tea                                          |                            |
|        |          | 40-49 years      | 192                          | Coffee, tea                                          |                            |
|        |          | 50-59 years      | 178                          | Coffee, tea                                          |                            |
|        |          | 60+ years        | 195                          | Coffee, tea, soft drinks chocolate,                   |                            |
|        |          | Overall          | 2.74 mg/kg/day               | Coffee, tea                                          |                            |

| Denmark | 1991 (7 day average) | 1-5 years | Mean (90th percentile) | 0.3 (0.7) | Tea | (Barone and Roberts, 1996) |
|         |                      | 6-9 years |                      | 0.6 (2.5) | Tea |
|         |                      | 10-14 years |                  | 0.7 (1.5) | Coffee, tea |
|         |                      | 15-19 years |                  | 2.1 (5.5) | Coffee, tea |
|         |                      | 20-24 years |                  | 4.8 (12.3) | Coffee, tea |
|         |                      | 25-34 years |                  | 7.9 (15.7) | Coffee, tea |
|         |                      | 35-49 years |                  | 9.9 (17.6) | Coffee, tea |
|         |                      | 50-64 years |                  | 8.9 (17.1) | Coffee, tea |
|         |                      | 65+ years |                  | 7.8 (15.0) | Coffee, tea |
|         |                      | Pregnant |                  | 5.8 (12.1) | Coffee, tea |

| UK      | 1988 (7 day average) | 1-5 years | Mean (90th percentile) | 2.29 (6.86) | Instant coffee, tea | (Barone and Roberts, 1996) |
|         |                      | 6-9 years |                  | 1.77 (4.23) | Brewed coffee, tea |                           |

*Risk Profile: Caffeine in energy drinks and energy shots*
<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Population group</th>
<th>Caffeine exposure (mg/kg/day)</th>
<th>Major Contributors (In descending order of importance)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>UK</td>
<td>2003</td>
<td>Pregnant women</td>
<td>Mean mg/day mg/kg/day*</td>
<td>Tea (62%), coffee (14%), cola drinks (12%), chocolate (8%), soft drinks</td>
<td>(Care Study Group, 2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Before pregnancy 238 3.5</td>
<td>Tea, brewed coffee, instant coffee</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5-12 weeks 139 2.1</td>
<td>Tea, brewed coffee, instant coffee</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Third trimester 153 2.3</td>
<td>Tea, brewed coffee, instant coffee</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Throughout pregnancy 159 2.4</td>
<td>Tea, brewed coffee, instant coffee</td>
<td></td>
</tr>
<tr>
<td>USA</td>
<td>1975 (14 day average)</td>
<td>0-11 months</td>
<td>Mean (90th percentile) 0.47 (0.51)</td>
<td>Tea</td>
<td>(Barone and Roberts, 1996)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1-5 years</td>
<td>1.21 (3.14)</td>
<td>Tea, coffee, soft drinks</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6-11 years</td>
<td>0.86 (2.04)</td>
<td>Tea, coffee, soft drinks</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>12-17 years</td>
<td>0.75 (1.79)</td>
<td>Tea, coffee, soft drinks</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>18+ years</td>
<td>2.64 (5.19)</td>
<td>Coffee, tea</td>
<td></td>
</tr>
<tr>
<td>USA</td>
<td>1989 (14 day average)</td>
<td>0-11 months</td>
<td>Mean (90th percentile) 0.32 (0.68)</td>
<td>Coffee, tea</td>
<td>(Barone and Roberts, 1996)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1-5 years</td>
<td>0.95 (2.10)</td>
<td>Coffee, tea, soft drinks</td>
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<tr>
<td></td>
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<td>6-11 years</td>
<td>0.67 (1.40)</td>
<td>Coffee, tea, soft drinks</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>12-17 years</td>
<td>0.65 (1.40)</td>
<td>Coffee, tea, soft drinks</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>18-24 years</td>
<td>1.10 (2.80)</td>
<td>Coffee, tea, soft drinks</td>
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<tr>
<td></td>
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<td>25+ years</td>
<td>2.40 (5.20)</td>
<td>Coffee, tea, soft drinks</td>
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<tr>
<td></td>
<td></td>
<td>Pregnant/nursing</td>
<td>0.91 (2.50)</td>
<td>Coffee, tea, soft drinks</td>
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</tr>
<tr>
<td>USA</td>
<td>1987-1988 (3 day average)</td>
<td>1-5 year</td>
<td>Mean (90th percentile) 1.33 (2.79)</td>
<td>Coffee, tea, soft drinks</td>
<td>(Barone and Roberts, 1996)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6-9 years</td>
<td>1.10 (2.38)</td>
<td>Coffee, tea, soft drinks</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>10-14 years</td>
<td>1.08 (2.03)</td>
<td>Coffee, tea, soft drinks</td>
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<td></td>
<td></td>
<td>15-19 years</td>
<td>0.98 (1.82)</td>
<td>Coffee, tea, soft drinks</td>
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<tr>
<td></td>
<td></td>
<td>20-24 years</td>
<td>1.79 (3.99)</td>
<td>Coffee, tea, soft drinks</td>
<td></td>
</tr>
</tbody>
</table>

Risk Profile: Caffeine in energy drinks and energy shots
<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Population group</th>
<th>Caffeine exposure (mg/kg/day)</th>
<th>Major Contributors (In descending order of importance)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>1994-1996, 1998 (2 day average)</td>
<td>25-34 years</td>
<td>3.13 (7.51)</td>
<td>Coffee, tea, soft drinks</td>
<td>(Frary et al., 2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35-49 years</td>
<td>3.69 (8.16)</td>
<td>Coffee, tea, soft drinks</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>50-64 years</td>
<td>3.81 (8.11)</td>
<td>Coffee, tea, soft drinks</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>65+ years</td>
<td>3.05 (6.69)</td>
<td>Coffee, tea, soft drinks</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pregnant women</td>
<td>1.47 (3.33)</td>
<td>Coffee, tea, soft drinks</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nursing women</td>
<td>2.63 (5.74)</td>
<td>Coffee, tea, soft drinks</td>
<td></td>
</tr>
<tr>
<td>USA</td>
<td>1994-1996, 1998 (2 day average)</td>
<td>2-5 years</td>
<td>0.4</td>
<td>Soft drinks, tea</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6-11 years</td>
<td>0.4</td>
<td>Soft drinks, tea</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>12-17 years Female</td>
<td>0.5</td>
<td>Soft drinks, tea</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>12-17 years Male</td>
<td>0.6</td>
<td>Soft drinks, tea</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>18-34 years Female</td>
<td>1.2</td>
<td>Coffee, soft drinks, tea</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>18-34 years Male</td>
<td>1.1</td>
<td>Coffee, soft drinks, tea</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>35-54 years Female</td>
<td>1.7</td>
<td>Coffee&gt;&gt;soft drinks, tea</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>35-54 years Male</td>
<td>1.8</td>
<td>Coffee&gt;&gt;soft drinks, tea</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>55-64 years Female</td>
<td>1.5</td>
<td>Coffee</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>55-64 years Male</td>
<td>1.8</td>
<td>Coffee</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>65+ years Female</td>
<td>1.3</td>
<td>Coffee&gt;&gt;tea</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>65+ years Male</td>
<td>1.3</td>
<td>Coffee</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pregnant women</td>
<td>0.8</td>
<td>Coffee, soft drinks, tea</td>
<td></td>
</tr>
</tbody>
</table>

Assuming average 67 kg body weight

Risk Profile: Caffeine in energy drinks and energy shots
APPENDIX 5: Cumulative probability curves of total caffeine exposure for children, teenagers and young males consuming 1-4 energy drinks or energy shots in addition to baseline dietary exposure.

An adverse effect level of 3 mg/kg bw/day is shown on each graph as a reference point. The portion of each curve to the right of the adverse effect level represents the proportion of the population group potentially at risk from adverse effects of caffeine. The exposure of any percentile may be read off the x-axis by extrapolating from the intersection of the selected percentile on the y-axis with the curve of one, two, three or four retail units consumed, where cumulative probability = 0.2 represents the 20th percentile, 0.4 = 40th percentile etc.

Figure 9: Cumulative probability curve of children (5-12 yrs) consuming 1-4 retail units of energy drinks or energy shots
Figure 10: Cumulative probability curve of teenagers (13-19 yrs) consuming 1-4 retail units of energy drinks or energy shots

Figure 11: Cumulative probability curve of young males (19-24 yrs) consuming 1-4 retail units of energy drinks or energy shots
Bibliography of Additional Readings

**Adolescents and Young Adults**


**Cardiovascular**


electrocardiographic, and bacterial contamination effects. Clinical Pharmacology and Therapeutics 93(3):267-274.


**Children**


Central Nervous System


Elderly

Arciero, P. J., and M. J. Ormsbee. 2009. Relationship of blood pressure, behavioral mood state, and physical activity following caffeine ingestion in younger and older women. Applied
Physiology, Nutrition, & Metabolism = Physiologie Appliquee, Nutrition et Metabolisme
34(4):754-762.

Fetal Development


**Pregnant Women**


Documents Submitted to Committee


