Scanning for New Evidence on Riboflavin to Support a Dietary Reference Intake Review – January 4, 2021

Riboflavin Status Across the Lifespan II

Blood Pressure, Hypertension, and Cognitive Function

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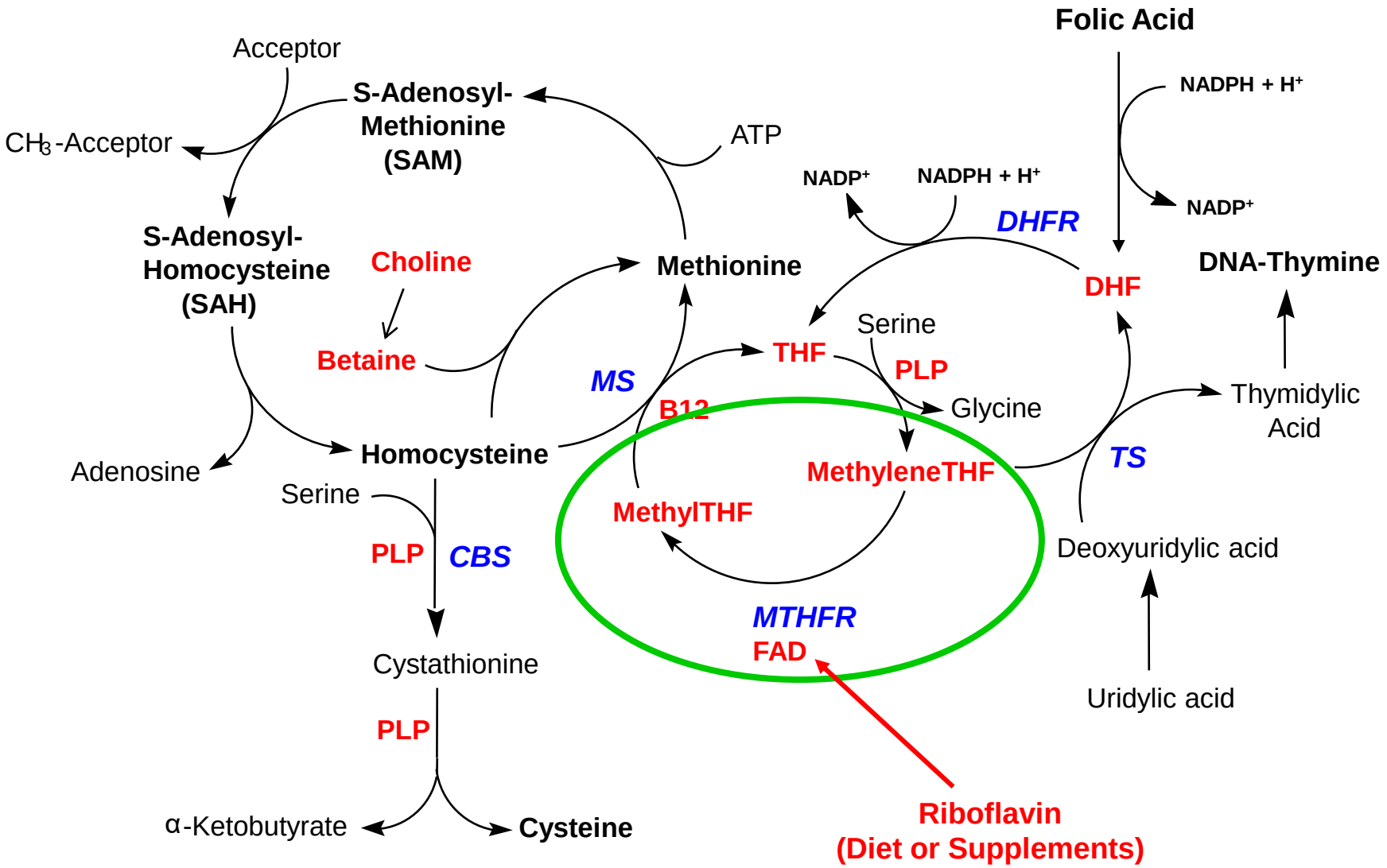
MTHFR and One-Carbon Metabolism



Characteristics of the *MTHFR* C677T Polymorphism

- Alanine --> Valine
- Thermolabile (activity reduced by exposure to heat)
- Reduced affinity for CH_2=THF and FAD
- Prevalence of TT form:
  - 10-15% in general population
  - Lower in Africans
  - Higher in Hispanics

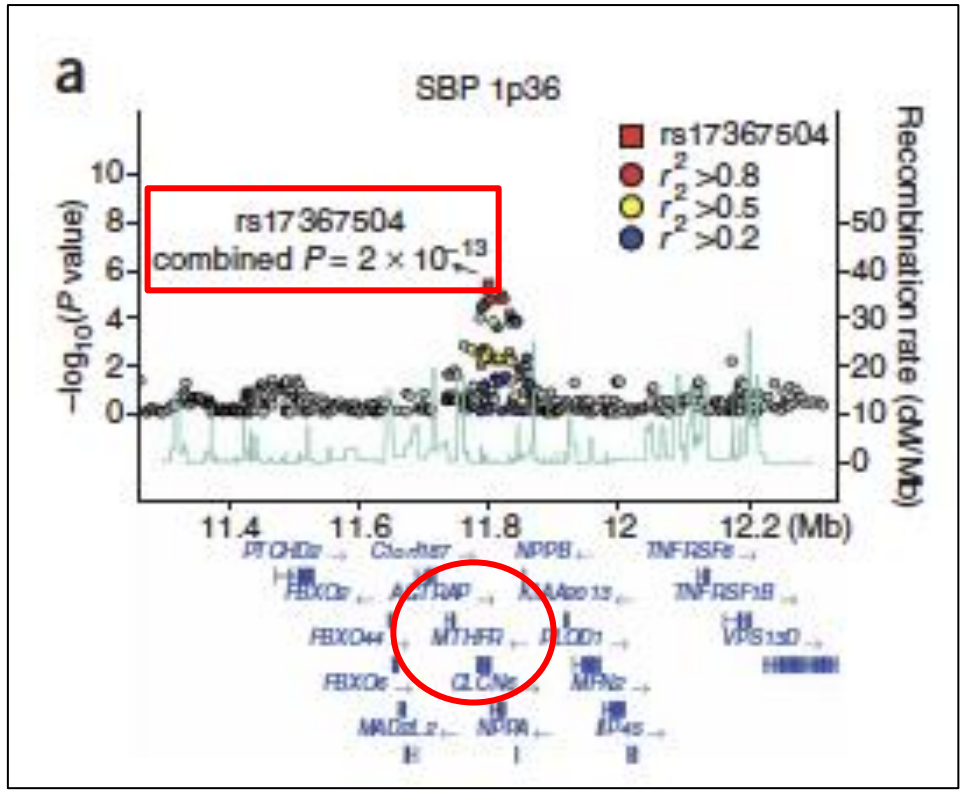
- Associated with …
  - Low folate status
  - Elevated homocysteine

MTHFR C677T and Disease Risk

Representative Meta-Analyses

Condition	Odds Ratio Associated with 677T Allele	Reference
Neural Tube Defects	1.72 (1.36, 2.17)	Yang et al, 2015
Congenital Heart Defects	1.35 (1.11, 1.64)	Zhang et al, 2018
Premature Coronary Heart Disease	1.17 (1.01, 1.34)	Hou et al, 2015
Ischemic Stroke	1.29 (1.18, 1.42)	Song et al, 2016
Acute Lymphoblastic Leukemia (ALL)	0.89 (0.80, 0.99)	Zhang et al, 2017
Colorectal Cancer	0.96 (0.94, 0.99)	Xu et al, 2017
Breast Cancer	1.17 (1.04, 1.30)	He and Shen, 2017
Ovarian Cancer	1.17 (1.05, 1.29)	He and Shen, 2017

MTHFR Genotype and Blood Pressure
Genome-Wide Association Study (GWAS)



Regional Association Plot

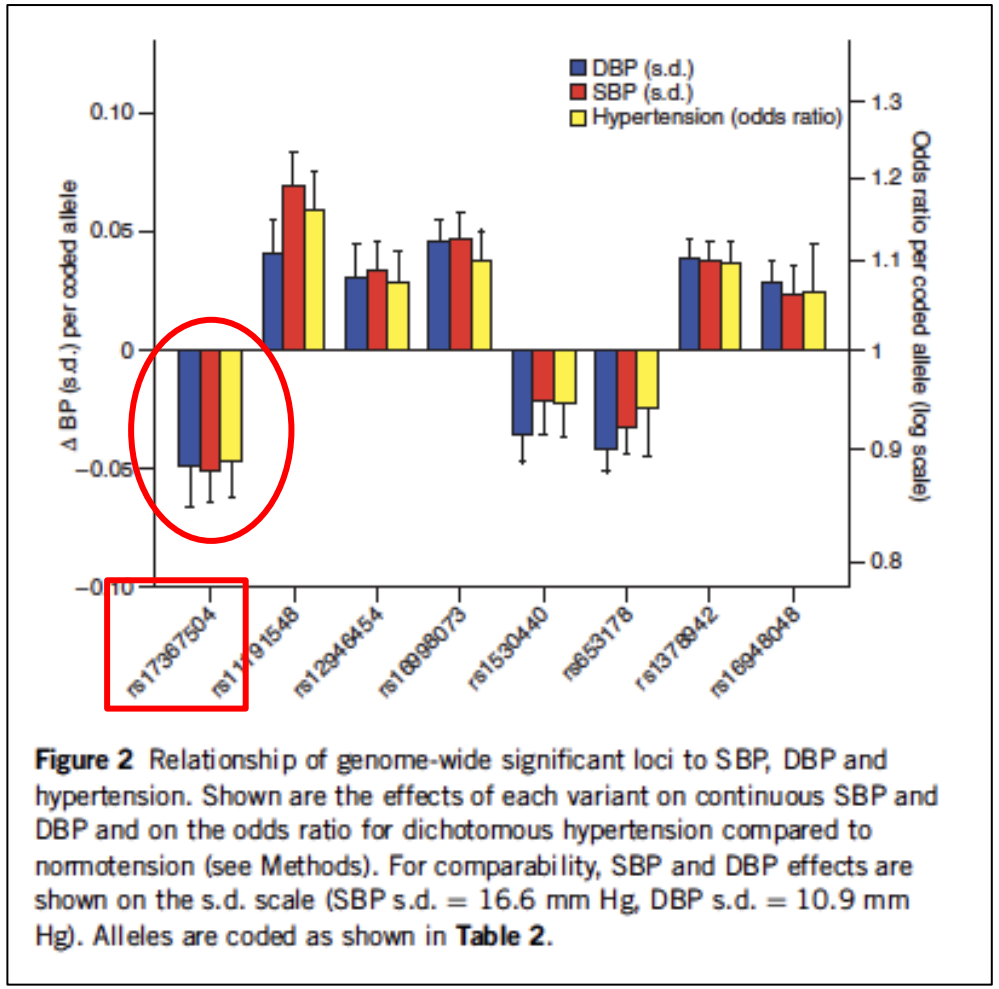


Figure 2 Relationship of genome-wide significant loci to SBP, DBP and hypertension. Shown are the effects of each variant on continuous SBP and DBP and on the odds ratio for dichotomous hypertension compared to normotension (see Methods). For comparability, SBP and DBP effects are shown on the s.d. scale (SBP s.d. = 16.6 mm Hg, DBP s.d. = 10.9 mm Hg). Alleles are coded as shown in **Table 2**.

Newton-Cheh et al, Nature Genetics, 2009

MTHFR 677TT Genotype, CVD and BP

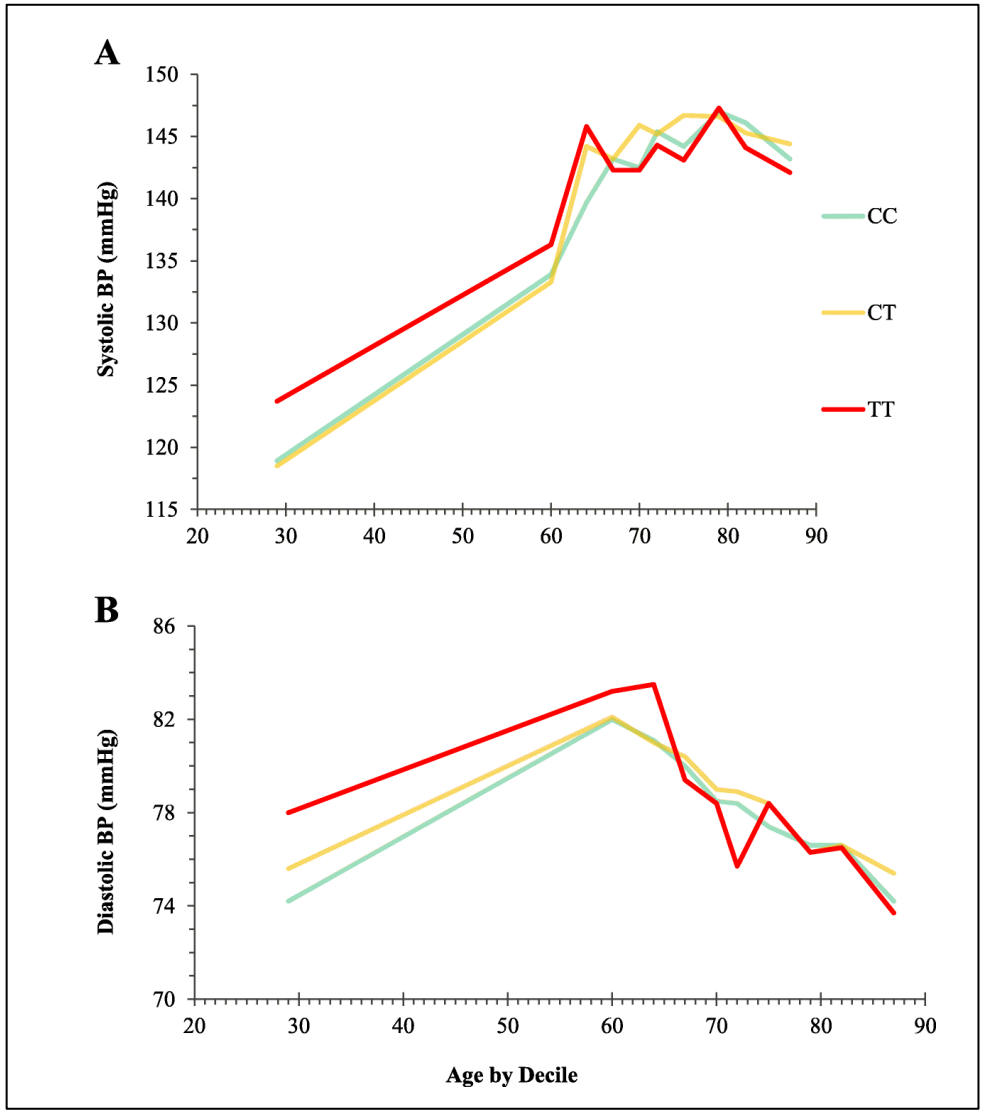
Author	n	Cohort	OR (95% CI)	Outcome
Klerk et al. 2002	23,920	CHD patients	**1.16** (1.05, 1.28)	↑ HD
Wald et al. 2002	16,849	CVD patients	**1.42** (1.11, 1.84) **1.65** (0.66 to 4.13)	↑ HD ↑ Stroke
Holmes et al. 2011	59,995	Stroke patients	**1.37** (1.25, 1.50)	↑ Stroke
Qian et al. 2007	2814 cases 3099 controls	Hypertensive patients	**1.24** (1.02, 1.50)	↑ BP
Niu et al. 2012	1520 cases 1334 controls	Hypertensive patients	**1.87** (1.31, 2.68)	↑ BP
Yang et al. 2014	5418 cases 4997 controls	Hypertensive patients	**1.59** (1.32, 1.92)	↑ BP
Wu et al. 2014	5207 cases 5383 controls	Hypertensive patients	**1.62** (1.32, 1.99)	↑ BP

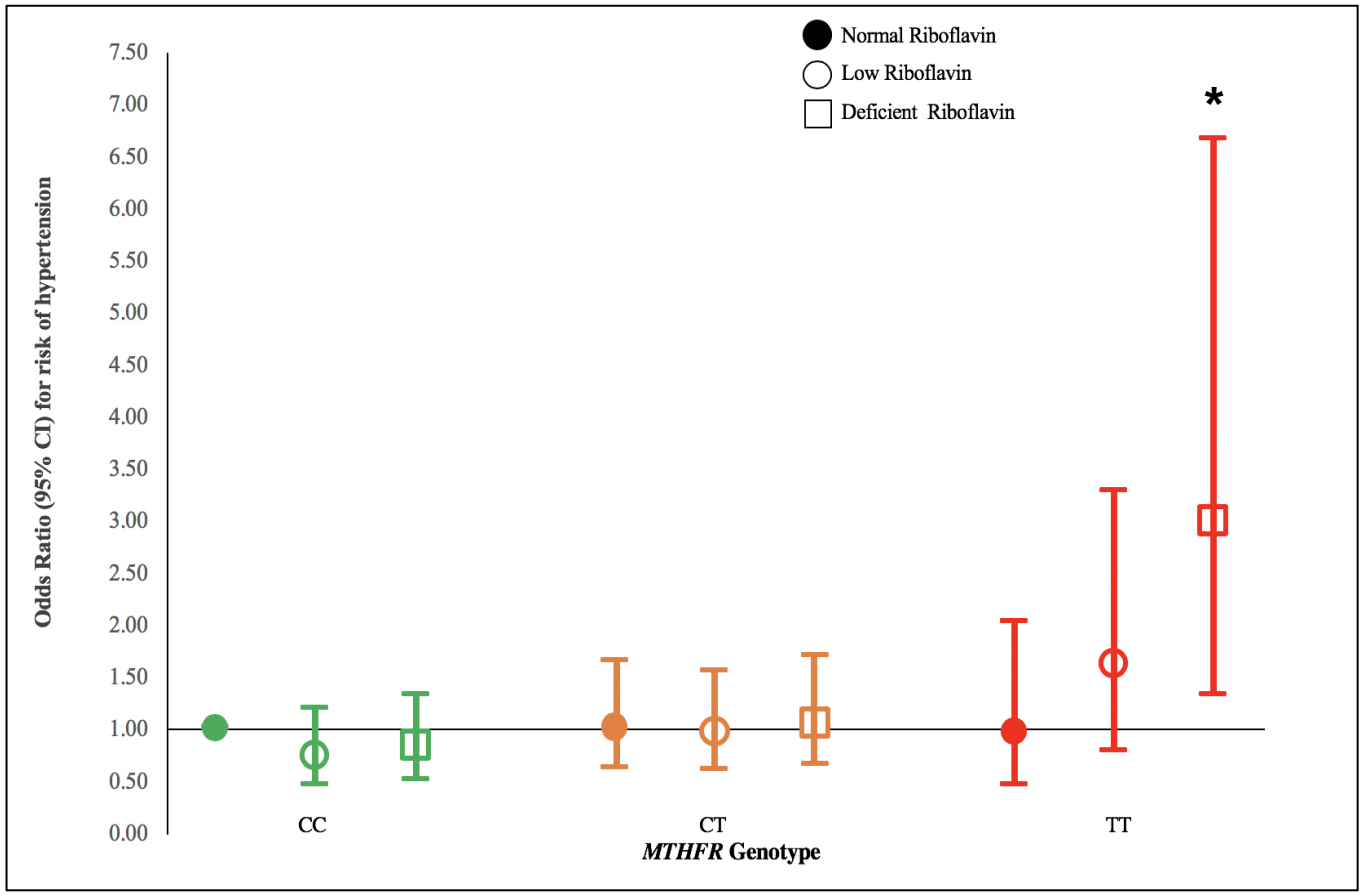
McNulty et al, Molecular Aspects of Medicine, 2017

Table courtesy of Helene McNulty

Effect of MTHFR Genotype on BP by Age

Effect Modification by Riboflavin Status



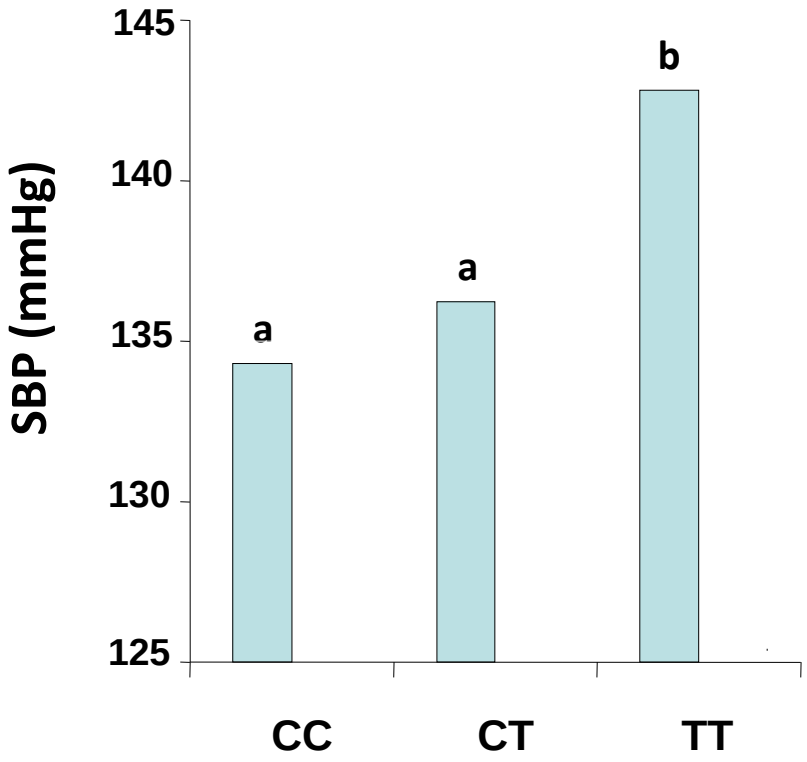


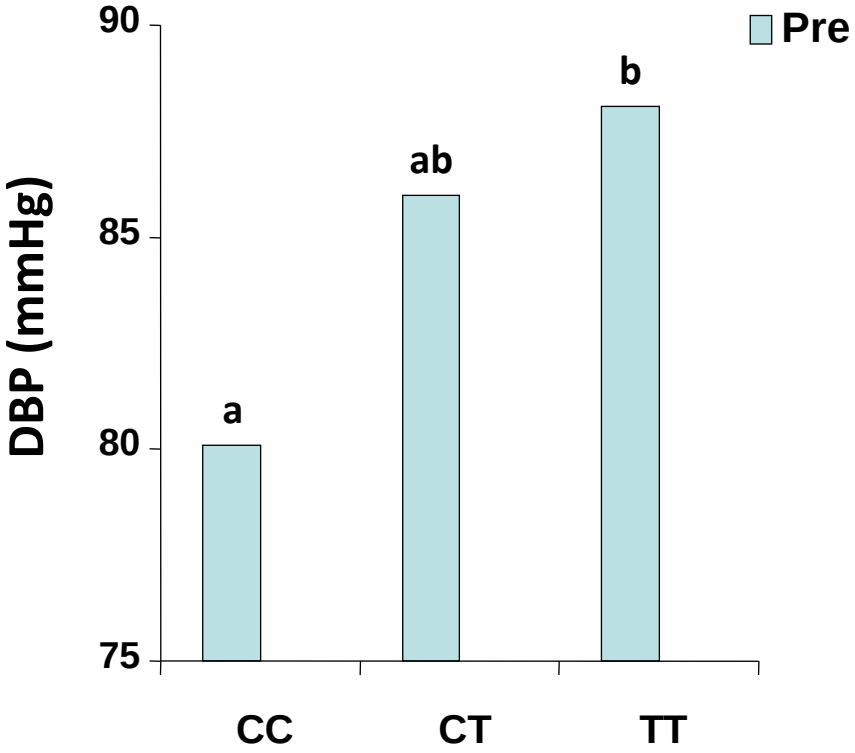
***OR 3.00 (1.34, 6.68), p = 0.007**

Ward et al, BMC Medicine, 2020

Figures and data courtesy of Helene McNulty

Blood Pressure in CVD Patients by *MTHFR* Genotype

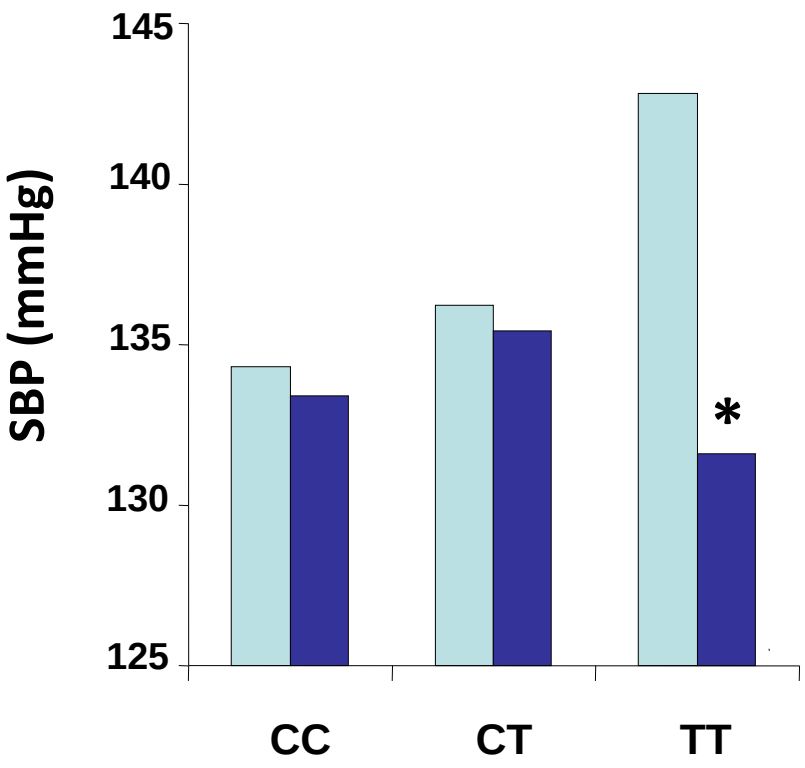


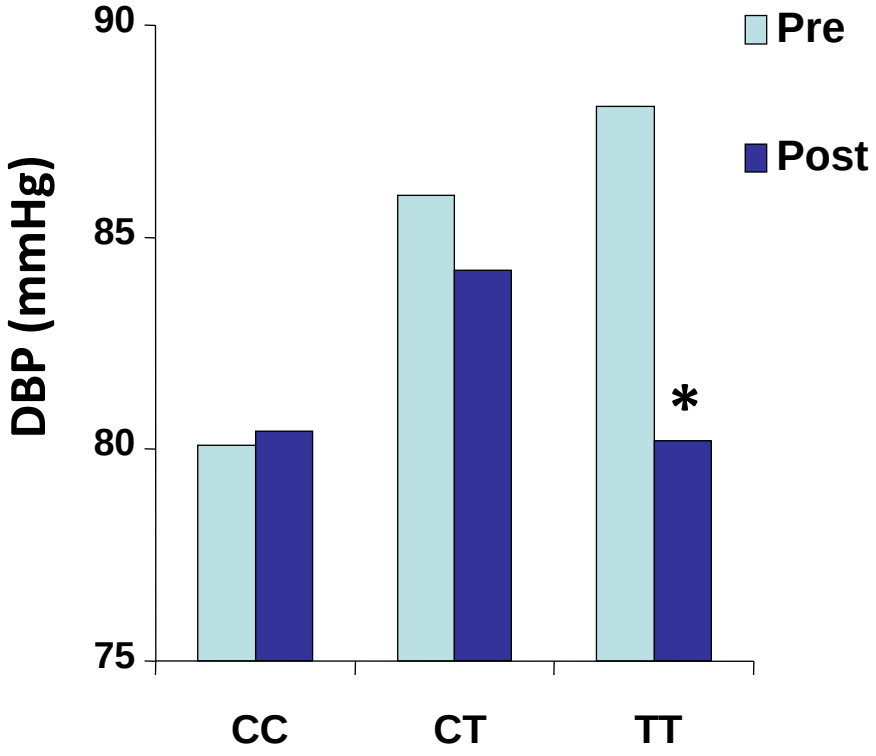


Horigan et al, Journal of Hypertension, 2010

Slide courtesy of Helene McNulty

Riboflavin Has a Novel Role in Treating Hypertension Specifically in the *MTHFR* TT genotype





Horigan et al, Journal of Hypertension, 2010

Slide courtesy of Helene McNulty

Lowering Blood Pressure with Riboflavin

Additional RCTs

The American Journal of CLINICAL NUTRITION

Riboflavin offers a targeted strategy for managing hypertension in patients with the *MTHFR* 677TT genotype: a 4-y follow-up[1–3]

Carol P Wilson, Mary Ward, Helene McNulty, J J Strain, Tom G Trouton, Geraldine Horigan, John Purvis, and John M Scott

Am J Clin Nutr 2012;95(3):766-72.

Hypertension

Journal of the American Heart Association

American Heart Association

Blood Pressure in Treated Hypertensive Individuals With the *MTHFR* 677TT Genotype Is Responsive to Intervention With Riboflavin : Findings of a Targeted Randomized Trial

Carol P. Wilson, Helene McNulty, Mary Ward, J.J. Strain, Tom G. Trouton, Birgit A. Hoeft, Peter Weber, Franz F. Roos, Geraldine Horigan, Liadhan McAnena and John M. Scott

Hypertension 2013;61:1302-8.

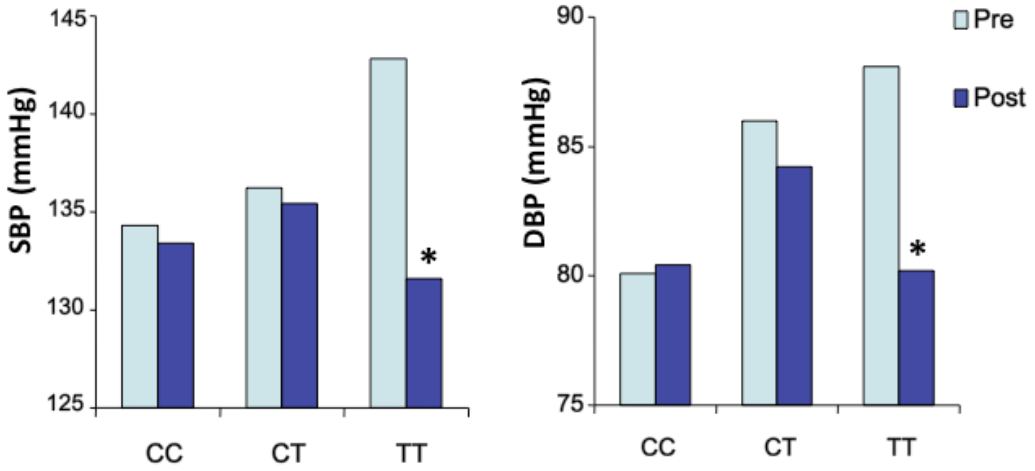
Wilson (2013) showed in hypertensive patients without overt CVD:

- Blood pressure in <u>treated</u> patients with the *MTHFR* TT genotype responded significantly to riboflavin supplementation (1.6 mg/d for 16 weeks)
- As a result of riboflavin supplementation, there was a marked improvement in blood pressure control which increased from 39% pre-intervention to 58% post-intervention (without any change in BP medications during the period of riboflavin intervention)

Slide (with modifications) courtesy of Helene McNulty

Lowering of Blood Pressure with Riboflavin

Comparison with Other BP Lowering Interventions



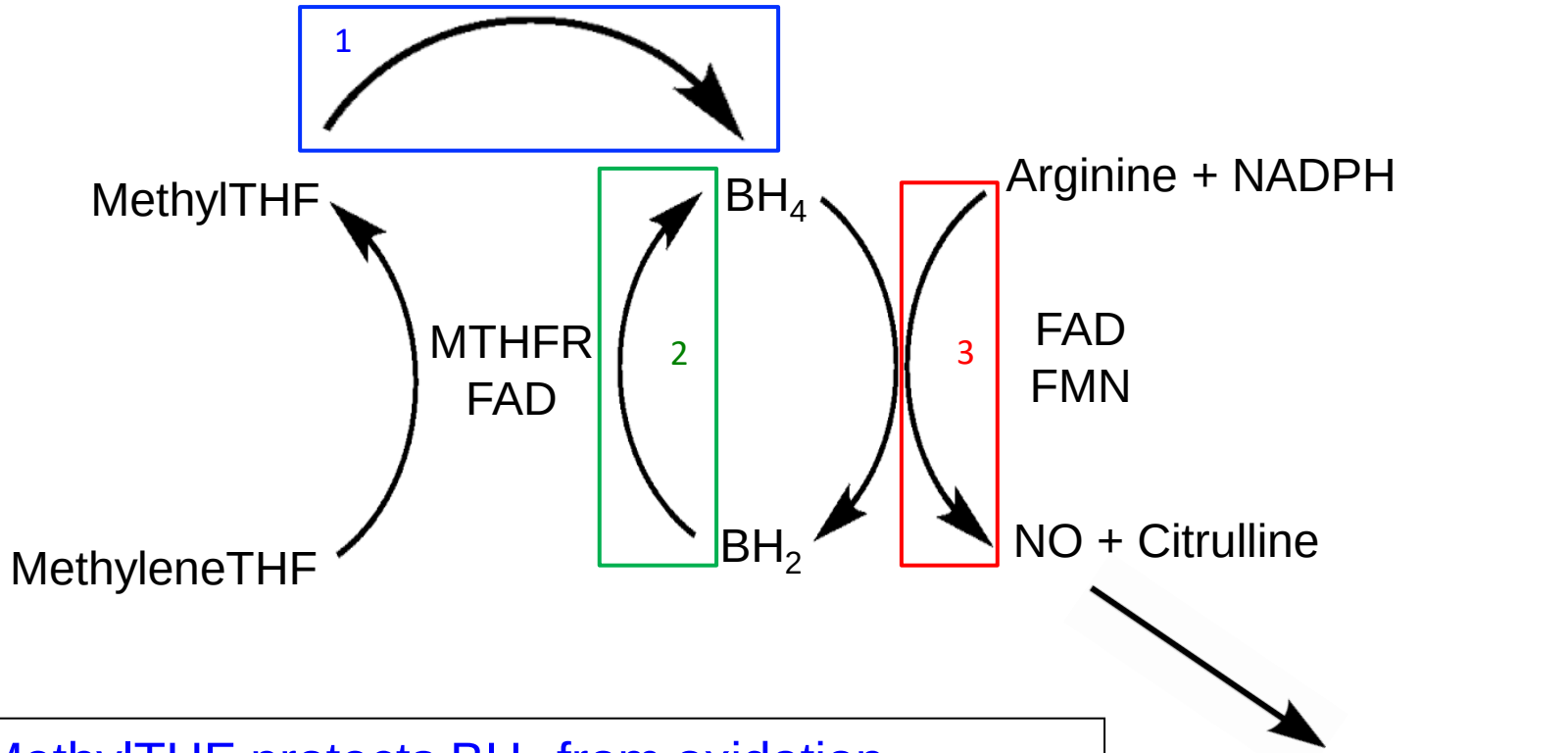
Lifestyle or Dietary Change	Systolic BP Decrease (mm Hg)
Weight loss (per 10 Kg)	5 – 20
Riboflavin supplements (*MTHFR* 677TT genotype)	**6 – 13 ***
Increased physical activity	4 – 9
Reduced sodium intake	2 – 8
Limiting alcohol intake	2 – 4
Dietary Approach to Stop Hypertension (DASH) Diet	3 – 5

Data (modified) from Chobanian et al. 2003

***Data from 3 published trials from Ulster: Horigan et al 2010; Wilson et al 2012 & 2013**

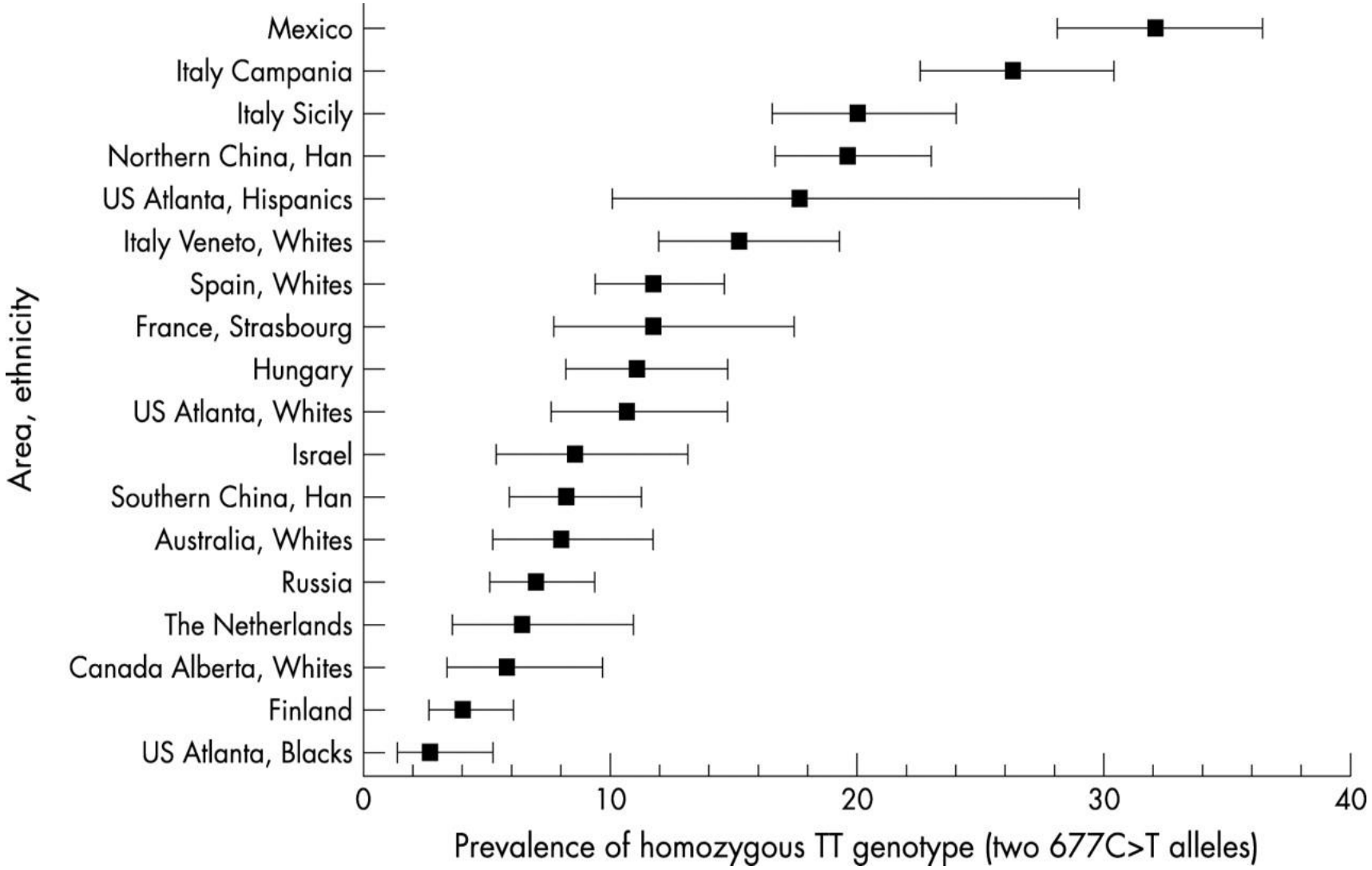
Figure and data courtesy of Helene McNulty

Folate, Riboflavin, Biopterin, and Nitric Oxide Interrelationships



[1] MethylTHF protects BH_4 from oxidation
[2] MTHFR/FAD catalyzes reduction of BH_2 to BH_4
[3] FAD/FMN serve as cofactors for NO synthesis

Frequency of *MTHFR* 677TT Genotype Worldwide



Wilcken et al, J Med Genet, 2003

Slide courtesy of Helene McNulty

Riboflavin (B2)
Global Deficiency

Prevalence of Functional Deficiency in Ireland and Canada*

	Erythrocyte Glutathione Reductase Activity Coefficient (EGRac)		
Population	***Normal* (≤1.30)**	***Suboptimal* (1.31 – 1.39)**	***Deficient* (≥1.40)**
Irish Adults			
NANS (n=1130)	461 (41%)	265 (23%)	404 (36%)
TUDA (n=5192)	2791 (52%)	970 (19%)	1503 (29%)
Canadian Adults			
Young (n=51)	17 (33%)	15 (30%)	19 (37%)
Older (n=226)	138 (61%)	31 (14%)	57 (25%)

NANS: National Adult Nutrition Survey of free living younger Irish adults aged 18-45 years.
TUDA: Trinity Ulster Department of Agriculture Aging Cohort Study of free living older Irish adults aged ≥60 years.
Canada Young Adults: Convenience sample of free-living women of childbearing age (25-45 years) sampled by University of British Columbia.
Canada Older Adults: convenience sample of elderly, institutionalized adults sampled by University of British Columbia.

***Data courtesy of Helene McNulty**

Conditions Associated with Deficiency

- Monotonous diets, low in animal source foods (meats, eggs, milk)
- Gastrointestinal infections

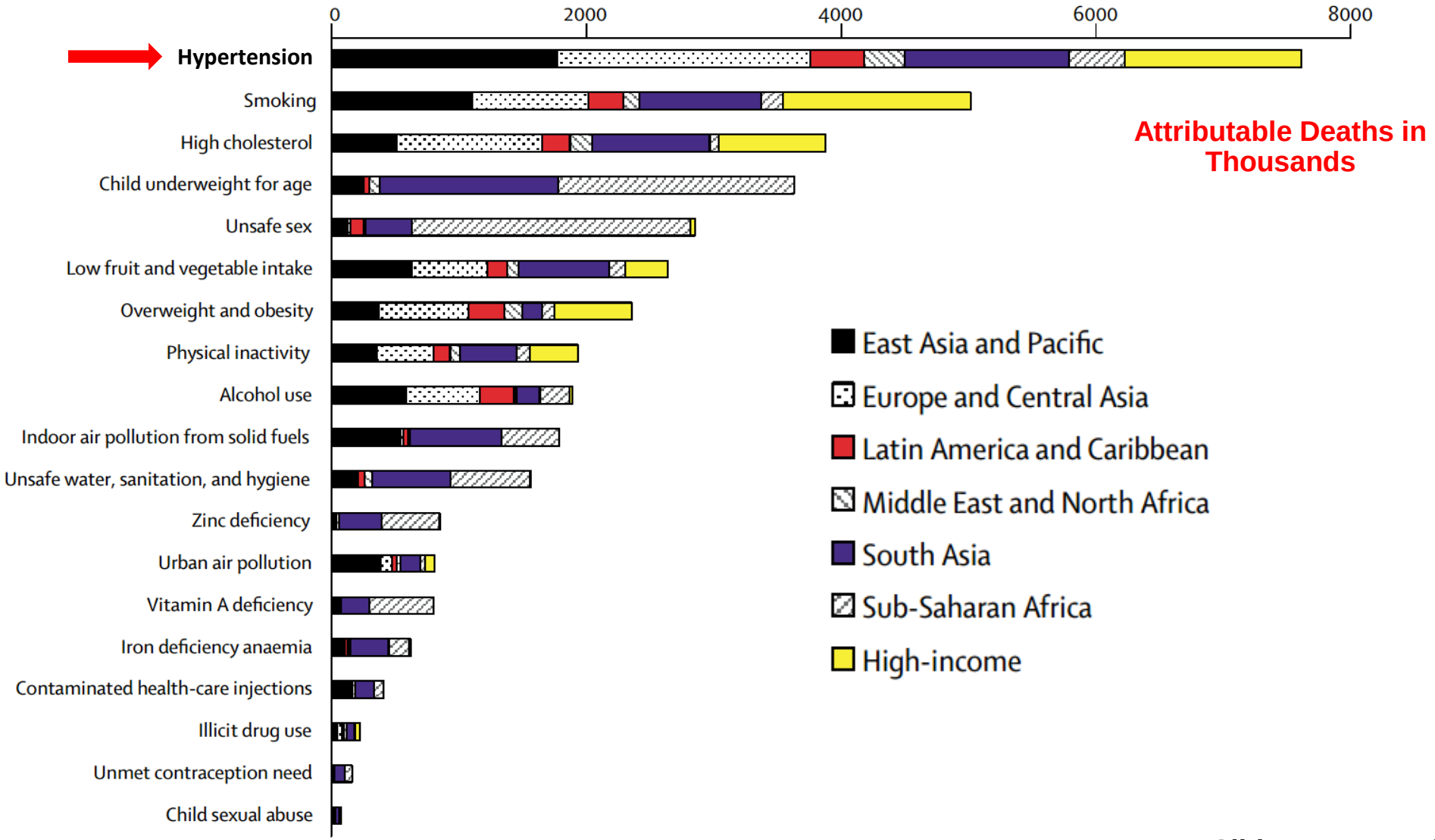
Countries/Regions with Reported Deficiency

- Côte d'Ivoire
- Cambodia (urban and rural)
- Canada (Vancouver area)
- Kenya
- Zambia

Titcomb & Tanumihardjo, Comp Rev Food Sci Food Safety, 2019

Hypertension – A Global Health Concern

Mortality Due to Global Risk Factors



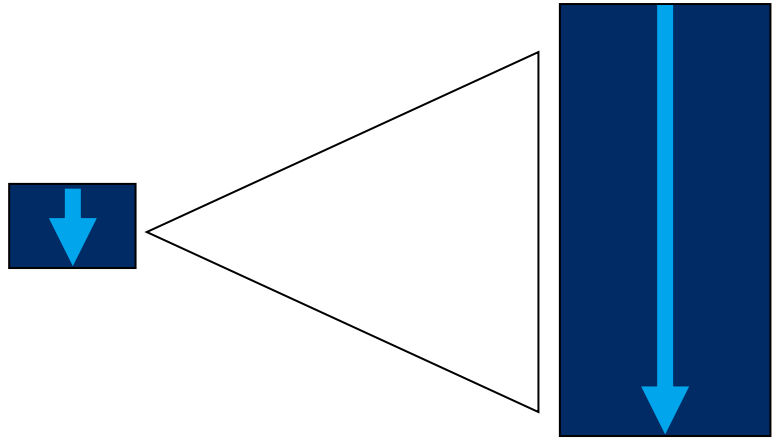
Lopez et al, Lancet, 2006

Slide courtesy of Helene McNulty

Potential Impact of Reducing BP

- Meta-analysis of 61 prospective studies including over 1 million adults

Each 2 mmHg decrease in SBP



10% reduction in risk of stroke mortality

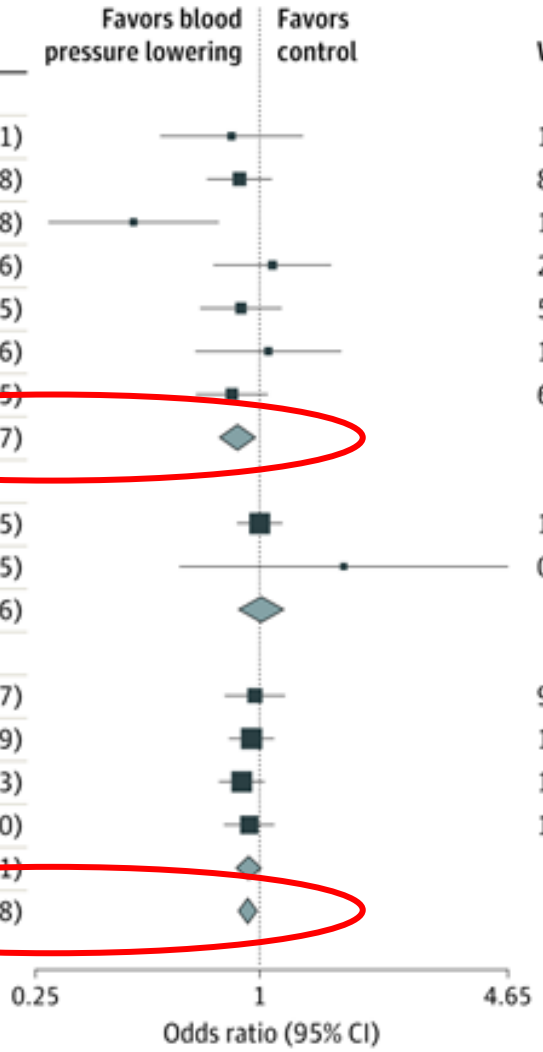
Potential public health significance of this gene-nutrient interaction on BP could be very significant

Lewington et al, Lancet, 2002

Slide courtesy of Helene McNulty

Blood Pressure Lowering and Risk of Dementia or Cognitive Impairment

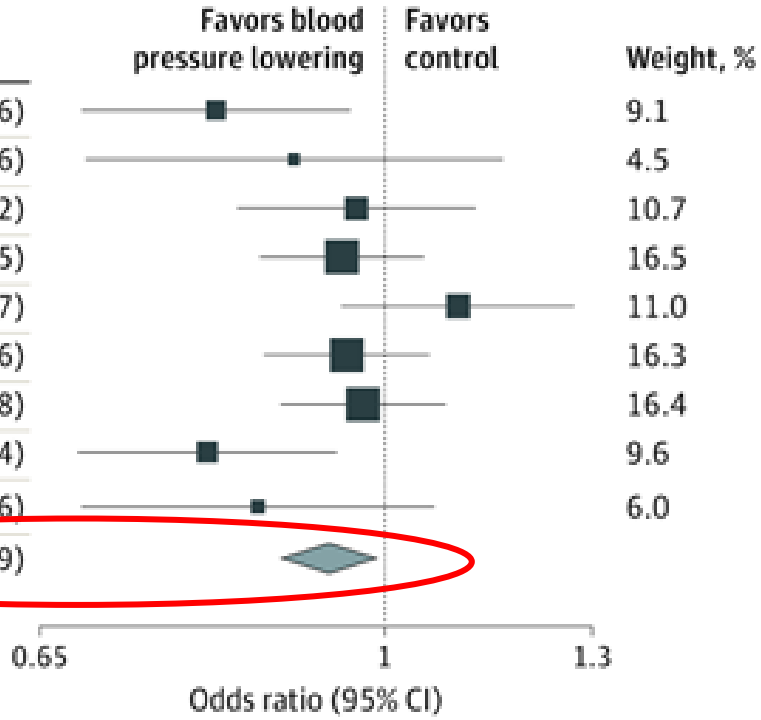
Study	Participants with dementia or cognitive impairment/total No.: Blood pressure lowering group	Control group	Absolute risk reduction (95% CI), %	Odds ratio (95% CI)	Weight, %
Dementia (criterion-referenced)					
SHEP,[22] 1994	37/2365	44/2371	0.29 (-0.45 to 1.03)	0.84 (0.54 to 1.31)	1.69
PROGRESS,[23] 2003	193/3051	217/3054	0.78 (-0.48 to 2.04)	0.88 (0.72 to 1.08)	8.14
Syst-Eur,[5] 2002	21/1485	43/1417	1.62 (0.54 to 2.70)	0.46 (0.27 to 0.78)	1.18
SCOPE,[24] 2003	62/2477	57/2460	-0.19 (-1.04 to 0.67)	1.08 (0.75 to 1.56)	2.48
HYVET-COG,[6] 2008	126/1687	137/1649	0.84 (-0.99 to 2.67)	0.89 (0.69 to 1.15)	5.17
ADVANCE,[25] 2009	39/5569	37/5571	-0.04 (-0.34 to 0.27)	1.05 (0.67 to 1.66)	1.61
SPRINT MIND,[12] 2019	149/4278	176/4285	0.62 (-0.18 to 1.43)	0.84 (0.67 to 1.05)	6.64
Random-effects model for subgroup ($Q_6 = 7.92$; $P = .24$; $I^2 = 0.0\%$)				0.87 (0.78 to 0.97)	
Dementia (clinical-based)					
PRoFESS,[26] 2008	408/8624	409/8646	0.00 (-0.63 to 0.63)	1.00 (0.87 to 1.15)	16.62
HOPE-3,[13] 2019	10/811	6/815	-0.50 (-1.46 to 0.46)	1.68 (0.61 to 4.65)	0.32
Random-effects model for subgroup ($Q_1 = 0.99$; $P = .32$; $I^2 = 0.0\%$)				1.01 (0.88 to 1.16)	
Dementia and mild cognitive impairment (composite)					
TRANSCEND,[7] 2011[a]	239/2694	245/2689	0.24 (-1.29 to 1.77)	0.97 (0.81 to 1.17)	9.41
ON TARGET (Dual),[7] 2011[a]	618/7807	326/3932.5	0.37 (-0.68 to 1.42)	0.95 (0.83 to 1.09)	16.75
ON TARGET (ARB),[7] 2011[a]	584/7797	326/3932.5	0.80 (-0.24 to 1.84)	0.90 (0.78 to 1.03)	16.44
SPS3,[27] 2014[a]	506/1323	535/1345	1.53 (-2.17 to 5.23)	0.94 (0.80 to 1.10)	13.55
Random-effects model for subgroup ($Q_3 = 0.57$; $P = .90$; $I^2 = 0.0\%$)				0.93 (0.87 to 1.01)	
Test for overall effect: $z = -2.50$; $P = .01$			0.39 (0.09 to 0.68)	0.93 (0.88 to 0.98)	
Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 12.14$; $P = .43$; $I^2 = 0.0\%$					



Hughes et al, JAMA, 2020

Blood Pressure Lowering and Risk of Dementia or Cognitive Impairment

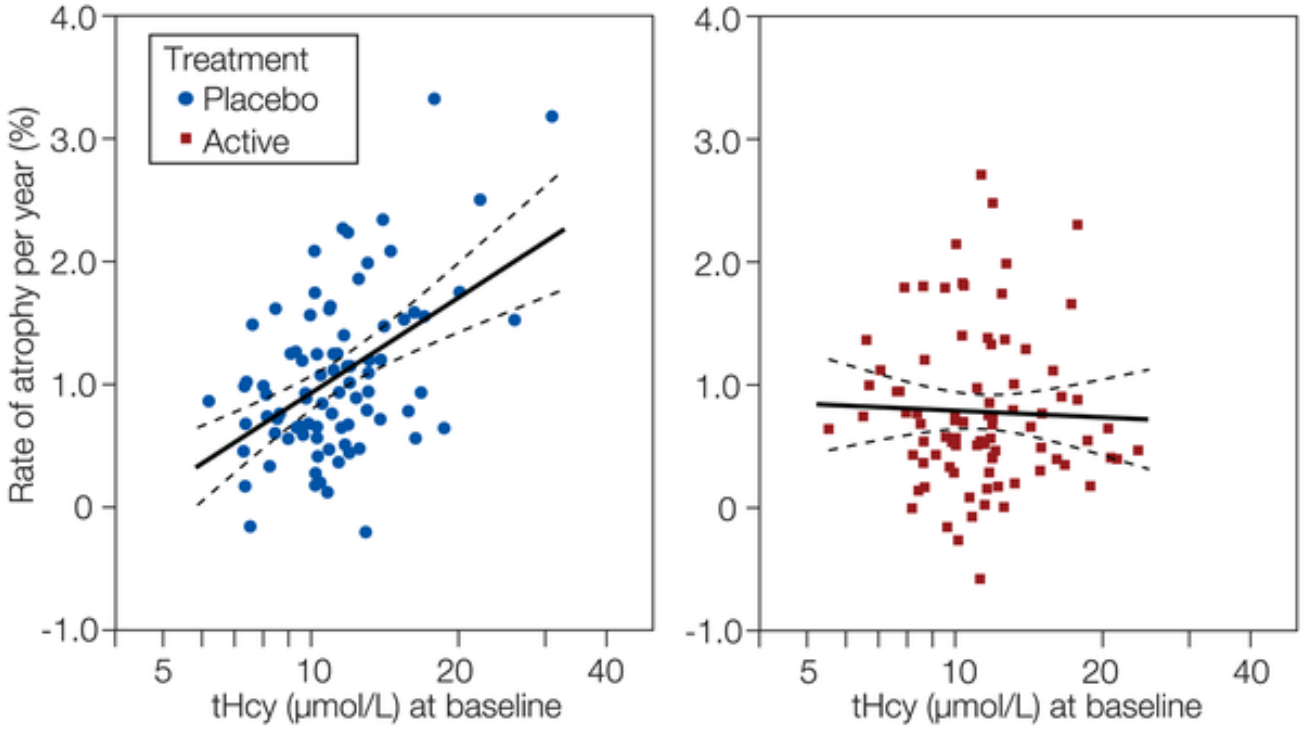
Study	Participants with dementia or cognitive impairment/total No.		Absolute risk reduction (95% CI), %	Odds ratio (95% CI)	Weight, %
	Blood pressure lowering group	Control group			
PROGRESS,[23] 2003	276/3051	334/3054	1.89 (0.39 to 3.39)	0.81 (0.68 to 0.96)	9.1
SCOPE,[24] 2003	113/2477	125/2460	0.52 (-0.68 to 1.71)	0.89 (0.69 to 1.16)	4.5
HYVET-COG,[6] 2008	485/1687	486/1649	0.72 (-2.36 to 3.81)	0.97 (0.83 to 1.12)	10.7
PRoFESS,[26] 2008	795/7531	832/7518	0.51 (-0.48 to 1.50)	0.95 (0.86 to 1.05)	16.5
TRANSCEND,[7] 2011[a]	454/2642	412/2589	-1.27 (-3.28 to 0.74)	1.10 (0.95 to 1.27)	11.0
ON TARGET (Dual),[7] 2011	1240/7461	657/3801	0.67 (-0.80 to 2.13)	0.95 (0.86 to 1.06)	16.3
ON TARGET (ARB),[7] 2011	1279/7566	657/3801	0.38 (-1.09 to 1.85)	0.97 (0.88 to 1.08)	16.4
SPRINT MIND,[12] 2019	287/4278	353/4285	1.53 (0.42 to 2.64)	0.80 (0.68 to 0.94)	9.6
HOPE-3,[13] 2019	584/811	612/815	3.08 (-1.20 to 7.37)	0.85 (0.68 to 1.06)	6.0
Test for overall effect: $z = -2.28$; $P = .02$ Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 12.60$; $P = .13$; $I^2 = 36.1\%$			0.71 (0.19 to 1.2)	0.93 (0.88 to 0.99)	

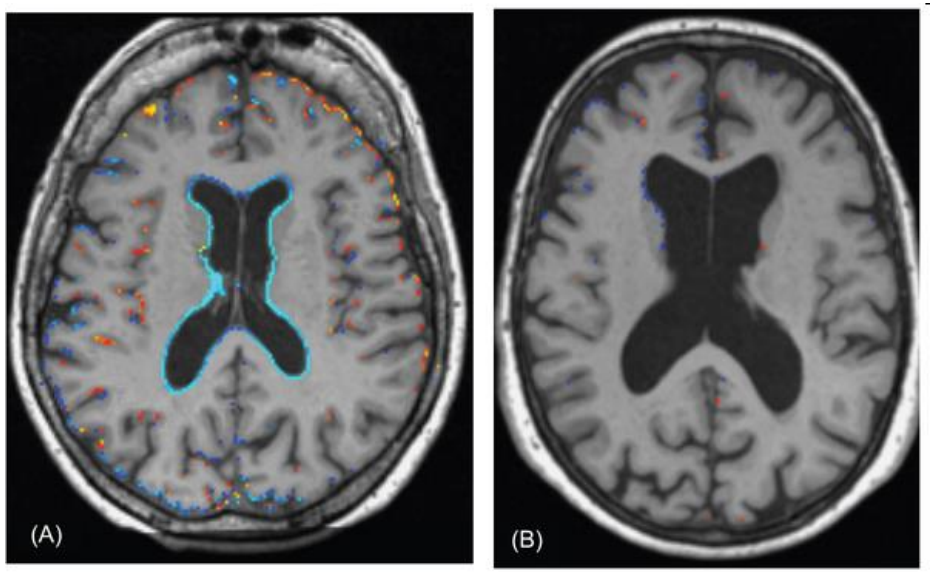


Hughes et al, JAMA, 2020

Effect of Homocysteine Lowering on Brain Atrophy

Folic Acid, B6, and B12





ΔHcy: 22 to 30 µmol/L
Atrophy Rate: 2.5%/yr

ΔHcy: 24 to 12 µmol/L
Atrophy Rate: 0.46%/yr

Smith et al, PLoS One, 2010

Question: What about riboflavin?

Riboflavin and Age-Associated Cognitive Decline

Data Limited to Dietary Intake

- High dietary intake pattern of riboflavin, folate, vitamin B12, and vitamin D associated with enhanced cognitive performance in healthy older adults (N=116; age 65-75 y). **Zwilling et al, 2019**
- High dietary intake of riboflavin in older adults (N=16,948; age 45-74 y at baseline) associated with lower cognitive impairment after ~20-year follow-up. **Sheng et al, 2019**
- Low dietary intake of riboflavin in older adults (N=237; ≥65 y) associated with increased rate of cognitive decline over ~6-year follow-up. **Araki et al, 2017**

No studies found assessing associations between riboflavin status and age-associated cognitive dysfunction, nor assessing the effect of riboflavin supplements on cognitive decline or risk of Alzheimer's disease and other forms of dementia.

A Brief Mention of ‘Cytoflavin’

- **Cytoflavin®:** Inosine (50 mg) + Succinic Acid (300 mg) + Nicotinamide (25 mg) + Riboflavin (5 mg)
  - Drug used primarily in Russia, most publications on Pubmed in Russian journals
  - Currently in clinical trials in Russia
  - Publications and trials focused on improving brain function and cognitive functions in:
    - Acute cerebrovascular accident (ischemic stroke)
    - Cerebrovascular disease
    - Toxic and hypoxic encephalopathy
    - Post-anesthetic depression of consciousness
    - Cognitive decline after major surgery
    - Head trauma, traumatic brain injury
    - Diabetic neuropathy
    - Elderly alcoholics
  - No publications or trials on treatment or prevention of age-related cognitive decline, Alzheimer’s disease, or other forms of dementia

Summary

- The active form of riboflavin, FAD, serves as a cofactor for the MTHFR enzyme, which is central to folate and one-carbon metabolism.

- The common MTHFR C677T polymorphism affects blood folate and homocysteine levels, and is associated with risk of NTDs, various cancers, vascular diseases (particularly stroke), and blood pressure/hypertension.

- The association between MTHFR C677T and blood pressure/hypertension is modified by riboflavin status, and in homozygous carriers of the variant form of MTHFR (677TT), riboflavin supplements significantly reduce systolic and diastolic blood pressure.

- Mid-life hypertension and hyperhomocysteinemia are associated with increased risk of late-life cognitive impairment and dementia. Lowering of blood pressure and/or homocysteine with riboflavin supplements in MTHFR 677TT individuals represents a potential, but untested strategy for reducing risk of cognitive decline and dementia in older adults.

- Studies assessing the influence of riboflavin on cognitive function in older adults are limited to dietary intake. No studies have assessed associations with riboflavin status or effects of riboflavin supplements.