Discussion of Research Recommendations:
Thiamin, Riboflavin, Niacin, Vitamin B6, Folate,
Vitamin B12, Pantothenic Acid, Biotin, and Choline

Dietary Reference Intake Research Synthesis Workshop

Issues
• New knowledge
• Common genetic variations influence DRIs
• Metabolomics and DRIs

Steven Zeisel Patrick Stover
Choline Folate
Thiamin B12
Riboflavin B6
Niacin Biotin
Pantothenic acid

DRI Report on Biotin  AI = 30 μg/day for adults

New Discoveries
- Histones are modified by biotin
  - Accounts for its effects on gene expression
- Holocarboxylase synthetase (HCS) deficiency
  - Infantile neurological, developmental and metabolic abnormalities
  - Resolves with pharmacological doses of biotin
  - Results in reduced Histone biotinylation

Provide “useful” indicators for setting an EAR?
DRI Report on Vitamin B₆

**EAR**: 1.1 - 1.7 mg/day adults
**UL**: 100 mg/day adults

**GAPS**
- Indicators for requirement
- Research on genetic variation & chronic disease prevention
- EAR for children, elderly, pregnant and lactating women
- Interaction with other vitamins

**New Discoveries**
- Plasma B₆ levels fall in inflammation
  - B₆ vs. CRP and IL6 negatively correlated (Clin Chem. 2006 Vol 52)
  - Sickle Cell Disease – elevated Hcy
  - Rheumatoid arthritis (Arth. Res. & Therapy (2005) 7:R1254)
- Pyridoxine supplementation corrects deficiency but not inflammation (Arth. Res. & Therapy (2005) 7:R1404)

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DRI Report on Folate

**EAR**: 320 - 520 µg (DFE)/day adults
**UL**: 1.0 mg/day adults

**Gaps and New Discoveries**
- Comprehensive risk/benefit resulting from fortification FA
  - NTDs, vascular disease, cancer, cognition, etc.
- Genetic variation
  - MTHFD1 and MTHFR for NTDs, plus others.
- Status indicator with cutoff point.
  - Homocysteine: Selnbub data.
- Bioavailability
- Interaction with B12 deficiency
- Role in differentiation & development
- Requirements vary by trimester in pregnancy
- Requirements for children, elderly, women of reproductive age.

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Two-phase regression model for the association between Ln Homocysteine concentrations and serum folate levels.

NHANESIII

J. Selnbub, Tufts Univ

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- Requirements for children elderly, women of reproductive age.

Gaps and New Discoveries:
- Interactions with other B-vitamins & choline
- Both affect methylation status
- Analytical methodology
- Mass spectrometry
- Development of mouse models for NTD prevention
- ? Ongoing

New Discoveries:
- Current RDA for folate is adequate for young women for all three MTHFR genotypes. (J. Nutr. (2003) 133:1272)
  - 43 subjects, 7 week depletion, 7 week repletion
- Reduced natural killer cell cytotoxicity in women with plasma FA; 78% of fasting participants exhibited plasma FA (2.3 nmol/L).
**DRI Report on Vitamin B₁₂**

**EAR = 2.0 – 2.4 μg/day adults**

**UL = none**

**Gaps:**
- Role in vascular disease
- Impact of genetic variation
- Requirements for the elderly
- Effect of folate on progression of B₁₂ deficiency
- Methods to detect status (indicators) (elderly, vegans)
- Interactions with other vitamins
- Efficacy of B₁₂ fortification

**New Discoveries**
- New methodologies for status are in development
- TC 776C G SNP affects indicators of B₁₂ status (HoloTC II and Hcy) (Blood (2002) 100:718)
- Plasma Hcy and MMA as indicators are consistent with values required to maintain hematological status

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**Two-phase regression models for the association between Ln MMA and Ln homocysteine concentrations and serum B₁₂ levels. NHANESIII**

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**"Perspectives"**

- Disease/pathology Outcome(s)
- Genetic Variation and Requirements
- Fetal/Stem Cell Programming
One-Carbon Metabolism

THF mutations/SNPs affect genome stability and methylation capacity

Methylation - DNA, proteins (histones), lipids, etc.

THYMYLATE

AdoMet/AdoHcy

Methionine

Homocysteine

5-methylTHF

5-methylTHF

MTHFR

Benefit and Risks of MTHFR Polymorphism

In utero Risk

“T” allele (A222V)

- NTDs
- In humans, (not in mice)
- Spontaneous abortion
- Not in HW equilibrium

Adult Benefit

“T” allele

- Physician’s Health Study – Colon Cancer Risk

Perspectives

Disease/pathology Outcome(s)
- Will a nutrient intake level present opposing benefit/risk outcomes for different diseases?

1. Genetic Variation and Requirements
2. Fetal/Stem Cell Programming
Penetrance

The probability of expressing a phenotype from a given genotype at a given time

Prevalence

A measure of the proportion of persons in the population with a certain SNP at a given time

**Diet and Genetic Variation**

**Polymorphism Impact Parameters**

**“Perspectives”**

1. Genetic Variation and Requirements

   - Few SNPs will be sufficiently penetrant to warrant genotype-specific recommendations.
     - miscarriage or non-HWEquilibrium (TCII, MTHFR, MTHFD1)
     - the use of supplemental folate has been suggested to reduce rates of human spontaneous abortion. *(Reprod. Biol. Endocrin. (2004) 2:7)*
   - Penetrant SNP-SNP interactions will have low prevalence.

   **Diet and Genetic Variation**

   **Polymorphism Impact Parameters**

   Gene-gene interactions

   The probability of expressing a phenotype from a given genotype at a given time

   A measure of the proportion of persons in the population with a certain SNP at a given time
**Interaction of cSHMT and MTHFR SNPs**

- 5-methylTHF
- AdoHyc
- AdoMet
- Methylation: DNA, proteins (histones), lipids, etc.
- THYMIDYLATE TS
- 5-methylTHF sequestration
- cSHMT

**Strain**

<table>
<thead>
<tr>
<th>Strain</th>
<th>AdoHyc (pmoles/ug protein)</th>
<th>AdoMet (pmoles/ug protein)</th>
</tr>
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<tbody>
<tr>
<td>Balb/c</td>
<td>0.6 +/- .16</td>
<td>2.9 +/- .9</td>
</tr>
<tr>
<td>129</td>
<td>0.4 +/- .17</td>
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**A genetic interaction between MTHFR and cSHMT in CVD Risk**

<table>
<thead>
<tr>
<th>Strain</th>
<th>MTHFR CT</th>
<th>MTHFR CT</th>
<th>MTHFR TT</th>
<th>cSHMT TT</th>
<th>cSHMT wild-type</th>
<th>cSHMT mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odds ratio (95% CI)</td>
<td>1.0</td>
<td>1.1</td>
<td>1.2</td>
<td>3.6</td>
<td>1.3</td>
<td>1.0</td>
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<tr>
<td>P value for interaction</td>
<td>&lt; .001</td>
<td></td>
<td></td>
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</tbody>
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**A common SNP (L474F) in cSHMT**

- L474F
- Impaired SUMO and Ubiquitin Conjugation
- Impaired SAM-dependent methylation
- Impaired thymidylate synthesis

**References**

- Pat Cassano
1. Disease/pathology Outcome(s)

2. Genetic Variation and Requirements
   - Framework for establishing impact/cutoffs of genetic variation (prevalence & penetrance) for subgroup recommendations.

3. Fetal/Stem Cell Programming

Fetal Origins of Adult Disease or “Barker” Hypothesis (1986)

Fetal environmental exposures, especially nutrition, act in early life to program risk for adult health outcomes.

**Early Nutrition Experiences** → **Risk Phenotype** → **CVD**
- obesity
- hypertension
- insulin resistance
- diabetes
- metabolic syndrome

**“Program” “Imprint”**

Sense → Adapt → Irreversible programming

**Can folate (program) gene expression?**

**A agr/v/a mice**

- IAP insertion leads to constitutive, ectopic agouti expression (yellow mouse)
- Methylation of the IAP element leads to less agouti expression (pseudoagouti coat color)
- Maternal folate & choline supplementation during gestation leads to increased embryonic IAP methylation and the pseudoagouti phenotype
- Rescue of SA?
cSHMT Deletion Induces:
- NTDs
- Programming of thymidylate synthesis

- Only cSHMT +/− exhibit NTDs
- cSHMT −/− display elevated TS
- TS Programming

B-vitamin Requirements
- Genetic Variation (EAR and UL)
- Epigenetic effects/critical windows (UL)
- (EAR, UL and new indicators)

Risk of inadequacy
Risk of excess

Increased intake

Inadequate intake