Genotyping and epigenetic imprinting--
Including assessment of effects of single nucleotide polymorphisms (SNP’s) on variability in requirements and/or Tolerable Upper Intake levels.

Methylenetetrahydrofolate reductase (MTHFR) and methionine synthesis

- Methylene-THF
- Methyl-THF
- THF
- Methionine synthase
- Methionine
The C677T Mutation in the MTHFR gene

- A polymorphic mutation in the structural gene at position 677 where cytosine is replaced by thymidine resulting in the substitution of alanine by valine in the enzyme protein (Frosst et al., 1995)

- Homozygosity for this mutation results in a less active and a more heat sensitive enzyme protein (Kang et al., 1988)

- Heat sensitivity is due to change in conformation, which results in dissociation of FAD. This dissociation is prevented by folate substrates (Guenther et al., 1999)

Total plasma homocysteine (tHcy) by C677T MTHFR genotype (Jacques et al., 1996)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>C/C</th>
<th>C/T</th>
<th>T/T</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>150</td>
<td>170</td>
<td>45</td>
</tr>
<tr>
<td>tHcy (umol/L)</td>
<td>8.7</td>
<td>8.4</td>
<td>9.9*</td>
</tr>
<tr>
<td>Folate &lt;15.4 (nmol/L)</td>
<td>9.8</td>
<td>9.5</td>
<td>12.1*</td>
</tr>
<tr>
<td>Folate ≥15.4 (nmol/L)</td>
<td>7.8</td>
<td>7.5</td>
<td>7.9</td>
</tr>
</tbody>
</table>

* P<0.05

Increase in Plasma Folate by Genotype

- Before Fortification
- After Fortification

* P<0.05 compared with the same genotype before fortification
* P<0.05 compared to wild type
* P<0.05 compared to wild type. Significance lost after adjustment for multiple comparisons.
MTHFR Variant in Folate Requirement

Research Techniques

Bioavailability studies
including stable isotope methods

Bioavailability Needs

Dietary Folate Equivalents (DFE's) misapplied to human milk folate bioavailability

"Ages 0 through 6 Months. The AI for infants 0 through 6 months of age, derived by using the average volume of milk of 0.78 L/day for this age group and the average folate concentration in human milk after 1 month of lactation (85µg/L), is 66 µg/day, which is rounded to 65 µg. This equals approximately 9.4 µg/kg of reference body weight. Because this is food folate, the amount is the same in dietary folate equivalents (DFE's)."
Need Research Strategy for setting UL's

- Sharper end points of adverse effects
- Dose response analyses with larger numbers
- Depletion - Repletion studies for adverse effects not done, often not feasible
- Need to validate surrogate markers of risk, e.g.:
  - Esterified Vitamin A
  - Circulating free folic acid