Meeting folate and related B-vitamin requirements through food: 

*Is it enough?*

*Role of fortification and dietary supplements*

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University of Ulster
Functional role of folate and related B vitamins

One-Carbon Metabolism

Transfer and utilisation of one-carbon units in

– amino acid metabolism
– methylation processes (e.g. when impaired, plasma homocysteine will be elevated)
– synthesis of DNA

The clinical sign of folate deficiency (megaloblastic anaemia) is the result of impaired synthesis of DNA
Meeting folate and related B-vitamin requirements through food: Is it enough?

This talk will address 3 key questions

• Can an optimal folate status be achieved through natural food folate sources alone?
• Is there evidence demonstrating beneficial health effects of fortification/supplementation with folic acid?
• Is there a role for Personalized Nutrition in this area?
Structure of pteroylglutamic acid
i.e. Folic acid
the folate form found in supplements and fortified foods
Food folates compared with folic acid

• **Folic acid** is
  – fully oxidised
  – a monoglutamate

• **Food folates** are
  – reduced molecules
  – predominantly **polyglutamates** but converted to **monoglutamates** for absorption

• **The potential to optimise folate** status by means of natural food folate sources only is **very limited**
  – unstable during cooking
  – incomplete bioavailability once ingested
3 routes to achieve optimal folate status

Natural food sources

Fortified Foods

Supplements
Intervention study to increase folate status in women by 3 different routes

Cuskelley et al. 1996 Lancet 347:657-659

• Folic acid-fortified foods and folic acid supplements were equally effective in optimising folate status

• Increased consumption of foods naturally rich in folate resulted in no significant response in folate status
## Impact of folic acid-fortified food on folate intake and status

Hoey et al *AJCN* 2007; 86: 1405-1413

<table>
<thead>
<tr>
<th></th>
<th>Non-Consumers</th>
<th>Low Consumers</th>
<th>Medium Consumers</th>
<th>High Consumers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$(n=97)$</td>
<td>$(n=111)$</td>
<td>$(n=118)$</td>
<td>$(n=115)$</td>
</tr>
<tr>
<td>(FA= 0µg/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Dietary Folate Intake</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total folate (µg/d)</td>
<td>186 (142, 223)</td>
<td>206 (173, 246)</td>
<td>259 (212, 310)</td>
<td>422 (333, 549)</td>
</tr>
<tr>
<td>Added folic acid (µg/d)</td>
<td>0 (0, 0)</td>
<td>25 (17, 33)</td>
<td>60 (50, 75)</td>
<td>208 (125, 291)</td>
</tr>
<tr>
<td>Natural folate (µg/d)</td>
<td>186 (142, 223)</td>
<td>179 (151, 215)</td>
<td>196 (150, 248)</td>
<td>197 (157, 238)</td>
</tr>
<tr>
<td><strong>Biomarker Folate Status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma Hcy (µmol/l)</td>
<td>11.5 (9.4, 13.9)</td>
<td>10.7 (8.9, 13.4)</td>
<td>9.6 (7.8, 11.2)</td>
<td>9.4 (7.7, 12.0)</td>
</tr>
<tr>
<td>RCF (nmol/l)</td>
<td>653 (532, 830)</td>
<td>697 (564, 857)</td>
<td>862 (680, 1082)</td>
<td>1040 (798, 1413)</td>
</tr>
<tr>
<td>Serum Folate (nmol/l)</td>
<td>15.1 (10.0, 21.1)</td>
<td>16.2 (11.6, 22.1)</td>
<td>22.6 (16.7, 30.5)</td>
<td>30.1 (21.5, 45.5)</td>
</tr>
</tbody>
</table>
## Impact of voluntary fortification and supplement use on dietary intakes and biomarker status of folate in Irish adults (NANS)


<table>
<thead>
<tr>
<th></th>
<th>Non-consumers FF or supp</th>
<th>Consumers FF</th>
<th>Supplement user</th>
<th>FF &amp; supplement user</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n=201</strong></td>
<td><strong>n=767</strong></td>
<td><strong>n=35</strong></td>
<td><strong>n=123</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Dietary folate intake

<table>
<thead>
<tr>
<th></th>
<th>Non-consumers FF or supp</th>
<th>Consumers FF</th>
<th>Supplement user</th>
<th>FF &amp; supplement user</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total folate ($\mu g/d$)</td>
<td>206 (160, 295)$^a$</td>
<td>311 (239, 427)$^b$</td>
<td>557 (365, 629)$^c$</td>
<td>582 (431, 746)$^c$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Folic acid ($\mu g/d$)</td>
<td>–</td>
<td>69 (32, 142)$^a$</td>
<td>200 (150, 400)$^b$</td>
<td>287 (220, 438)$^b$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Natural folate ($\mu g/d$)</td>
<td>206 (160, 295)</td>
<td>223 (176, 284)</td>
<td>239 (177, 310)</td>
<td>246 (185, 309)</td>
<td>0.983</td>
</tr>
</tbody>
</table>

### Biomarker status of folate

<table>
<thead>
<tr>
<th></th>
<th>Non-consumers FF or supp</th>
<th>Consumers FF</th>
<th>Supplement user</th>
<th>FF &amp; supplement user</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC Folate (nmol/L)</td>
<td>702 (538, 936)$^a$</td>
<td>885 (697, 1194)$^b$</td>
<td>1000 (804, 1444)$^{bc}$</td>
<td>1159 (828, 1519)$^c$</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Serum folate in a general Danish population (adults 30-60 y)

Thuesen et al. 2010 Brit J Nutr 103: 1195-1204

<table>
<thead>
<tr>
<th>Folate (n=6371)</th>
<th>Prevalence &lt; 6.8 nmol/L</th>
<th>Prevalence &lt; 4.0 nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>n/n_total</td>
<td>%</td>
</tr>
<tr>
<td>All</td>
<td>31.4</td>
<td>2003/6371</td>
</tr>
<tr>
<td>Men</td>
<td>32.6</td>
<td>1019/3123</td>
</tr>
<tr>
<td>Women</td>
<td>30.3</td>
<td>984/3248</td>
</tr>
<tr>
<td>P</td>
<td>0.045</td>
<td>0.792</td>
</tr>
</tbody>
</table>

**Age (years)**

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Prevalence &lt; 6.8 nmol/L</th>
<th>n/n_total</th>
<th>Prevalence &lt; 4.0 nmol/L</th>
<th>n/n_total</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>43.4</td>
<td>139/320</td>
<td>7.5</td>
<td>24/320</td>
</tr>
<tr>
<td>35</td>
<td>38.5</td>
<td>253/658</td>
<td>7.5</td>
<td>49/658</td>
</tr>
<tr>
<td>40</td>
<td>38.0</td>
<td>474/1246</td>
<td>5.6</td>
<td>70/1246</td>
</tr>
<tr>
<td>45</td>
<td>32.5</td>
<td>424/1304</td>
<td>5.8</td>
<td>75/1304</td>
</tr>
<tr>
<td>50</td>
<td>28.4</td>
<td>379/1334</td>
<td>4.6</td>
<td>61/1334</td>
</tr>
<tr>
<td>55</td>
<td>24.0</td>
<td>244/1017</td>
<td>3.0</td>
<td>30/1017</td>
</tr>
<tr>
<td>60</td>
<td>18.3</td>
<td>90/492</td>
<td>3.3</td>
<td>16/492</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
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</tbody>
</table>
Options to achieve optimal folate status:

*natural folate sources vs folic acid*

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Strategy shown to be effective in</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural food folates</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Folic acid supplementation</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Folic acid-fortification</td>
<td>yes</td>
<td>yes</td>
</tr>
</tbody>
</table>
All countries in blue fortify flour with iron and folic acid except Australia which does not include iron, and Venezuela, the United Kingdom, the Philippines, and Trinidad and Tobago which fortify with iron only and do not include folic acid.
Current global recommendations for preventing NTDs

To be commenced prior to conception and during the first 12 weeks of pregnancy:

• Recurrence: 4 mg/d folic acid
• First Occurrence: 0.4 mg/d folic acid
The neural tube closes 21-28 days after conception.... just when most women are beginning to suspect that they might be pregnant!

Recent evidence\(^1\) from pregnant women sampled at 14 wk (n=296) showed that

- 84% were taking FA in pregnancy BUT
- Only 1 in 5 had commenced FA before conception as recommended

\(^{1}\)McNulty et al. *Hum Reprod* 2011; 26: 1530-1536
Red cell folate at 14 GW according to time of commencement of folic acid supplement

McNulty et al. *Hum Reprod* 2011; 26: 1530-36
Rates of NTDs per 10 000 births: 1988-98

Current folic acid fortification worldwide

the evidence

• Mandatory folic acid fortification has been in place for several years in a number of countries worldwide
  – This measure has reduced the incidence of NTD (e.g. in the US\textsuperscript{1} and Canada\textsuperscript{2} by between 27\% and 50\%)

• By contrast, there has been no change in NTD rates in Europe\textsuperscript{3} since the introduction of folic acid recommendations to prevent NTD in women of reproductive age

\textsuperscript{1}Honein et al \textit{JAMA} 2001;285:2981-6
\textsuperscript{2}De Wals et al \textit{N Engl J Med} 2007; 357: 135-142
\textsuperscript{3}Botto et al \textit{BMJ} 2005; 330:571
Optimal folate and related B vitamin status

*Known and Emerging Health Benefits*

- Maternal health in pregnancy: conclusive
- Foetal development: conclusive
- Prevention of heart disease/stroke: convincing
- Cancer prevention: promising
- Bone health: possible role
- Cognitive function in ageing: possible role
Elevated homocysteine as a risk factor for CVD

Evidence until 2004

- a similar magnitude of risk to that of elevated cholesterol
- an independent risk factor, but may enhance the effect of “conventional” risk factors
- estimated that a lowering of homocysteine by $3 \, \mu\text{mol/l}$ would reduce the risk of
  - coronary heart disease by 11-16%
  - stroke by 19-24%

Evidence 2004 – 2010

- Publication of several RCTs (secondary prevention trials)

2. Wald et al. 2002 *BMJ*; 325:1202-08
Randomized Trials of folic acid and risk of stroke: a meta-analysis
Wang et al 2007 *Lancet*;369:1876-82

<table>
<thead>
<tr>
<th></th>
<th>Relative risk (95% CI)</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td><strong>Overall</strong></td>
<td>0.82 (0.68-1.00)</td>
<td>0.045</td>
</tr>
<tr>
<td><strong>Duration of intervention</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤36 months</td>
<td>1.00 (0.83-1.21)</td>
<td>0.95</td>
</tr>
<tr>
<td>&gt;36 months</td>
<td>0.71 (0.57-0.87)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Homocysteine lowering</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20%</td>
<td>0.89 (0.55-1.42)</td>
<td>0.62</td>
</tr>
<tr>
<td>≥20%</td>
<td>0.77 (0.63-0.94)</td>
<td>0.012</td>
</tr>
<tr>
<td><strong>History of stroke</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1.04 (0.84-1.29)</td>
<td>0.71</td>
</tr>
<tr>
<td>No</td>
<td>0.75 (0.62-0.90)</td>
<td>0.002</td>
</tr>
</tbody>
</table>
Decline in stroke related mortality in the US and Canada

Yang et al 2006 Circulation;113:1335-43
Is \textit{MTHFR} 677C→T Polymorphism a risk factor for CVD?

- Homozygosity (TT genotype) results in lower MTHFR enzyme activity and increased homocysteine concentrations \textit{in vivo}

- Meta-analyses\textsuperscript{1-4} estimate excess risk of CVD (by 14-21\%) risk in individuals with the TT genotype, but large geographical variation between countries

\textsuperscript{1}Wald DS et al. \textit{BMJ} 2002; \textbf{325}: 1202–1206.
\textsuperscript{2}Klerk et al. \textit{JAMA} 2002; \textbf{288}: 2023–2031.
\textsuperscript{3}Lewis et al. \textit{BMJ} 2005; \textbf{331}: 1053–1056.
\textsuperscript{4}Holmes et al. \textit{Lancet} 2011; \textbf{378}: 584-594
Methylenetetrahydrofolate reductase (MTHFR)

**SUBSTRATE:** 5,10 methylenetetrahydrofolate

**PRODUCT:** 5 methyltetrahydrofolate

**COFACTOR:** Flavin Adenine Dinucleotide (FAD)

**PRECURSOR:** Riboflavin (vitamin B2)

- Polymorphic mutations in MTHFR
  - MTHFR 677C→T Polymorphism
    - C to T substitution at base pair 677
    - Alanine/valine change in the amino acid sequence
    - Functionally defective enzyme
# Genotype-specific response to riboflavin

<table>
<thead>
<tr>
<th></th>
<th>CC (n = 27)</th>
<th>CT (n = 26)</th>
<th>TT (n = 34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>10.7</td>
<td>12.2</td>
<td>17.6</td>
</tr>
<tr>
<td>Riboflavin 1.6mg/d 12 weeks</td>
<td>10.9</td>
<td>11.8</td>
<td>13.0*</td>
</tr>
</tbody>
</table>

*McNulty et al. 2006 Circulation; 113(1): 74-80*
Genome-wide association study identifies eight loci associated with blood pressure


This gene-nutrient interaction may have a novel role in BP

Horigan et al. 2010 *Journal of Hypertension*; 28: 478-486.
This gene-nutrient interaction has a novel role in BP

Blood pressure in treated hypertensive individuals with the MTHFR 677TT genotype responded significantly to riboflavin.
Genetic risk and a novel gene-nutrient interaction in BP
Some unanswered questions

• How important is MTHFR genotype relative to other determinants of blood pressure in adults at all ages?
  • And what about anti-hypertensive drugs?

• Can MTHFR genotype increase the risk of developing hypertension?
  – Does diet matter?

• Effect of MTHFR genotype on BP by age currently under investigation in a large sample of Irish adults
CVD mortality risk increases as BP rises

Chobanian AV et al. *JAMA* 2003;289:2560-2572
Impact of BP reduction

- Meta-analysis of 61 prospective studies including over 1 million adults\(^1\) aged 40 to 69 years

2 mmHg decrease in SBP  \rightarrow  10% reduction in risk of stroke mortality

- Potential public health significance of this gene-nutrient interaction on BP

# Lifestyle factors targeted to reduce BP

Modified from Chobanian *et al.* 2003 JNC 7 report

<table>
<thead>
<tr>
<th>Lifestyle factor</th>
<th>SBP decrease (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight loss (per 10 kg)</td>
<td>5 - 20</td>
</tr>
<tr>
<td>Riboflavin (genotype-specific)</td>
<td>6 - 13</td>
</tr>
<tr>
<td>Physical activity</td>
<td>4 - 9</td>
</tr>
<tr>
<td>Sodium reduction</td>
<td>2 - 8</td>
</tr>
<tr>
<td>Limit alcohol</td>
<td>2 - 4</td>
</tr>
</tbody>
</table>

Modified from Chobanian *et al.* 2003 JNC 7 report
Applications

Optimisation of riboflavin status could be achieved through:

- supplementation

- fortification
  - specific foods
  - population-wide

- dietary changes emphasising riboflavin rich foods such as dairy products
Sub-optimal riboflavin status
NDNS data of British adults aged 19-64y and a convenience sample in Northern Ireland

<table>
<thead>
<tr>
<th></th>
<th>NDNS Males 19-64y</th>
<th>NI sample: Males 19-64y (n=215)</th>
<th>NDNS Females 19-64y</th>
<th>NI Sample: Females 19-64y (n=220)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGRac</td>
<td>1.38 ± 0.17</td>
<td>1.35 ± 0.15</td>
<td>1.40 ± 0.19</td>
<td>1.39 ± 0.18</td>
</tr>
</tbody>
</table>

EGRac, erythrocyte glutathione reductase activation coefficient, a functional indicator of riboflavin status.

(Note: A higher EGRac ratio indicates lower riboflavin status)
Deficient and sub-optimal vitamin B12 status

High prevalence of deficient B12 status in older people despite good dietary intake

- Pernicious anaemia (intrinsic factor deficiency) explains < 2% of cases

- Food-bound B12 malabsorption (owing to hypochlorhydria)
  - Mild (pre-clinical) B12 deficiency
  - Very common; as high as 45% in some studies
  - Factors: atrophic gastritis, PPI use
Optimal folate and related B vitamin status

Known and Emerging Health Benefits

- Maternal health in pregnancy
- Foetal development
- Prevention of heart disease/stroke
- Cancer prevention
- Bone health
- Cognitive function in ageing

Evidence

- conclusive
- conclusive
- convincing
- promising
- possible role
- possible role
Meeting folate and related B-vitamin requirements through food: Is it enough?

*3 key questions addressed*

• Can an optimal folate status be achieved through natural food sources alone?
  – No

• Is there evidence demonstrating beneficial health effects of fortification/supplementation with folic acid? Yes
  – Proven effect in preventing NTD
  – Probable effect in stroke prevention

• Is there a role for Personalized Nutrition in the case of folate and related B vitamins
  – Yes
My thanks to……..

**NICHE at Ulster**
- Mary Ward
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- Kristina Pentieva
- Catherine Hughes
- Adrian McCann

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- Conal Cunningham
- Miriam Casey

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*The EURRECA and BOND project teams (international)*

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- Geraldine Horigan (2006)
- Breige McNulty (2007)
- Carol Wilson (2010)
- Rosie Reilly (current)

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- John Purvis
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