Vitamin B12, Folic Acid Metabolism and Neural Tube Defects

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Closure of the Neural Tube

21 – 28 days gestation (3 to 4 weeks pregnant)

Incomplete closure – more than spina bifida

Fails to close completely: Rachischisis
None survive

Fails to close in brain region: Anencephaly
None survive

Fails to close in spinal region: Spina Bifida
Many survive

Folic acid helps the neural tube to close properly
Professor Richard (Dick) Smithells 1924-2002

Professor of Paediatrics, Leeds

- 1962-65 published several papers on vitamin deficiency as a cause of neural tube defects (NTDs)

- 1981-83 published the results of multivitamin intervention studies in women at risk of NTD recurrence

- 1991 The report of the MRC randomised controlled trial fully endorsed his work

Professor Dick Smithells receiving the James Spence Medal (1992) for outstanding contribution to the advancement of paediatric knowledge
“The results suggest that the more severe NTDs may be associated with a more marked vitamin depletion and that vitamin B12 deficiency might be implicated in the aetiology of anencephaly.”
One of these two B12 dependent enzymes controls the ability of folate to enter cells and function properly.
INTESTINE

Enteral system [LIVER]

CIRCULATION

TISSUES

Gene control and signalling

Food folates
Mainly CH₃THF

Fortified food or supplements

Folic acid

THF=tetrahydrofolate

Cell Division

Methionine Synthase

Homocysteine

Methionine

B12

THF

Folic acid

Folic acid

Folic acid

Folic acid

Gene control and signalling

Cell Division

THF=tetrahydrofolate
Could NTDs be reduced further with vitamin B12?

• Biologically plausible: - Folic acid supplementation could correct abnormalities in DNA synthesis caused by B12 deficiency

• B12 deficiency (PA) has a serious impact on pregnancy
  • Early pregnancy recurrent losses and infertility

• B12 was not included in Smithells’ trials or in the MRC trial - (4µg in Hungarian treatment group - none in multi-mineral placebo).

• Supporting observational data on maternal serum and amniotic fluid
Effects of B12 deficiency on reproduction

• Pernicious anemia (severe B12 deficiency)

1. **Cause of infertility**
   - Jackson et al. Lancet 1967
   - Hall and Davidson 1968
   - Bennett 2001

2. **Cause of early pregnancy loss**
   - Bennett 2001
   - Reznikoff-Etievant et al. 2002
   - Zetterberg et al. 2002

3. **Cause of pre-term delivery**
   - Bondevik et al. 2001
   - Ronnenberg et al. 2002
Studies on vitamin B12 status and NTDs

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Sample</th>
<th>Time of sampling</th>
<th>Cases / controls</th>
<th>Cases/controls pmol/L OR highest to lowest quantile</th>
<th>95% CI</th>
<th>Significant (yes/no) ; P (if given)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suarez 2003</td>
<td>Texas-Mexico border (1995-2000)</td>
<td>Serum B12</td>
<td>Post-partum</td>
<td>225/378</td>
<td>317/367 (median)</td>
<td>3.0</td>
<td>1.4-6.3</td>
</tr>
<tr>
<td>Molloy 2009 (3 cohorts)</td>
<td>(i) Ireland (1983-1984)</td>
<td>Serum B12</td>
<td>15 weeks Median</td>
<td>95/265</td>
<td>155/179 (median)</td>
<td>3.14</td>
<td>1.46-6.72</td>
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<tr>
<td></td>
<td>(ii) Ireland (1986-1990)</td>
<td>Plasma B12</td>
<td>15 weeks Median</td>
<td>76/222</td>
<td>180/221 (median)</td>
<td>2.45</td>
<td>1.12-5.32</td>
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<td></td>
<td>(iii) Ireland (1986-1990)</td>
<td>Plasma B12</td>
<td>15 weeks Median</td>
<td>107/414</td>
<td>199/232 (median)</td>
<td>2.75</td>
<td>1.43-5.28</td>
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<tr>
<td>Ray 2007</td>
<td>Canada (1993-2004)</td>
<td>Serum holoTC</td>
<td>15-20 weeks</td>
<td>89/422</td>
<td>68/81 (geometric mean)</td>
<td>2.9</td>
<td>1.2-6.9</td>
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<td>Zhang 2008</td>
<td>China (Shanxi) (2004-2005)</td>
<td>Serum B12</td>
<td>20 weeks median</td>
<td>84/110</td>
<td>73/91 (geometric mean)</td>
<td>4.96</td>
<td>1.94-12.7</td>
</tr>
<tr>
<td>Mills 1992</td>
<td>Finland (1983-89)</td>
<td>Serum B12</td>
<td>6-16 weeks</td>
<td>78/150</td>
<td>356/384 (mean)</td>
<td></td>
<td>No</td>
</tr>
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<td>Christensen 1999</td>
<td>Canada (pre-1998)</td>
<td>Non pregnant</td>
<td></td>
<td>59/88</td>
<td>298/350 (mean)</td>
<td></td>
<td>Borderline; p=0.05</td>
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<tr>
<td>Gene</td>
<td>Enzyme</td>
<td>Association with NTDs</td>
<td>Reference</td>
<td></td>
<td></td>
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<tr>
<td>MTR</td>
<td>Methionine Synthase D919G</td>
<td>No independent association. May interact with other genes as a maternal risk factor</td>
<td>Brody et al., 1999; Christensen et al., 1999; Johanning et al., 2000; Doolin et al., 2002; Zhu et al., 2003</td>
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<tr>
<td>MTRR</td>
<td>Methionine synthase reductase I22M</td>
<td>Several studies show case or maternal risk associations and possible interactive effects with low B12 or other genes</td>
<td>Wilson et al., 1999; Zhu et al., 2003; Relton et al., 2004b; O’Leary et al., 2005b; van der Linden et al., 2006; Candito et al., 2008.</td>
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<tr>
<td>TCII</td>
<td>Transcobalamin II R259P</td>
<td>Significant association in a small study of NTD mothers but no risk association in larger studies. However, several studies show changes in transcobalamin II concentrations in serum or in amniotic fluid of NTD affected mothers</td>
<td>Pietrzyk and Bik-Multanowski, 2003; Ray and Blom, 2003; Swanson et al., 2005; Boyles et al., 2006. Pangilinan et al 2008</td>
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<td>TCII RE88Del</td>
<td>ECD320; Transcobalamin Receptor</td>
<td>Low frequency high penetrance factor. Cause of elevated MMA in neonatal screen</td>
<td>Pangilinan et al 2010 Quadros et al 2010</td>
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<td>CUBN</td>
<td>Cubilin Rs1907362 rs7070148 rs2273737</td>
<td>Two studies of candidate genes: (i) analysis of 45 candidate genes found rs1907362 (ii) Analysis of 82 candidate genes found rs 7070148 and rs 2273737 among 10 top independent association signals</td>
<td>Franke et al 2009 Pangilinan et al 2012</td>
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</table>
The G220R polymorphism is a highly significant risk factor for NTDs.

The RR genotype was not detected in nearly 1,000 controls, indicating a low frequency, high penetrance factor.

E88Del is also a low frequency, high penetrance factor – but the analysis did not survive correction for multiple testing.

Further work is required to firmly establish the disease conferring variant.
Maternal Vitamin B₁₂ Status and Risk of Neural Tube Defects in a Population With High Neural Tube Defect Prevalence and No Folic Acid Fortification

Anne M. Molloy, PhD®, Peadar N. Kirke, FFPhM®, James F. Troendle, PhD®, Helen Burke, BScS®, Marie Sutton, MB, MPH®, Lawrence C. Brody, PhD®, John M. Scott, ScD®, James L. Mills, MD, MS®

Conclusion

1. Low maternal B12 in early pregnancy is significantly associated with increased risk for NTD

2. Women should aim to enter pregnancy with vitamin B12 status above 250 pmol/L

3. There is no evidence that improving B12 beyond this level would be protective
<table>
<thead>
<tr>
<th>Dietary Folate Equivalents</th>
<th>All Ages</th>
<th>18-50 y</th>
<th>51-64 y</th>
<th>≥65 y</th>
<th>18-50 y</th>
<th>51-64 y</th>
<th>≥65 y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>312 (228, 448)</td>
<td>372 (280, 506)</td>
<td>351 (265, 497)</td>
<td>314 (214, 470)</td>
<td>260 (192, 349)</td>
<td>297 (225, 423)</td>
<td>277 (198, 391)</td>
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<tr>
<td>Natural</td>
<td>223 (173, 286)</td>
<td>274 (204, 344)</td>
<td>271 (213, 325)</td>
<td>223 (184, 274)</td>
<td>189 (151, 231)</td>
<td>207 (171, 268)</td>
<td>210 (158, 248)</td>
</tr>
<tr>
<td>Total Folic Acid</td>
<td>64 (14, 167)</td>
<td>74 (20, 175)</td>
<td>68 (9, 205)</td>
<td>71 (12, 183)</td>
<td>52 (13, 135)</td>
<td>66 (24, 196)</td>
<td>63 (12, 177)</td>
</tr>
<tr>
<td>Fortified Foods</td>
<td>50 (9, 118)</td>
<td>58 (12, 125)</td>
<td>60 (8, 169)</td>
<td>64 (11, 180)</td>
<td>35 (6, 87)</td>
<td>40 (9, 109)</td>
<td>47 (0, 138)</td>
</tr>
</tbody>
</table>

| Consumers of Folic Acid, % | | | | | | | |
| Fortified Foods | 79 | 80 | 80 | 81 | 78 | 80 | 74 |
| Supplements | 14 | 14 | 8 | 8 | 16 | 20 | 14 |

| Vitamin B-12 Intakes, μg/d | | | | | | | |
| Total | 4.2 (2.9, 6.1) | 5.0 (3.4, 7.0) | 4.8 (3.3, 6.9) | 4.8 (2.9, 6.6) | 3.5 (2.4, 4.8) | 4.2 (3.2, 6.0) | 4.1 (2.8, 5.6) |
| Natural | 3.7 (2.5, 5.1) | 4.3 (3.0, 5.9) | 4.4 (2.8, 6.1) | 4.2 (2.8, 5.6) | 2.9 (2.0, 4.0) | 3.8 (2.7, 4.8) | 3.7 (2.7, 4.8) |
| Total Synthetic | 0.3 (0.0, 0.8) | 0.3 (0.0, 0.8) | 0.2 (0.0, 0.9) | 0.2 (0.0, 1.2) | 0.2 (0.0, 0.7) | 0.3 (0.0, 0.9) | 0.2 (0.0, 0.8) |
| Fortified Foods | 0.1 (0.0, 0.5) | 0.2 (0.0, 0.5) | 0.2 (0.0, 0.8) | 0.2 (0.0, 1.0) | 0.1 (0.0, 0.4) | 0.1 (0.0, 0.5) | 0.1 (0.0, 0.4) |

| Consumers of Synthetic Vitamin B-12, % | | | | | | | |
| Fortified Foods | 65 | 67 | 63 | 65 | 64 | 63 | 60 |
| Supplements | 14 | 14 | 7 | 8 | 15 | 19 | 14 |
The proportion of women aged 18-50 years with optimal B12 or RCF status by voluntary fortified food consumption.
Some Conclusions

• Up to 17% of women of child-bearing age do not have adequate vitamin B12 status. The consequences of maternal B12 deficiency (or inadequate status) to the mother or to the neonate are still not well defined.

• Diets chronically low in B12 are very common in some areas of the world, and more prevalent than expected in developed countries. The effects of such diets on reproduction need to be considered by the relevant care authorities.
Some Questions

• Should women be encouraged to take B12 supplements

• How much Vitamin $B_{12}$ daily would be required to move them to higher status, offering optimum protection?

• Why have women got low serum B12 status if they appear to be consuming the RDA — is the RDA for B12 wrong?
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