Vitamin B12 deficiency in phenylketonuria and impact on brain function

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• B12 not involved with phe catabolism

• metabolism of B12 not affected by high phe levels
case report

Farriaux et al, Semin Hop Paris, 1983

- PKU patient with macrocytic anaemia & megaloblastic bone marrow
- methylmalonic acid in urine
- low normal serum B12 level
Case report

• 18 yr old woman
  – poorly compliant PKU
  – slowly progressive spastic paraparesis
  – megaloblastic anaemia
  – serum B12 65.8 pmol/l (150-700)
  – rapid improvement in anaemia with oral $B_{12}$, incomplete resolution of neurological disease

Hanley et al, Lancet 1993
• is B12 deficiency common in PKU?

• if so, are there significant consequences?
• Digestion & Absorption
  – R binder
  – intrinsic factor
  – cubulin receptor
• Transport
  – TC I/II/III
• Cellular processing
  – lysosomal
  – cytoplasmic methylation
  – mitochondrial adenosylation
B12

- total body stores: 2.5 mg (50% in liver)
- recommended daily intake:
  - children: 0.7 µg
  - adults & adolescence: 2 µg
  - pregnancy & lactation: 2.6 µg
recommended daily intake:
children: 0.7 µg
adults & adolescence: 2 µg
pregnancy & lactation: 2.6 µg
### Vitamin B₁₂ µg

<table>
<thead>
<tr>
<th></th>
<th>Per 100g of tablets</th>
<th>Per 5 tablets*</th>
<th>Per tablet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B₁₂ µg</td>
<td>58.8</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

**Recommended Daily Intake:**
- Children: 0.7 µg
- Adults & Adolescence: 2 µg
- Pregnancy & Lactation: 2.6 µg
- reduced purine synthesis
  - nucleotide synthesis, integrity, transcription
- reduced methyl donors
  - DNA methylation
  - non genomic methylation; >100 SAM mediated methylation in numerous neural pathways (eg myelin basic protein, membrane phospholipids)
- increased homocysteine
  - vascular disease
  - DNA damage in CNS
- increased methylmalonate
  - ?adverse effects
B12 deficiency

- functional deficiency of methionine synthase and methylmalonyl mustase
- also disturbs a number of cytokines and growth factors
  - TNF alpha
  - nerve growth factor
  - IL-6
  - epidermal growth factor
- deceased mRNAs of
  - glial fibrillary acidic protein
  - myelin basic protein
laboratory abnormalities

- low plasma B12
- macrocytic / megaloblastic anaemia
- increased blood homocysteine
- increased urine/plasma methylmalonate
vitamin B12 deficiency

- peripheral neuropathy
- subacute combined degeneration of the cord (posterior & lateral columns)
- autonomic dysfunction
- optic atrophy
- psychiatric illness
neurological findings

- symmetrical distal sensory manifestations & ataxia
- diminished vibration sense & proprioception
- limb reflexes exaggerated, diminished or absent
- spastic paraparesis (lateral column involvement)
• 37 adults & adolescents with PKU
  – 6 (16%) low B12 (<150 pmol/l)
  – 6 (16%) borderline (150-200 pmol/l)
  – 1 had low Hb, & 8 MCV >94fl
  – MMA and hct not measured
  – no neurological abnormalities

• recommended routine measurement of B12, folate MMA & total Hct

case report

• 19 yr old male with PKU, presented with tiredness poor memory & concentration. Pale smooth red tongue. Normal neurological examination

• Past medical history
  – stopped diet at 14 and lost to follow up
  – ‘maintained dietary compliance’

Aung et al, JIMD 1997
• mebalobastic/macrocytic anaemia (Hb 7.3g/dl)
• serum $\mathrm{B}_{12}$ 125 pg/ml (200-900)
• serum folate 3.4 ng/ml (1.5-20.6)
• normal Schilling test

• started on oral B12 – after 1 month anaemia resolved

Aung et al, JIMD 1997
Hypothesis: stopping or relaxation diet predisposes to B12 deficient because of poor dietary intake.
Food sources of vitamin B12:

Eggs, meat, poultry, shellfish, milk and milk products
<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Mean B12 (ng/l)</th>
<th>SD</th>
<th>P value</th>
<th>&lt;180 ng/l</th>
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</thead>
<tbody>
<tr>
<td>Normal population</td>
<td>1676</td>
<td>411.9</td>
<td>149</td>
<td></td>
<td></td>
</tr>
<tr>
<td>unrestricted</td>
<td>31</td>
<td>275</td>
<td>95</td>
<td>&lt;0.001</td>
<td>6</td>
</tr>
<tr>
<td>relaxed</td>
<td>30</td>
<td>333</td>
<td>128</td>
<td>0.003</td>
<td>3</td>
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<tr>
<td>strict</td>
<td>22</td>
<td>469</td>
<td>200</td>
<td>0.77</td>
<td>1</td>
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</tbody>
</table>

Robinson et al, J Pediatr 2000
case series

- 31 adult patients, (24 taking PreKUnil®)
- age range 18-43
- None had overt clinical B\textsubscript{12} deficiency but 13 had one neurological symptom (9 with paraesthesia)
- 24 (77%) biochemical evidence of low B\textsubscript{12}
  - 9 serum cbl <200 pmol/l (1 < 150 pmol/l)
  - 15 serum holoTC <50 pmol/l
- 9 (29%) plasma homocysteine > 12µmol/l
- 11 (39%) took less than the daily recommended B\textsubscript{12} and 19 (61%) B\textsubscript{6}

Hyas et al, JIMD 2006
• Definition of vitamin $B_{12}$ deficiency?
  – Low serum cobalamin
    • Large between-laboratory variation
  – Low holotranscobalamin
  – Biochemical or haematological abnormalities
  – Clinical abnormalities
75 PKU patients (1-37 yr)

Increased concentrations in 10 (15%): 5 of serum mma/3 hcy/2 both

- Normal concentrations of Hcy/MMA (49)
- Unknown concentrations of Hcy/MMA (8)

Increased concentrations in 2 (25%): 1 of serum mma/1 hcy

- Normal concentrations of Hcy/MMA (6)
- Unknown concentrations of Hcy/MMA (0)

Vugteveen et al, Mol Gen Metab, 2011
• serum MMA or hcy
  – is a reliable indicator of B12 deficiency (rather than serum vit B12)
  – should be monitored regularly in PKU

Vugteveen et al, Mol Gen Metab, 2011
Low B12 in PKU – does it matter?

• Brisk reflexes
• Resting/intention tremor
• Depression
• MRI abnormalities
<table>
<thead>
<tr>
<th>Subject</th>
<th>Age at diagnosis</th>
<th>Blood Phe at diagnosis (μmol/l)</th>
<th>Age diet stopped (yr)</th>
<th>IQ at school (WISC)</th>
<th>Late neurological illness</th>
<th>Clinical features</th>
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<tbody>
<tr>
<td>1</td>
<td>1 wk</td>
<td>3630</td>
<td>18</td>
<td>94</td>
<td>13</td>
<td>Quadriparesis, ataxia, epilepsy</td>
</tr>
<tr>
<td>2</td>
<td>5 wk</td>
<td>&gt;1200</td>
<td>18</td>
<td>ND</td>
<td>20</td>
<td>Paraparesis</td>
</tr>
<tr>
<td>3</td>
<td>6 wk</td>
<td>2880</td>
<td>18</td>
<td>64</td>
<td>20</td>
<td>Epilepsy</td>
</tr>
<tr>
<td>4</td>
<td>1 yr</td>
<td>1420</td>
<td>7</td>
<td>95</td>
<td>25</td>
<td>Quadriparesis, dystonia, epilepsy</td>
</tr>
<tr>
<td>5</td>
<td>2 yr</td>
<td>1026</td>
<td>7</td>
<td>45</td>
<td>20</td>
<td>Quadriparesis, sensory changes</td>
</tr>
<tr>
<td>6</td>
<td>15 mo</td>
<td>1360</td>
<td>7</td>
<td>45</td>
<td>15</td>
<td>Tremor, paraparesis, dystonia</td>
</tr>
</tbody>
</table>

B12, hct, MMA not reported

Thompson et al, Lancet 1990
Final case report

- Born in 1978
- Poor control after 4 years of age
- IQ at 8 yrs 91.
- Well apart from recurrent ear infections

• 14 yrs myringoplasty under nitrous oxide GA
• Irritable and disorientated after procedure
• After 1 week walking became clumsy
• After 3 weeks confused, episodes of urinary incontinence

• Examination:
  – Parkinsonian facies
  – Resting tremor
  – Lower limb hypertonia
  – Extensor plantar & sustained ankle clonus

• Investigations
  – Phe 1490 µM
  – Hb 8.4 g/dl; MCV 114.9 fl
  – Red cell folate 112 ng/ml (150-600)
  – B12 < 110 pg/ml (150-700)
  – MR increased periventricular signal on T2

Nitrous oxide forms complex with cobalt(I) in methylcobalamin resulting in inactivation of the enzyme.

Formation of active enzyme requires new protein synthesis, as well as cobalamin supply

Those with low or marginal cobalamin are at risk.
Conclusions

• Those off diet at risk
• Unclear whether major causative factor in those with severe neurological deterioration
• Serum assay is an insensitive marker & anaemia may not be present - measure MMA & hct
• Ensure adequate intake & continue long term follow up of all adults