THE VITAMIN B12 STORY: WHY IS IT STILL A CONCERN?

E Haddad, DrPH, RD
6th International Congress on Vegetarian Nutrition
Loma Linda, CA
Feb 26, 2013
Objectives

- Review the reasons why vitamin B12 nutritional status continues to be a relevant issue
- Examine how dietary patterns influence B12 status in participants of the Calibration Study sub-study of the Adventist Health Study (AHS-2)
- Derive guidelines for practice
- Identify areas of future research
B12 status is of critical concern because

- Deficiency and borderline status are common both in developing and developed countries.
- Borderline B12 status is more common than previously thought.
- B12 nutritional status is important throughout the life cycle especially in
  - Pregnancy and the neonatal period
  - Elderly
- In the elderly, low status worsens functional decline in cognition and cardiovascular disease.
Recommendation for individuals ages 50 and older

“Consume foods fortified with vitamin B12 such as fortified cereals and dietary supplements.”
Attached to central cobalt:

α axial ligand: 5,6-dimethylbenzimidazole nucleotide

β axial ligand (X): any of several moieties such as methyl, 5’-deoxyadenosyl, hydroxyl or cyanide

Ultimate source: Synthesis by bacteria and archaea
Role in humans

- **Biochemical role:** cofactor for 2 different enzymes
  - *Methionine synthase*
    - homocysteine $\rightarrow$ methionine
  - *Methylmalonyl-CoA mutase*
    - methylmalonyl CoA $\rightarrow$ succinyl CoA

- **Physiological**
  - Protein synthesis, DNA synthesis, hematological development, etc.
  - Development and initial myelination of the central nervous system
B12 uptake in GI tract

**Mouth** (saliva)
- Haptocorrin (HC)

**Stomach**
- Release of protein-bound B12
- Haptocorrin HC
- Intrinsic factor IF

**Jejunum** (pancreatic secretions)
- Binding to IF

**Ileal receptor** (cubilin, amnionless)
- Uptake into intestinal cell
The efficiency of absorption of a single oral dose of vitamin B-12 across a range of intakes.
Causes of B12 Deficiency*

- **Severe malabsorption**
  - Pernicious anemia (autoimmune gastritis)
  - Gastric or ileal surgery
  - Inflammatory bowel disease
  - Imerslund-Gräsbeck and other syndromes

- **Mild malabsorption**
  - Protein-bound cobalamin malabsorption
  - Atrophic gastritis
  - Use of drugs that block stomach acid

- **Dietary**
  - Vegetarian or vegan
  - Breast-feeding infants of B12 deficient mothers

- **Recreational or occupational abuse of nitrous oxide or nitrous oxide anesthesia**

*Adapted from Stabler, SP, New Engl J Med 368:2, 2013.
How common is B12 deficiency?

- In the elderly population (age 60+)
  - National Health and Nutrition Examination Survey (NHANES)
    - Deficiency ~ 6%
    - Marginal depletion ~20%

- In Vegetarians
  - Review by Pawlak et al (2013)
    - Children ~ 25-86%
    - Adolescents ~ 21-41%
    - Pregnant women ~ 62%
    - Elderly ~ 11-90%
Who are the AHS-2 “Calibration Study” participants?

A group of participants that were

- Randomly selected from the AHS-2 cohort
  - By church
  - By subject within church
- Attended a clinic
  - Medical exam
  - Bio-specimens
    - Blood
    - Urine
    - Adipose tissue
- Six 24-hour dietary recalls
Processing of biospecimens
# AHS-2 Calibration Study

## Dietary patterns (n= 949)

<table>
<thead>
<tr>
<th>Diet group</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegan</td>
<td>9.3%</td>
</tr>
<tr>
<td>No dairy, eggs, meat, poultry or fish</td>
<td></td>
</tr>
<tr>
<td>Lacto-ovo vegetarian</td>
<td>27.9%</td>
</tr>
<tr>
<td>Dairy and eggs but no meat, poultry or fish</td>
<td></td>
</tr>
<tr>
<td>Pesco vegetarian</td>
<td>11.5%</td>
</tr>
<tr>
<td>Fish, dairy and eggs but no meat or poultry</td>
<td></td>
</tr>
<tr>
<td>Semi vegetarian</td>
<td>4.4%</td>
</tr>
<tr>
<td>Meat, poultry or fish 1-3 times per month</td>
<td></td>
</tr>
<tr>
<td>Non-vegetarian</td>
<td>46.8%</td>
</tr>
<tr>
<td>Meat, poultry and/or fish &gt;1 time per week</td>
<td></td>
</tr>
</tbody>
</table>
Subject characteristics by diet pattern

<table>
<thead>
<tr>
<th>Subjects (N=949)</th>
<th>Vegan N=82</th>
<th>Lacto-ovo N=245</th>
<th>Pesco N=101</th>
<th>Semi N=39</th>
<th>Non-vegetarian N=412</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, (y) Mean±SD</td>
<td>63.7±12.7</td>
<td>60.1±14.0</td>
<td>57.2±12.8</td>
<td>56.9±13.3</td>
<td>56.4±12.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male, %</td>
<td>26.8</td>
<td>38.4</td>
<td>30.7</td>
<td>35.9</td>
<td>31.9</td>
<td>0.274</td>
</tr>
<tr>
<td>Female, %</td>
<td>73.2</td>
<td>61.6</td>
<td>69.3</td>
<td>64.1</td>
<td>68.1</td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White, %</td>
<td>58.5</td>
<td>75.1</td>
<td>40.6</td>
<td>69.3</td>
<td>47.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Black, %</td>
<td>41.5</td>
<td>24.9</td>
<td>59.4</td>
<td>30.7</td>
<td>52.3</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m2 Mean±SD</td>
<td>25.3±5.8</td>
<td>25.9±5.7</td>
<td>27.4±5.8</td>
<td>26.9±4.7</td>
<td>30.2±7.3</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Vitamin B-12 Status Measurements

- Serum vitamin B-12 (B-12)
  - Enzyme immunoassay (Monobind, CA)
- Serum holotranscobalamin (holoTC)
  - Enzyme immunoassay (Axis-Shield, UK)
- Plasma homocysteine (tHCY)
  - Enzymatic colorimetric assay (Pointe Scientific, MI)

*Have not yet measured methyl malonic acid!
Cobalamin (vitamin B-12) and its binding proteins in human plasma

- Apohaptocorrin
- Analog-haptocorrin
- B-12-haptocorrin
- B12-Holotranscobalsmin
- Apotranscobalamin

Total B12
Biomarkers of B-12 status according to age, gender and race

<table>
<thead>
<tr>
<th></th>
<th>Serum B-12 (pg/mL)</th>
<th>Serum HoloTC (pg/mL)</th>
<th>Plasma tHCY (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;60 y</td>
<td>352 ± 192</td>
<td>97.9 ± 49.4</td>
<td>13.1 ± 4.6</td>
</tr>
<tr>
<td>&gt;60 y</td>
<td>371 ± 222</td>
<td>107.7 ± 55.4</td>
<td>14.7 ± 4.2</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>330 ± 195</td>
<td>93.5 ± 50.2</td>
<td>15.2 ± 4.8</td>
</tr>
<tr>
<td>Female</td>
<td>376 ± 210</td>
<td>106.7 ± 53.0</td>
<td>13.1 ± 4.2</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>363 ± 244</td>
<td>97.6 ± 53.6</td>
<td>14.0 ± 4.5</td>
</tr>
<tr>
<td>Black</td>
<td>358 ± 146</td>
<td>105.4 ± 51.9</td>
<td>13.5 ± 4.6</td>
</tr>
</tbody>
</table>

P-values: Age <60 y vs. >60 y: P=0.164, Gender Male vs. Female: P=0.001, Race White vs. Black: P=0.753.
# Mean serum B-12 and HoloTC

<table>
<thead>
<tr>
<th>Diet Type</th>
<th>Serum B-12, ng/L</th>
<th>Serum Holotranscobalamin, ng/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegan</td>
<td>378</td>
<td>144</td>
</tr>
<tr>
<td>Lacto-ovo</td>
<td>379</td>
<td>145</td>
</tr>
<tr>
<td>Pesco</td>
<td>372</td>
<td>152</td>
</tr>
<tr>
<td>Semi</td>
<td>305</td>
<td>124</td>
</tr>
<tr>
<td>Non-vegetarian</td>
<td>352</td>
<td>134</td>
</tr>
</tbody>
</table>

\[ P=0.195 \quad P=0.065 \]
<table>
<thead>
<tr>
<th>Diet Group</th>
<th>Serum B12 &lt;148 pmol/L (&lt;200 pg/mL)</th>
<th>Serum HoloTC &lt;40 pmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegan</td>
<td>13.4%</td>
<td>5.0%</td>
</tr>
<tr>
<td>Lacto-ovo</td>
<td>9.4%</td>
<td>7.6%</td>
</tr>
<tr>
<td>Non-vegetarian</td>
<td>5.8%</td>
<td>6.7%</td>
</tr>
</tbody>
</table>

P = 0.0048

P = 0.325
Percentage of individuals with high plasma homocysteine according to diet group

**Homocysteine ≥ 15 µmol/L**

- **Vegan**: 45.2%
- **Lacto-ovo**: 30.3%
- **Pesco**: 32.1%
- **Semi**: 31.4%
- **Non-vegetarian**: 33.8%

*P=0.238*
“Percent deficient” varies with various cut-off points

**Cut-off points**

- **< 148 pmol/L**
  - Vegan: 13%
  - Lacto-ovo: 9%
  - Non-vegetarian: 6%

- **< 200 pmol/L**
  - Vegan: 27%
  - Lacto-ovo: 23%
  - Non-vegetarian: 25%

- **< 222 pmol/L**
  - Vegan: 43%
  - Lacto-ovo: 39%
  - Non-vegetarian: 39%
Vitamin B12 supplement users (%) according to diet group

- Vegan: 27.5%
- Lacto-ovo: 13.5%
- Non-vegetarian: 11.1%
Effect of B12 supplement use on % “deficient” according to diet pattern

<table>
<thead>
<tr>
<th></th>
<th>Non-user B-12 supplement (n = 728)</th>
<th>User B-12 supplement (n=111)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum B-12 &lt; 200 pg/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegan</td>
<td>13.3</td>
<td>13.6</td>
</tr>
<tr>
<td>Lacto-ovo</td>
<td>9.4</td>
<td>9.7</td>
</tr>
<tr>
<td>Non-vegetarian</td>
<td>6.1</td>
<td>3.6</td>
</tr>
<tr>
<td>Serum HoloTC &lt; 40 pg/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegan</td>
<td>5.2</td>
<td>4.5</td>
</tr>
<tr>
<td>Lacto-ovo</td>
<td>8.3</td>
<td>3.1</td>
</tr>
<tr>
<td>Non-vegetarian</td>
<td>7.6</td>
<td>1.8</td>
</tr>
<tr>
<td>Plasma homocysteine &gt;15µmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegan</td>
<td>47.2</td>
<td>40.0</td>
</tr>
<tr>
<td>Lacto-ovo</td>
<td>31.6</td>
<td>21.4</td>
</tr>
<tr>
<td>Non-vegetarian</td>
<td>29.4</td>
<td>28.8</td>
</tr>
</tbody>
</table>
Mean ± SD dietary intake of B12 from foods, fortified foods, and supplements according to diet patterns

Dietary Vitamin B12 (Mean ± SE)

- Non-vegetarian
- Semi
- Pesco
- Lacto-ovo
- Vegan

P=0.001
Response of serum B12 and HoloTC to increasing dietary B12 consumption

Serum B12

Serum HoloTC

P<0.05
Response of plasma homocysteine to increasing dietary B12 consumption

Plasma homocysteine

Dietary B12, µg/day

2-4 4-6 6-10 10-25 25-100 100+

Response of plasma homocysteine to increasing dietary B12 consumption
Percent showing “deficient” and “borderline” status with increasing intake of dietary B12

<table>
<thead>
<tr>
<th></th>
<th>Vegan</th>
<th>Lacto-ovo</th>
<th>Non-vegetarian</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Deficient</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum B12 &lt; 148 pmol/L (&lt;200 pg/ml)</td>
<td>18%</td>
<td>8%</td>
<td>7%</td>
</tr>
<tr>
<td></td>
<td>22%</td>
<td>11%</td>
<td>7%</td>
</tr>
<tr>
<td></td>
<td>7%</td>
<td>7%</td>
<td>7%</td>
</tr>
<tr>
<td></td>
<td>0%</td>
<td>10%</td>
<td>20%</td>
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<tr>
<td></td>
<td>24%</td>
<td>23%</td>
<td>22%</td>
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<tr>
<td></td>
<td>17%</td>
<td>17%</td>
<td>25%</td>
</tr>
<tr>
<td></td>
<td>7%</td>
<td>9%</td>
<td>10%</td>
</tr>
<tr>
<td><strong>Borderline</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum B12 148-221 pmol/L (200-300 pg/ml)</td>
<td>24%</td>
<td>23%</td>
<td>22%</td>
</tr>
<tr>
<td></td>
<td>17%</td>
<td>17%</td>
<td>25%</td>
</tr>
<tr>
<td></td>
<td>7%</td>
<td>9%</td>
<td>10%</td>
</tr>
</tbody>
</table>
Low B12 and high homocysteine

- Increased risk
  - Vascular disease, thrombosis and stroke
  - Dementia and cognitive impairment

- Prospective
  - Framingham Heart Study (Morris MA et al, JAGS 60:1457, 2012)
  - Plasma B12 from 187 to 256.8 pmol/L predict more rapid cognitive decline
Summary
Calibration Study (AHS-2)

- Vitamin B12 deficiency is common in all participants of the study and more so in the vegetarian group.
- Elevated concentrations of homocysteine are common in all dietary groups.
- Reported vitamin B12 supplement use shows low frequency.
- Dietary intake of vitamin B12 from food, fortified food and supplements assessed by 2 sets of 3 weighted 24-hour recalls shows highest intake in vegans.
- The prevalence of vitamin B12 deficiency decreases with increasing dietary intake, but not in all individuals.
Where do we go from here?

- **Individuals**
  - Daily intake of a reliable source of vitamin B12
- **Health professionals**
  - Assess B12 status (Measure B12 and homocysteine or methylmalonic acid)
  - Determine and treat causes of low vitamin B12 status
  - High dose B12 therapy may improve neurologic function in the absence of B12 deficiency
- **Future studies**
  - How to effectively improve vitamin B12 status and lower homocysteine
  - How to reduce the ravages of cognitive impairment
  - Determine the mechanisms of apparent B12 resistance in the elderly and in those with diabetes and renal disease
  - Does RDA and EAR for B12 need to be increased?
Thanks

- The lab team
  - Lian Liu, Natalie Kazzi, Rawiwan Sirirat

- Statistician
  - Michael Batech

- Principle Investigator
  - Gary Frazer

- Grant Support
  - NIH AHS2 grant
  - McClean Fund, Department of Nutrition, LLU

- Inspiration
  - UD Register