Vitamin A: the first vitamin

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Outline: Vitamin A (and E)

- Background & Definitions
- Deficiency
  - WHO
- Toxicity
- Measurement
- Interpretation of results
  - Reference Intervals
  - Standardization
1912: “Vitamine”

- Casimir Funk
- 1884-1967

- Vital amine

- Described a growth factor present in food which was essential for life.

- Funk, C. (1912) J. State Med. 20, 341-368
1916: “Vitamine A & B”

THE DIETARY FACTORS OPERATING IN THE PRODUCTION OF POLYNEURITIS.*

BY E. V. McCOLLUM AND CORNELIA KENNEDY.

(From the Laboratory of Agricultural Chemistry of the University of Wisconsin, Madison.)

(Received for publication, February 29, 1916.)

- Elmer Vernon McCollum
- It became clear that there was more than one growth factor
- McCollum divided them into two classes:
  - ‘fat-soluble A’
  - ‘water-soluble B’.

http://www.mc.vanderbilt.edu/biolib/hc/nutrition/nh3.html
Definitions

Vitamin:
- An organic compound required as a nutrient, which cannot be synthesized in adequate amounts, and therefore must be obtained in the diet.

Vitamin A:
- 1960 - vitamin A was recognised to exist in 3 parent forms retinal, retinol and retinoic acid.
- Now the term vitamin A is used as “the generic description for retinoids exhibiting qualitatively the biological activity of retinol”[i].

Vitamin A

1. Intake
   - Retinyl esters (animal products)
   - Carotenoids esp β-carotene (pigmented fruit & vegetables)

2. Transportation
   - In plasma by retinol binding protein and pre-albumin

3. Storage
   - Retinol is the storage & transport form of vitamin A.
   - 90% liver

4. Action
   - Eye – retinal
   - Hormone activity – Retinoic acid has hormonal activity with nuclear (RAR) receptors and is fundamental to cellular division and differentiation
   - Immunity
   - Skin
Role of Vitamin A: the eye

- Retinol is oxidised in the rods of the eyes to retinal.
- Retinal complexes to opsin to form rhodopsin = dim light vision
- When retinal is depleted in the retina, opsin is destabilised and catabolised = permanent destruction of the rod cells.

Diagram of eye: http://www.bartsandthelondon.nhs.uk/llibrary/eye_structure.jpg
Vitamin A: Deficiency (VAD)

VAD and Cystic Fibrosis

- Historically, vitamin A deficiency is associated with the oldest genetic disease known to man
- Dorothy Anderson 1938 linked vitamin A deficiency with the fatty liver
- Essentially a Caucasian disease

At RCH approximately 50% of requests are for CF patients
Vitamin A Deficiency (VAD)

- Listed by WHO as a major health issue especially in developing countries
- Main cause of preventable childhood blindness
- Increased risk of morbidity and mortality
- Affects the most vulnerable - pre school children and pregnant women
- Worldwide public health problem – 190 million pre-school children vitamin A deficient
WHO: Vitamin A deficiency

1987
- WHO estimated that VAD endemic in 39 countries.
- Based on the ocular manifestations of xerophthalmia or
- Deficient serum (plasma) retinol concentrations (<0.35 µmol/L).

1995
- WHO updated these estimates
- VAD a public health significance in 60 countries
- Likely to be a problem in an additional 13 countries.

2009
- The current estimates reflect 1995 to 2005
- VAD is of public health significance in preschool children in:
  - 45 countries based on the prevalence of night blindness
  - 122 countries based on biochemical vitamin A deficiency (serum retinol <0.70 µmol/L).
Causes of death among pre-school children in non-Industrialized countries, 2000

- Malnutrition 60%
- ARI 20%
- Vitamin A deficiency increases risk of mortality with 23%
- Other 29%
- Perinatal 22%
- Measles 5%
- Diarrhoea 12%
- Malaria 8%
- HIV 4%

Ref.: WHO 2002

http://www.euro.who.int/ppt/nut/vad.pdf
Global Prevalence of VAD: Prevalence of serum retinol <0.70 µmol/L by region 1995-2005

<table>
<thead>
<tr>
<th>WHO region</th>
<th>Preschool-age children&lt;sup&gt;a&lt;/sup&gt;</th>
<th></th>
<th>Pregnant women</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prevalence&lt;sup&gt;b&lt;/sup&gt; (%)</td>
<td>Number affected (millions)</td>
<td>Prevalence (%)</td>
<td>Number affected (millions)</td>
</tr>
<tr>
<td>Africa</td>
<td>44.4 (41.3-47.5)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>56.4 (52.4-60.3)</td>
<td>13.5 (8.9-18.2)</td>
<td>4.18 (2.73-5.63)</td>
</tr>
<tr>
<td>Americas</td>
<td>15.6 (6.6-24.5)</td>
<td>8.68 (3.70-13.7)</td>
<td>2.0 (0.4-3.6)</td>
<td>0.23 (0.04-0.41)</td>
</tr>
<tr>
<td>South-East Asia</td>
<td>49.9 (45.1-54.8)</td>
<td>91.5 (82.6-100)</td>
<td>17.3 (0.0-36.2)</td>
<td>6.69 (0.00-14.0)</td>
</tr>
<tr>
<td>Europe</td>
<td>19.7 (9.7-29.6)</td>
<td>5.81 (2.87-8.75)</td>
<td>11.6 (2.6-20.6)</td>
<td>0.72 (0.16-1.29)</td>
</tr>
<tr>
<td>Eastern Mediterranean</td>
<td>20.4 (13.2-27.6)</td>
<td>13.2 (8.54-17.9)</td>
<td>16.1 (9.2-23.1)</td>
<td>2.42 (1.38-3.47)</td>
</tr>
<tr>
<td>Western Pacific</td>
<td>12.9 (12.3-13.5)</td>
<td>14.3 (13.6-14.9)</td>
<td>21.5 (0.0-49.2)</td>
<td>4.90 (1.00-11.2)</td>
</tr>
<tr>
<td>Global</td>
<td>33.3 (31.3-35.4)</td>
<td>190 (178-202)</td>
<td>15.3 (7.4-23.2)</td>
<td>19.1 (9.30-29.0)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Population subgroups: Preschool-age children (<5 years); Pregnant women (no age range defined).

<sup>b</sup> Numerator and denominator excludes countries with a 2005 GDP ≥US$ 15 000.

<sup>c</sup> 95% Confidence Intervals.
## Preschool Children with VAD

<table>
<thead>
<tr>
<th>Country</th>
<th>year</th>
<th>Prev/data</th>
<th>mean +/- SD umol/L</th>
<th>Vit A &lt;0.7 umol/L</th>
<th>Vit A &lt;0.35 umol/L</th>
<th>Current xerophthalmia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>1997</td>
<td>Low</td>
<td>1.29 +/- 0.28</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cambodia</td>
<td>2000</td>
<td>N</td>
<td>-</td>
<td>22.3%</td>
<td>2.2%</td>
<td>0.7%</td>
</tr>
<tr>
<td>China</td>
<td>2000</td>
<td>N</td>
<td>1.06 +/- 0.33</td>
<td>12.2%</td>
<td>-</td>
<td>0.14%</td>
</tr>
<tr>
<td>India</td>
<td>2003</td>
<td>SR</td>
<td>-</td>
<td>60%</td>
<td></td>
<td>0.3</td>
</tr>
<tr>
<td>Sri Lanka</td>
<td>1996</td>
<td>N</td>
<td>0.86</td>
<td>35%</td>
<td>9%</td>
<td>0.8%</td>
</tr>
<tr>
<td>Vietnam</td>
<td>1998</td>
<td>S</td>
<td>1.03 +/- 0.30</td>
<td>10.8%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Malaysia</td>
<td>2000</td>
<td>N</td>
<td>1.33 +/- 0.44</td>
<td>3.5%</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
Vitamin A deficiency in Viet Nam

- <0.35 µmol/L associated with symptoms
- Supplementation associated with immunization program
- Supplementation Schedule:
  6-11, 12-17, 18-23, 24-29, 30-36 months of age
- Classified by WHO (2007) as sub-clinical for vitamin A deficiency
<table>
<thead>
<tr>
<th>Sibling</th>
<th>Age</th>
<th>Sex</th>
<th>Vit A µmol/L</th>
<th>Vit D nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12y</td>
<td>M</td>
<td>0.8</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>7y</td>
<td>M</td>
<td>0.6</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>5y</td>
<td>F</td>
<td>0.6</td>
<td>17</td>
</tr>
<tr>
<td>4</td>
<td>1y</td>
<td>M</td>
<td>0.6</td>
<td>10</td>
</tr>
</tbody>
</table>
Vitamin A: Toxicity
Vitamin A: Toxicity

- Levels not well defined
- Acute:
  - 20 – 100x the RDI
- Chronic: Daily intakes of:
  - 25 000 IU for 6 years
  - 100,000 IU for 6 months
  - Serum levels may be in RI
- Fasting retinyl ester concentrations >10% of total circulating vitamin A
- Vitamin A (retinol esters) could be a biomarker for toxicity

*Am J Clin Nutr* 2006;83:191–201
The case for monitoring

- Narrow therapeutic range
- Supplementation should be monitored
- Retinol stored in the liver as retinoic acid
- Post prandial levels increase
Vitamin case 2: 17 y.o. male with CF

<table>
<thead>
<tr>
<th>Test</th>
<th>Vit A µmol/L</th>
<th>Vit E µmol/L</th>
<th>Chol mmol/L</th>
<th>Trig mmol/L</th>
<th>Ratio µmol/mmol</th>
<th>Vit D nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>66</td>
</tr>
<tr>
<td>2007</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>17</td>
</tr>
<tr>
<td>2009</td>
<td>4.5</td>
<td>22</td>
<td>2.9</td>
<td>1.6</td>
<td>4.9</td>
<td>83</td>
</tr>
<tr>
<td>RI</td>
<td>0.9-2.5</td>
<td>13-24</td>
<td>3.1-5.4</td>
<td>0.9-2.0</td>
<td>0.9-7.1</td>
<td>50-160</td>
</tr>
</tbody>
</table>
Measurement

Serum vitamin A and E analysis: comparison of methods between laboratories enrolled in an external quality assurance programme

Ronda Greaves¹, Lisa Jolly², Gerald Woollard³ and Kirsten Hoad⁴

Sample collection

i. Serum or plasma
ii. Protect from light
iii. Freeze post separation at -20°C until analysis
iv. Stable with freeze-thaw cycles
v. Fasting sample ideal

ii. Effects of Preanalytical Variables on Clinical Laboratory Tests; AACC press
Vitamin A (& E) analysis

Agilent HPLC 1200 & 1100 series

Sample preparation
- Protein precipitation
- Liquid extraction with hexane

HPLC
- Reverse phase C18
- Isocratic/gradient
- Methanolic MP
- Vitamin A: 325 nm
- Vitamin E: 292 nm

Degasser
Quaternary pump
Autosampler
Column Oven
PDA or UV/Vis detector
Chromatography

α Tocopherol (R1, R2 & R3 = CH3)

Retinol
Fat soluble vitamins with MS

- e.g. Waters application
  - UPLC & PDA + MS
  - Gradient elution
    - MP A = 10%ACN + 90%H₂O
    - MP B = 50%ACN + 50%H₂O
  - 5 min per sample

- No RCPA-QAP enrolled laboratories using MS

- Potential future direction for analysis

Waters application note: *Analysis of fat soluble vitamins using UPLC-PDA-SQD*
Interpretation of Results
Adult reference intervals

Results from 2007 RCPA-QAP vitamin A & E questionnaire
Method Standardisation

- Common Calibrator
- Common Method
- Common Reference Intervals

With standardization, results from one lab can be directly compared to another lab - improved patient care.
Summary of vitamin A

- The first vitamin
  - Deficiency linked to the oldest genetic disease known to man still in existence
  - First in the vitamin alphabet
  - (But actually not the first vitamin recognised!)
  - The leading vitamin deficiency worldwide

- WHO recognizes VAD to be in epidemic levels in some developing countries

- WHO levels of 0.7 and 0.35 umol/L well defined - ? method

- Supplementation should be monitored because of narrow therapeutic range

- Method standardization essential for accurate interpretation.
  
  "With standardization, results from one lab can be directly compared to another lab = improved patient care."

Members of the AACB vitamins working party 2009

AACB vitamins working party aiming to improve laboratory performance through:

- Support of QAP
- Consensus method
- Standardization
- Peer support

L to R: Chris Salonikas, Lisa Jolly, Kirsten Hoad, Ronda Greaves, Trevor Walmsley, Lambro Johnson, Gerald Woollard & Scott Briscoe (not pictured)