Outline

- Discovery and definition
- Vitamin A and E
- Vitamin D
- External QA vitamin programs
Discovery and definition
1912: “Vitamine”

Casimir Funk
1884-1967

Vital amine

Describes 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
Vitamins today!

Vitamin:
- A  Retinyl acetate
- B1  Thiamine nitrate
- B2  Riboflavine
- B3  Nicotinamide
- B5  Pantothenic acid
- B6  Pyridoxine HCl
- B12  Cyanocobalamin
- B9  Folic acid
- C  Ascorbic acid
- D3  Cholecalciferol
- E  d-α-tocopheryl acid succinate

Biotin

Trace metals / elements

Definition of a vitamin

- An organic compound required as a nutrient.
- It cannot be synthesized in adequate amounts.
- Therefore it must be obtained from the diet.
- What is considered a vitamin varies between organisms.

- NB: Vitamin D is needed in the human diet only in certain circumstances
### Water & Fat soluble vitamins

#### WATER
- **B group vitamins**
  - Vitamin B1 - thiamine
  - Vitamin B2 - ribofavine
  - Vitamin B6
  - Vitamin B12
  - Vitamin B9 - folate
- Vitamin C

#### FAT
- Carotenoids
- Vitamin A
- Vitamin D
- Vitamin E
- Vitamin K
- Coenzyme Q10
Vitamin A & E
Vitamin A

Retinol carried in plasma by retinol binding protein

Prevents night blindness:

- Retinal complexes to opsin to form rhodopsin = dim light vision
- When retinal is depleted in the retina, opsin is destabilised & catabolised = permanent destruction.
Vitamin A: Deficiency

- 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 – 254 million pre-school children vitamin A deficient
Causes of death among pre-school children in non-Industrialized countries, 2000

- Malnutrition: 60%
- Perinatal: 22%
- Diarrhoea: 12%
- Measles: 5%
- HIV: 4%
- Malaria: 8%
- ARI: 20%
- Other: 29%

Vitamin A deficiency increases risk of mortality with 23%

Ref.: WHO 2002

http://www.euro.who.int/ppt/nut/vad.pdf
Vitamin A deficiency in Viet Nam

- <0.3 µmol/L associated with symptoms
- Supplementation associated with immunisation 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

http://www.who.int/vaccines/globalsummary/immunization/countryprofileresult.cfm?C='vnm
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
### Vitamin E

![Chemical Structure of Vitamin E](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>R¹</th>
<th>R²</th>
<th>R³</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Tocopherol</td>
<td>CH₃</td>
<td>CH₃</td>
<td>CH₃</td>
</tr>
<tr>
<td>β-Tocopherol</td>
<td>CH₃</td>
<td>H</td>
<td>CH₃</td>
</tr>
<tr>
<td>γ-Tocopherol</td>
<td>H</td>
<td>CH₃</td>
<td>CH₃</td>
</tr>
<tr>
<td>δ-Tocopherol</td>
<td>H</td>
<td>H</td>
<td>CH₃</td>
</tr>
</tbody>
</table>

Also 4 x Tocotrienols (3 double bonds in the phytol side chain)
Vitamin A & E analysis

Sample preparation
- Protein precipitation
- Liquid extraction with hexane

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

Agilent HPLC 1200 & 1100 series
- Degasser
- Quaternary pump
- Autosampler
- Column Oven
- UV/Vis detector
- EzChrome software
Commercial Chromatograms

Chromsystems

Recipe: http://www.recipe.de/

Varian application note
## Commercial sources

### Standards
- Bio-Rad
- Chromsystems
- Sigma
- NIST
- Other

### Commercial Kits
- Bio-Rad
- Chromsystems
- Recipe
- Other

### Internal QC
- Bio-Rad
- Chromsystems
- Recipe
- In house
- Other

### Columns
- Alltech
- Phenomenex
- Varian
- Waters
- Other
Adult reference intervals

Results from 2007 RCPA-QAP vitamin A & E questionnaire
Vitamin D
Vitamin D Metabolism

7-dehydrocholesterol $\xrightarrow{25$-Hydroxylase\ Liver}$ 25OHD $\xrightarrow{1$-Hydroxylase\ Kidney}$ 1,25(OH)$_2$D $\xrightarrow{24$-Hydroxylase}$ 24,25(OH)$_2$D$_3$ $\xrightarrow{1,24,25$-Hydroxylase}$ 1,24,25(OH)$_3$D$_3$
1,25 di OH Vitamin D

- Regulates absorption of calcium
- Important for bone growth & development
- Two forms:
  - Vitamin D2 = ergocalciferol, is ingested in foods
  - Vitamin D3 = cholecalciferol, is produced in the skin on exposure to sunlight.
- These forms are converted in the liver to 25 OH vitamin D and then in the kidney to the active 1,25 di OH vitamin D.
1,25 Vitamin D (Calcitriol)

Dietary intake

\[
\text{ECF} \quad \downarrow [\text{Ca}^{2+}] \rightarrow \uparrow \text{PTH} \\
+ \\
\downarrow [\text{PO}_4^{2-}] \\
= \uparrow 1,25 \text{ Vit D}
\]

\[\uparrow \text{Ca}^{2+} \text{ absorption in the gut by stimulating synthesis of binding proteins}\]

\[\uparrow \text{ osteoclastic activity} = \uparrow [\text{Ca}^{2+}] \text{ and } [\text{PO}_4^{2-}]\]

\[\uparrow \text{Ca}^{2+} \text{ and } \text{PO}_4^{2-} \text{ reabsorption}\]

Overall effect – \[\uparrow \text{ plasma } [\text{Ca}^{2+}] \text{ and } \uparrow \text{ plasma } [\text{PO}_4^{2-}]\]
Vitamin D: Automated analysis

- Roche Cobas e601
  - 25 OH Vit D3 only

- Liaison
  - 25 OH Vit D3
  - >80% cross reactivity with 25 OH Vit D2
Chromatography + MS (+MS)

- Gold standard
- TAT a problem
- Expertise required
- Up front cost high

\[
300,000.00 \text{ AUD} = 4,168,904,855.85 \text{ VND}
\]

1 AUD = 13,896.35 VND
1 VND = 0.0000719613 AUD
25-Hydroxyvitamin D - Which Assay?

JA Grant,1,2, MJ Whiting3, RF Greaves4, MJ Black5, AM Wootton2

1Biochemistry Department, The Royal Melbourne Hospital, Parkville Vic 3050 Australia; School of Medical Sciences, RMIT University, Bundoora Vic 3083 Australia;
2SouthPath, Flinders Medical Centre, Bedford Park, SA 5042 Australia; 3Complex Biochemistry Department, The Royal Children's Hospital, Parkville Vic 3052 Australia;
4Clinical Biochemistry Department, Alfred Pathology Service, Melbourne Vic 3004 Australia.

Vitamin D, an important role in calcium homeostasis. Deficiency is associated with defects in bone mineralization, and may predispose to a range of proliferative and immune diseases. In the Western world, vitamin D deficiency is common, particularly in the elderly. As a consequence of vitamin D status is essential, both to identify patients at risk and to monitor intake and effective treatment. A number of different 25-hydroxyvitamin D (25(OH)D) assays are used in clinical practice, and are generally categorized by their relative sensitivity and specificity.

Vitamin D exists in two forms: chemically similar to vitamin D3 (cholecalciferol) or vitamin D2 (ergocalciferol) but with different binding affinities and biological activity. Until recently, only 25(OH)D was used for this purpose in Australia. However, pharmaceutical preparations of vitamin D3 are now available.

Analysis of 25(OH)D is complicated by the need to detect both 25-hydroxyvitamin D3 (25(OH)D3) and 25-hydroxyvitamin D2 (25(OH)D2). Poor agreement between 25(OH)D2 and 25(OH)D3 serum concentrations is not uncommon, particularly in patients taking vitamin D3 supplements. To overcome this problem, an isotopic dilution mass spectrometry (IDMS) method has been developed, with potential as a reference method for 25(OH)D analysis.

Introduction

120 de-identified routine 25(OH)D serum samples were collected over a 12-week period in 2000. Of these, 107 were obtained from The Royal Melbourne Hospital (RMH) Pathology Service from a predominantly adult female population and 13 were from Melbourne Health (MH) Pathology Service from a mixed gender adult population. An additional 22 samples were collected from a clinical trial subjecting high doses of vitamin D3, to provide samples containing substantial levels of 25(OH)D3. Approval for the use of all samples was granted by the Royal Children's Hospital Human Research Ethics Committee and the MH Human Research Ethics Committee. 25(OH)D3, 25(OH)D2, and total 25(OH)D concentrations were determined using LC-MS/MS.

25(OH)D were measured using the following five measurement systems:

- DAS80 automated immunoassay system
- OS army blood samplers (SIA)
- IDS system RIA
- diagnosis (Lancet) automated immunoassay system
- DRI-EAS automated immunoassay system

All samples were divided into three groups, according to the assay used for 25(OH)D2 and subjected to three linked quality control schemes to ensure accuracy (R2) and R2. Results from samples containing 25(OH)D3 and 25(OH)D2 were evaluated using LC-MS/MS using HPLC-MS (Clinical laboratory) with Microsoft Excel 2003.

Discussion

When samples contained only 25(OH)D3, all immunoassays demonstrated a similar slight negative bias compared to LC-MS/MS, with the current RIA being the most negative bias when compared to the immunoassay results.

Agreement with LC-MS/MS was more variable when samples contained significant levels of 25(OH)D3.

The RIA assay generally underestimated 25(OH)D3, which was expected as the antibody specificity for 25(OH)D3 is stated as zero in the kit insert. This assay was marketed as a 25(OH)D3 assay and is unreliable for monitoring 25(OH)D in patients taking vitamin D3.

The two DRI-EAS assays (OS and LMS) demonstrated a positive proportional bias in this group, which is expected because the antibodies are specific to 25(OH)D3, as stated above. The DRI-EAS assay showed an unexpectedly good correlation given the antibody specificity for 25(OH)D3, at 75%. It is proposed that these assays may detect additional metabolites, such as 24,25(OH)2D3, which have been detected in the assay of subjects taking high doses of vitamin D3. It is unclear whether the 24,25(OH)2D3 produced much higher levels than the 25(OH)D3, as the antibody used in the kit is stated to be the same.

While most routine samples contained only 25(OH)D3, 25(OH)D3, serum levels varied widely, and the RIA should still be as a reference method, and we should continue to assess the accuracy of routine 25(OH)D assays in the presence of this metabolite.

Conclusion

An assay de-identified samples with significant levels compared to LC-MS/MS in samples containing 25(OH)D3, only. Agreement was more variable in samples containing 25(OH)D2. The presence of this metabolite in 18% of routine patients indicates that 25(OH)D is still low as a vitamin D supplementation and must be considered in the context of the metabolite.
Conclusion: Vitamin D is stable in whole blood stored at room temperature in sunlight for up to 96 hours. Presented at the AACB ASM in 2005.
External QA vitamin programs
RCPA-QAP Vitamin program

Countries:
- Australia
- New Zealand
- South Africa
- Thailand
- USA
- Singapore
- Israel

Analytes:
- Vitamin A
- Vitamin E
- Vitamin B1
- Vitamin B6
- Beta carotene
- Total carotenoids
VITAMIN QUESTIONNAIRE
July 2006

The AACB Vitamin Working Party assists the QAP with the Vitamin Quality Assurance Program by providing expert advice, reviewing the program and suggesting future direction.

At this year’s meeting a number of suggestions for vitamins to be added to the program were proposed. To assess the demand for new vitamins, the Vitamin Working Party and the QAP would appreciate your feedback on the following questions. Please return to the QAP Office by 30 July 2006.

Laboratory Name: ___________________________ Lab. No. ___________________________

Your Name: ________________________________
Response to questionnaire:

- Total respondents = 66

- Strongest interest in Vitamins B1, B6 & C

- EQA material for Vit B1 & B6 available from SKML

- Decision to pilot Vitamin B1/B6 program 2008. Program in operation from 2009


- Coenzyme Q10 under consideration.
Members of the AACB vitamins working party 2009

- Chris Salonikas
- Lisa Jolly
- Kirsten Hoad
- Ronda Greaves
- Trevor Walmsley
- Lambro Johnson
- Gerald Woollard
- Scott Briscoe (not pictured)

Ronda.greaves@rch.org.au