Methodologies and Cut-offs for Measuring or Evaluating Vitamin D Status

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Outline:
  - Measurements of Vitamin D status
    - Assays for 25-hydroxyvitaminD and concerns
  - Public health & clinical implications of poor vitamin D status measurements
    - Confusions on “optimal” 25-hydroxyvitaminD cut-offs
25-hydroxyvitamin D [25(OH)D]
- major circulating form of vitamin D
- half life ~ 2-3 weeks

1,25-dihydroxyvitamin D [1,25(OH)₂D]
- biologically active form of vitamin D
- half life ~ 4-6 hours

Figure source: Holick MF. *Ann Epidemiol*. Feb 2009; 19(2): 73–78.
Serum (total) 25(OH)D, referred as calcidiol or calcifediol, comprises the sum of 25(OH)D$_2$ and 25(OH)D$_3$

- Vitamin D$_2$ ergocalciferol (made from ergosterol)
- Vitamin D$_3$ cholecalciferol (made from 7-dehydrocholesterol in the skin)

Serum 25(OH)D concentration is directly related to the body’s storage of vitamin D

- Note: 1,25(OH)$_2$D concentration correlates more with kidney function
Antibody-based methods
- use a kit or an automated clinical chemistry platform
  - Binding protein: e.g., CPBA
  - Immunoassays: e.g., RIA (Diasorin, IDS); EIA (IDS); CLIA (Diasorin); ECL (Roche); ELISA (Eagles)

Liquid chromatography (LC)-based methods
- use automated equipment featuring either UV or mass spectrometric (MS)-detection
  - HPLC-UV
  - GC-MS
  - LC-MS/MS

The performance of these assays in clinical and research laboratories remains controversy
Assay Performance Concerns

- Each assay presented technical problems
  - Inter-laboratory variability is high
  - High variability between assays
  - Reference standards
  - Some assays are unable to measure D2 or D3 (only total D); some over- or under-estimate D2 or D3

- These concerns led to the establishment of the VDSP (Vitamin D Standardization Program) in November 2010 & DEQAS (Vitamin D External Quality Assessment Scheme)

Figure 2. Seasonal differences in 25-hydroxyvitamin D levels for the HPLC-APCI-MS, RIA and CLIA assays.

http://www.plosone.org/article/info:doi/10.1371/journal.pone.0011555

Figure 4. Bland Altman plots for the difference in 25-hydroxyvitamin D level between the assays.
Figure 3. Cumulative proportion of the subjects who are classified as insufficient using a 50 nmol/L (20 ng/mL) cut-off.

http://www.plosone.org/article/info:doi/10.1371/journal.pone.0011555
**Vitamin D Standardization Program (VDSP)**

- Established by the National Institutes of Health (NIH) Office of Dietary Supplements (ODS) in November 2010
  - an international collaborative effort to standardize the laboratory measurement of vitamin D status

- **VDSP Goal**
  - To improve the detection, evaluation, and treatment of vitamin D deficiency and insufficiency by promoting the standardized laboratory measurement of serum total 25(OH)D
  - VDSP promotes the standardized laboratory measurement of total 25(OH)D to improve clinical and public health practice worldwide

DEQAS (Vitamin D External Quality Assessment Scheme)

- An accuracy based Proficiency Testing Scheme for 25(OHD) assays
- Aim of DEQAS is to monitor/improve performance of 25-OHD assays
- Accuracy based PT schemes can be used to monitor:
  - Accuracy of individual 25(OH)D results
  - Inter-laboratory imprecision
  - Trueness of 25(OH)D methods

http://www.deqas.org/
Despite Standardization Efforts, 25(OH)D Variability Persists

Mean 25(OH)D Values for Various Methods

Data from DEQAS April 2013 distribution
DEQAS initially used gas chromatography-mass spectrometry (GC-MS) as its “gold standard,” and an all-laboratory trimmed mean (ALTM) as the value to judge performance.

More recently, it uses the National Institute of Standards and Technology (NIST) reference standards calibrated using a “validated” LC-MS/MS method.
Research dollars may be wasted due to incorrect findings resulted from inaccurate and imprecise assays
The problem of misclassification of the exposure

- Is vitamin D deficiency a risk factor for disease X?

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<th>Vitamin D deficiency</th>
<th>Disease X</th>
<th>No disease</th>
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<td>Normal vitamin D</td>
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Disease is twice as common in those with vitamin D deficiency compared to normal vitamin D (30/45, cf. 5/15)

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Misclassified due to imprecise assay

No evidence of increased disease risk in relation vitamin D deficiency

Source: Prof. Robyn Lucas, Telethon Kids Institute/ANU, Australia
Vitamin D deficiency was defined by a physical manifestation
- i.e., the presence of bone disease, either rickets or the adult equivalent, osteomalacia
- The serum level of 25(OH)D to define vitamin D "deficiency / insufficiency / sufficiency" remains controversial

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<tr>
<th>Organization</th>
<th>Deficiency</th>
<th>Insufficiency</th>
<th>Sufficiency</th>
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<tr>
<td>Endocrine Society</td>
<td>&lt;20 ng/mL</td>
<td>21-29 ng/mL</td>
<td>30-100 ng/mL</td>
</tr>
<tr>
<td>National Kidney Foundation Guidelines*</td>
<td>≤15 ng/mL</td>
<td>16-30 ng/mL</td>
<td>&gt;30 ng/mL</td>
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*For children with chronic kidney disease

25(OH)D conversion factor 1 ng/mL = 2.5 nmol/L

References:
IOM committee integration of optimal bone health outcomes (y-axis) and achieved serum 250HD levels (x-axis) revealing congruence of benefit between 16 and 20 ng/ml [40 to 50 nmol/L]. BMD, Bone mineral density. [Reproduced from IOM (Institute of Medicine): Dietary reference intakes for calcium and vitamin D, p 293. Washington DC: The National Academies Press 2011 (2), with permission.]
Figure 3. Relationships between prediagnosis blood 25-(OH)D concentrations and risks for colorectal, prostate, and breast cancer in individual nested case–control studies included in the dose–response meta-analyses.
Both high and low levels may increase health risks

Mandatory food fortification is a BIG decision

- Shifting the mean of the population shifts some people to high levels

Source: Prof. Robyn Lucas, Telethon Kids Institute/ANU, Australia
Respected Groups Have Differing Daily Intake Recommendations

- **Ministry of Health**
  - Nutrient Reference Values for Australia and New Zealand
  - 200-600 IU

- **CPME**
  - Comité Permanent des Médecins Européens Standing Committee of European Doctors
  - 600-800 IU

- **Institute of Medicine of the National Academies**
  - Food and Nutrition Board
  - 600-800 IU

- **International Osteoporosis Foundation**
  - 800-1,000 IU

- **The Endocrine Society**
  - 1,500-2,000 IU
Relationship between doses of vitamin D3 supplementation and net changes in serum 25(OH)D concentrations in RCTs by baseline vitamin D status among adults

Clinical Impact = Confusion
Conclusions

- High variability between commercially available 25(OH)D assays and high inter-laboratory variability
- Lack of standardization of 25(OH)D assays hamper research efforts and resulted in negative public health and clinical impacts
- There is no well defined and international accepted definition of optimal serum 25(OH) D value/cut-off for bone or other nutritional and/or health outcomes
- Systematic, evidence-based approach that assesses the strength of evidence for both benefits and risks is needed to make clinical or public health recommendations
CRITICAL HEALTH APPLICATIONS
That Depend on the Dietary Reference Intakes (DRIs)

NUTRITION MONITORING
Assess nutritional health on a national level
- U.S. National Health and Nutrition Examination Survey (NHANES) and What We Eat in America (WWEIA) analyses
- Canadian Community Health Survey (CCHS) analyses

DIETARY GUIDELINES
U.S. Dietary Guidelines for Americans
USDA Food Patterns
Canada’s Food Guide

HEALTH PROFESSIONALS
Dietary counseling and education
Healthy diets for institutions (hospitals, long-term care, prisons)

NUTRITION RESEARCH
Study how diet can help prevent diseases
Provide a frame of reference in research

ASSISTANCE PROGRAMS
Guide the design of healthier federal nutrition assistance programs
- School Meals, WIC, SNAP, Child and Adult Care programs
- Administration on Aging programs

NUTRITION LABELING
May be used for Nutrition Facts label and Supplement Facts label
Key tools to help consumers make healthier food choices

FOOD POLICIES
National, state/province, and local food policies to improve health
Wellness policies in schools

MILITARY
Ensure nutrient needs are met for armed forces
Plan meals
Procure food, including military rations

GLOBAL NUTRIENT STANDARDS
Provide a framework that is used by many other countries and international organizations when setting their own standards

FOOD AND SUPPLEMENT INDUSTRIES
Develop healthy foods and safe supplements

CURRENT DRIs CAN RESULT IN:
HEALTHIER AMERICANS
HEALTHIER CANADIANS
HEALTHIER GLOBAL POPULATION

INSTITUTE OF MEDICINE
OF THE NATIONAL ACADEMIES

for more information, visit www.iom.edu/dri
Acronyms

- CLIA, chemiluminescence immunoassay
- CPBA, competitive protein-binding assay
- ECL, electrochemiluminescence assay
- EIA, enzyme immunoassay
- ELISA, enzyme-linked immunosorbent assay
- RIA, radioimmunoassay