Vitamin D: Past, Present, and Future in Diagnostics

Matt Silverman, PhD
Outline of talk

• History and properties
• Biological roles
• Pathophysiology
• Challenges to overcome
• Efforts toward standardization
Vitamin D
The hormone we pretend is a vitamin

1919 – Sir Edward Mellanby conducted experiments showing that cod liver oil reversed rickets in dogs

Conclusion: “Rickets is a deficiency disease which develops in consequence of the absence of some accessory food factor or factors.”
Rickets/Osteomalacia through the ages

• 1\textsuperscript{st} and 2\textsuperscript{nd} century: Greek physicians Soranus of Ephesus and Claudius Galenus

• 1645: Dr. David Whistler "De morbo puerile anglorum, quem patrio idiomate indigenae vocant the Rickets" (Concerning the disease of English children, which in English it is called "Rickets")

• 1650: Dr. Francis Glisson "De Rachitide Sive Morbo Puerili, qui Vulgo The Rickets dicitur, Tractatus"

A: Images taken from radiopaedia.org
B: Image taken from Indian Journal of Endocrinology and Metabolism 2012; 16(2)
Identifying the cure

• With the industrial revolution came an increase in rickets in polluted areas where people stayed inside and had poor diets.
• Cod liver oil had been used as a medicine for some time, and in 1824 started being used to treat rickets.
• In the late 1800s Dr. Theobald Adrian Palm realized the inverse correlation between rickets and sunlight.
• By the early 1900s, it was established that both dietary supplements and UV light could reverse rickets.
Vitamin D and Rickets

• Vitamin D is an important component in calcium homeostasis
• Regulates between 200 and 600 genes in various cell types; totaling more than 1000 targets
• A variety of other diseases are now starting to be linked to vitamin D deficiency:
  • Diabetes
  • Cardiovascular
  • Certain cancers
• High vitamin D can cause overcalcification of tissues and hypertension
Calcium regulation overall picture

8.6-10.3 mg/dL
PTH – Parathyroid Hormone
Genetic Regulation – Vitamin D Receptor

• Calcium and phosphate transporters
• Osteoblast and osteoclast differentiation
• Bile acid metabolism
• Differentiation of keratinocytes
• Development and cycling of dermal hair follicles
• Functions of key cell types involved in immunity
A class of hormones: Do they all matter?
Two types of sources

**Fungi**

Ergosterol → UV / sunlight → Ergocalciferol (Vitamin D$_2$)

**Animals**

7-dehydrocholesterol → UV / sunlight → Cholecalciferol (Vitamin D$_3$)
Two types of sources

• 20-30 minutes of sunlight/week

• Food
  • Egg yolk
  • Fish
  • Mushrooms
  • Cheese
  • Anything with vitamin D added
Mechanism of activation

Vitamin D

25(OH)D

1,25(OH)2D
Transport

• Very hydrophobic class of molecules

• Vitamin D binding protein (VDBP)
  • Increases in pregnancy, oral contraceptive use, and hormone replacement therapy
  • Decreases in renal disease, proteinuria, ICU patients, malnourished patients
  • Exist in three isoforms: Gc1F, GC2, GC1S, and each have different affinities for the 25(OH)D metabolite

• Albumin also transports
Ethnic Group Variation

• African Americans tend to have lower 25(OH)D levels than light skinned ethnic groups, but have normal bone strength
• There is a disputed theory that different ratios of VDBP isomers may contribute to this discrepancy
• Vitamin D bioavailability may not completely correlate with total vitamin D levels
Pathology inhibiting synthesis

• Skin damage/burns
  • Limit D3 production

• Liver damage
  • Vitamin D-25-hydroxylase

• Kidney failure
  • 25-Hydroxyvitamin D-1α-Hydroxylase
  • Elevated calcium, phosphate, or 1,25(OH)2D
# Typical reference levels/Daily Intake

<table>
<thead>
<tr>
<th>Compound</th>
<th>RI</th>
<th>Half-life</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D</td>
<td>0.2-20 ng/mL</td>
<td>1-2 days</td>
</tr>
<tr>
<td>25(OH)D</td>
<td>10-65 ng/mL</td>
<td>2-3 wks</td>
</tr>
<tr>
<td>1,25(OH)2D</td>
<td>15-60 pg/mL</td>
<td>4-6 hrs</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Recommended Daily Dose</th>
<th>Upper limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants (0-6 months)</td>
<td>400 IU (10μg)/day</td>
<td>1000 IU</td>
</tr>
<tr>
<td>(6-12 months)</td>
<td>400 IU/day</td>
<td>1500 IU</td>
</tr>
<tr>
<td>Toddlers (1-3 years)</td>
<td>600 IU/day</td>
<td>2500 IU</td>
</tr>
<tr>
<td>Children (4-8 years)</td>
<td>600 IU/day</td>
<td>3000 IU</td>
</tr>
<tr>
<td>(9-70 years)</td>
<td>600 IU/day</td>
<td>4000 IU</td>
</tr>
<tr>
<td>&gt;70 years</td>
<td>800 IU/day</td>
<td>4000 IU</td>
</tr>
<tr>
<td>Pregnant/Lactating</td>
<td>600 IU/day</td>
<td>4000 IU</td>
</tr>
</tbody>
</table>

Recommended daily intake increased in the last decade.
Low D effects

• Osteomalacia/Rickets
• A few studies have suggested low vitamin D reduces insulin response
• Increase in cardiovascular disease risk factors, such as LDL and triglycerides
• Increase in blood pressure
• Increased inflammation/heart failure
• Endothelial dysfunction
• Coronary heart disease, stroke, some cancers...
High D effects

• Tends to come from overdose of vitamins
• Long term- calcification of soft tissue
• Short term
  • Fatigue
  • Nausea/vomiting
  • Anorexia
  • Diarrhea/constipation
Asthma and vitamin D

- A few studies have linked vitamin D status with asthma development
- Vitamin D has been shown to have both utero and post-natal effects on lung development and immune system development/function
- Deficiency results in increased Th2 lymphocytes cells and reduction in T regulatory cells
  - Activation of pro-inflammatory cytokine production
- Both high and low values (<20 ng/mL and >30 ng/mL) have correlated with asthma risk in different studies
- A few studies have shown positive correlation between vitamin D supplementation and improvement of asthma control
Cancer

• Again, light correlation is a motivating factor; patients with skin cancer have a lower risk of other cancers
• Vitamin D promotes cell differentiation and apoptosis
• Cancers with hints of correlation:
  • Colorectal
  • Breast
  • Prostate
  • Pancreatic
Diabetes

• Again, studies are inconsistent
• North-south gradient, as well as seasonal, have been reported (linking to sunlight)
• A few studies showed reduction in risk of type 1 onset with vitamin D supplements as infants
• No protection seen from vitamin D supplements during pregnancy
Celiac disease

• More common at northern latitudes, suggesting relationship with sunlight
• Often vitamin D supplementation is suggested simply in response to poor nutrition in CD patients
• Maternal vitamin D or neonatal vitamin D show no relation to risk of development
Obesity

• Low vitamin D is associated with obesity
• Efforts to establish causation tend to suggest obesity contributes to lower vitamin D levels, rather than the reverse
• Given vitamin D is a lipophilic hormone, a likely explanation is that increased adipose tissue reduces availability of vitamin D
Medication that lower vitamin D

• Certain anticonvulsants and antiretroviral drugs used for HIV
  • Enhance catabolism of active forms
• Phenytoin
• Phenobarbitone
• Carbamazepine
• Isoniazid
• Rifampicin
• Theophylline

Additional medications
• Foscarnet
• Pentamidine
• Recombinant growth hormone
• Glucocorticoids
  • Inhibit intestinal calcium absorption
• Ketoconazole
  • Block activation
Active/Inactive form specificity

• In order to properly use reference ranges for inactive/active metabolites, assays need to be specific

• Multiple active and inactive forms, both D2 and D3 variants, adds to the complexity
## Reference Ranges and Cross-Reactivity

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Typical Ranges</th>
<th>Architect (Abbott)</th>
<th>LIAISON (DiaSorin)</th>
<th>iSYS (IDS)</th>
<th>Elecsys (Roche)</th>
<th>Centaur (Siemens)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D3</td>
<td>18-29 nM</td>
<td>0.3%</td>
<td>&lt;1%</td>
<td>2.7%</td>
<td>5%</td>
<td>0.3%</td>
</tr>
<tr>
<td>D2</td>
<td></td>
<td>0.1%</td>
<td>&lt;1%</td>
<td>2.7%</td>
<td>6%</td>
<td>0.2%</td>
</tr>
<tr>
<td>25(OH)D3</td>
<td>8-165 nM</td>
<td>105%</td>
<td>100%</td>
<td>100%</td>
<td>98%</td>
<td>106%</td>
</tr>
<tr>
<td>25(OH)D2</td>
<td>&lt;7 nM</td>
<td>52%</td>
<td>104%</td>
<td>100%</td>
<td>81%</td>
<td>97%</td>
</tr>
<tr>
<td>1,25(OH)2D3</td>
<td>48-168 pM</td>
<td>13%</td>
<td>17%</td>
<td>nd</td>
<td>5%</td>
<td>3%</td>
</tr>
<tr>
<td>1,25(OH)2D2</td>
<td></td>
<td>nd</td>
<td>40%</td>
<td>nd</td>
<td>6%</td>
<td>1%</td>
</tr>
<tr>
<td>24R,25(OH)2D3</td>
<td>&lt;30 nM</td>
<td>112%</td>
<td>nd</td>
<td>&gt;100%</td>
<td>121%</td>
<td>nd</td>
</tr>
<tr>
<td>3-epi-25(OH)D3</td>
<td>&lt;22 nM</td>
<td>3%</td>
<td>&lt;1%</td>
<td>nd</td>
<td>93%</td>
<td>1%</td>
</tr>
</tbody>
</table>
Mass spec specificity

• Several vitamin D metabolites have the same molecular weight, but likely have different physiological roles
  • 1,25(OH)2D
  • 24,25(OH)2D

• Mass to charge ratios measured by mass spec will be the same
• 24,25(OH)2D form will likely be hundreds of times higher concentration
3-epi-25(OH)D

• Supresses PTH
• Doesn’t seem to have the same effect as the 1,25 form on calcium uptake
• Detected in 23% of infants under 1 year
• Contributes to 9-61% of total 25(OH)D measured by LC-MS/MS
• Methods need to take this into account when measuring vitamin D in infants
Neonatal blood spot testing

• Need to distinguish between 25(OH)D form and 1-epi form
• Newer LC-MS/MS and ESI-MS methods able to specifically measure 25(OH) form
  • Two step derivatization
  • Enhances detectability of 25(OH)D
Saliva Testing

• Most circulating vitamin D metabolites are bound to plasma proteins CDBP and albumin

• It is generally assumed that non-protein-bound form concentrations are more reflective of functional activity

• In theory, saliva concentrations would more accurately reflect non-bound form, but is present in lower than picomolar concentrations

• LC-MS/MS method can get a LLOQ of 5 pM from 1 mL of saliva, using PTAD derivatization and methylamine addition to mobile phase

• In theory, could potentially be useful in patients with abnormal plasma proteins: pregnancy, liver damage, renal failure
Current 25(OH) reference method

- Isotope dilution LC-MS/MS
- Separate from C-3 isomers in 12 minutes
Thoughts to consider

• Vitamin D is inconsistently being linked to a wide range of diseases
• The discovery of biological roles that other vitamin D metabolites play makes testing method very important
• We could likely see an increase in the scenarios where vitamin D testing is requested, as well as an increase in the types of tests performed