Vitamin D

Saurabh Mehta, M.B.B.S., Sc.D.
Assistant Professor of Global Health and Nutrition
Division of Nutritional Sciences
Cornell University
September 15, 2011

What is Vitamin D?
Rickets
Vitamin D and Disease
Nobel Prize 1903
Sun Beds and Sanatoria
History of Vitamin D

Metabolism
- Systemic transport of calcitriol to distal target organs
- Binding of 1,25(OH)2D3 to nuclear receptor or membrane receptor at target organs
- Biological responses in intestines, parathyroid glands, bone, kidney
- Excess 25(OH)D3 is metabolized to inactive metabolites (P-450)
- Excreted into the bile

Assessment: Vitamin D Status
- Serum calcidiol concentration (25(OH)D)
  - Reflects vitamin D from solar synthesis, diet, supplements
    - Optimal level: 35–55 ng/mL
    - Deficiency: 10-15 ng/mL
  - Fairly long circulating half-life (15 days)
  - However, does not indicate vitamin D stored in body tissues
- Circulating 1,25(OH)2D is less optimal
  - Short half-life (15 hours)
  - Tight regulation by parathyroid hormone, calcium, phosphate

Vitamin D Cutoff

Why Vitamin D?
**Vitamin D and Bone Health**

**Fracture Risk**
- Meta-Analysis (age ≥ 65 years)
  - Vitamin D in doses > 400 IU/day
    - RR for hip fracture: 0.82 (0.65, 0.97) [5 RCTs; n=31872]
    - RR for nonvertebral fracture: 0.80 (0.72, 0.89) [9 RCTs; n=33265]
  - Nonvertebral fracture prevention started with achieved 25(OH)D levels of at least 75 nmol/L
  - No effect of vitamin D at a dose of 400 IU or less

**Fall Prevention**
- Meta-Analysis (age ≥ 65 years)
  - Vitamin D in doses of 700-1000 IU/day
    - RR for falls: 0.81 (0.71, 0.92) [7 RCTs; n=1921]
    - No effect of vitamin D in doses less than 700 IU/day
    - Optimal fall prevention with achieved mean 25(OH)D levels of 75-100 nmol/L
    - Better lower extremity function (8-foot walk, repeated sit-to-stand) with higher 25(OH)D levels

**NHANES**
- Third NHANES between 1988-1994
  - Comparing those in the lowest quartile of serum 25(OH)D levels with those in the highest quartile:
    - Prevalence of Hypertension, OR: 1.30
    - Diabetes Mellitus, OR: 1.98
    - Obesity, OR: 2.29
    - High serum triglycerides, OR: 1.47
Mechanisms

- Improves insulin secretion and sensitivity
- Inhibits vascular smooth-muscle cell proliferation
- Modulates inflammation
- Downregulates the renin-angiotensin system
- If vitamin D levels are low and PTH levels are high, calcium is resorbed from bones - may end up in arterial walls

Hypertension

- Results from the Nurses’ Health Study and the Health Professionals Follow-up Study
  - RR of Incident Hypertension (4-8 years of follow-up) in those with <15 ng/mL compared to those with >=30 ng/mL: 3.18 (1.39, 7.20)
    - Men: 6.13 (1.00, 37.80)
    - Women: 2.67 (1.05, 6.79)
  - Meta-Analyses of 10 trials
    - Vitamin D supplementation nonsignificantly reduced SBP but had no effect on DBP

Diabetes

- Equivocal evidence
- Observational studies:
  - Lower incident diabetes risk in the highest vs. the lowest vitamin D status group
  - Framingham Heart Study: Vitamin D levels inversely correlated with serum insulin levels
- Trials:
  - No effect of vitamin D supplementation on glycaemia or incident diabetes

Cardiovascular Disease

- MI, CVD-related death, Stroke
- Observational:
  - Higher risk in lowest vitamin D groups
  - RR: 1.53 (1.00, 2.36) for levels 10 to <15 ng/mL
- Trials:
  - No effect of supplementation when results pooled; though individual studies have suggested lower risk for outcomes such as ischemic heart disease

Cardio-Metabolic Outcomes

- Cardiovascular Disease, Metabolic Syndrome, and Type 2 Diabetes
- Systematic Review of 28 studies with n=99745
  - RR: 0.57 (0.48, 0.68) for those in the highest vitamin D group compared to those in the lowest vitamin D group

Vitamin D and Cancer
Vitamin D and Cancer

- Vitamin D metabolites prevent disjunction of cells, a key step in cancer etiology.
- Higher levels of serum 25(OH)D associated with lower risk of:
  - Colon CA
  - Breast CA
  - Ovarian CA
  - Renal CA
  - Pancreatic CA

RCT of Vitamin D and Cancer Risk

- 1179 healthy postmenopausal women aged >55 years in a 9-county rural area of Nebraska.
- Randomly assigned to 1400-1500 mg supplemental calcium alone, calcium plus 1100 IU of vitamin D, or placebo per day.
- RR for incident cancer in the Ca plus D group: 0.40 (0.20, 0.82).
- RR for incident cancer diagnosed after the first 12 months in the Ca plus D group: 0.23 (0.09, 0.60).

Cardiovascular Disease Mortality

- Third NHANES
  - 3408 U.S. individuals aged 65 and older.
  - Hazard Ratio (HR) for those with 25(OH)D levels <10 ng/mL compared to those with >=40 ng/mL: 2.36 (1.17, 4.75).
- Tuscany, Italy
  - 1006 adults
  - HR for CVD mortality for those with levels <10.5 ng/mL compared to those with >26.5 ng/mL: 2.64 (1.14, 4.79).

Cancer Mortality

- Estimated that 1000 IU/day can lead to:
  - 7% reduction in mortality rate for males in the U.S.;
  - 9% for U.S. females;
  - 14% and 20% for males and females in Western Europe living below 59 degrees.

All-Cause Mortality

- Third NHANES
  - 3408 U.S. individuals aged 65 and older.
  - Hazard Ratio (HR) for those with 25(OH)D levels <10 ng/mL compared to those with >=40 ng/mL: 1.83 (1.14, 2.94).
- Tuscany, Italy
  - 1006 adults
  - HR for CVD mortality for those with levels <10.5 ng/mL compared to those with >26.5 ng/mL: 2.11 (1.22, 3.64).
Vitamin D in Pregnant Women

Marya et al

- Randomized 100 Indian women to 600,000 IU of vitamin D twice, once each in the 7th and 8th month of pregnancy and 100 to no vitamin D supplements
- Infants of supplemented mothers had greater intruterine growth, higher birth weight, crown-heel length, head circumference, arm circumference, and skin-fold thickness


Maternal Vitamin D Deficiency increases the Risk of Preeclampsia

| Table 2: Association between maternal vitamin D and preeclampsia
<table>
<thead>
<tr>
<th>Maternal Vitamin D Status</th>
<th>Cases</th>
<th>Controls</th>
<th>Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deficient</td>
<td>200</td>
<td>300</td>
<td>1.5 (1.2-1.9)</td>
</tr>
<tr>
<td>Sufficient</td>
<td>180</td>
<td>200</td>
<td>1.2 (0.9-1.6)</td>
</tr>
</tbody>
</table>

*Adjusted for maternal smoking status, body mass index, parity, and infant birth weight

Finkelstein et al
Vitamin D and Immune System

- Vitamin D deficiency in laboratory studies:
  - Mononuclear cells - decreased ability to ingest E. coli
  - Neutrophils - impaired motility
  - Phagocytic capacity of macrophages decreased
  - Inflammatory response is depressed
  - Cell-mediated immunity is impaired

  - Abe E et al. Proc Natl Acad Sci USA 1984;81:7112-16

Vitamin D and Immune System

- Vitamin D supplementation in laboratory studies:
  - Enhanced secretion of Hydrogen peroxide by Monocytes
  - Increased cytotoxicity shown by macrophages
  - Increase in cell-mediated immunity shown by mice supplemented with Vitamin D for 8 weeks

  - Abe E et al. Proc Natl Acad Sci USA 1984;81:7112-16
Vitamin D and Innate Immunity

Role in Immunity

- Mammalian TLR recognize microbial patterns such as lipopolysaccharide and bacterial DNA
- Innate immunity
  - Activation of TLRs triggers direct antimicrobial activity against intracellular bacteria
- Adaptive immunity
  - Activation of TLRs activates MAPKs and NF-κB pathways, leading to pro-inflammatory gene expression that regulate the adaptive immune response (by production of cytokines and costimulatory molecules)

Role of the Vitamin D Pathway in Induction of Cathelicidin mRNA and Antimicrobial Activity


Conclusions

- TLR2/1 on stimulation leads to up-regulation of VDR and the hydroxylase enzyme for conversion of 25D to 1,25D
- If enough 25D, converted to 1,25D and induces cathelicidin
- Consequent direct antimicrobial effect
**Vitamin D and Tuberculosis**

**Randomized Trials**

- Nursyam et al 2006: RCT in Indonesia
  - 67 TB patients randomized to receive vitamin D (0.25 mg/day) or placebo in a double blind design
  - All vitamin D recipients showed sputum conversion compared to 77% of placebo recipients (p=0.002)
  - More subjects with radiological improvement in the vitamin D group

- Randomized placebo-controlled trial in UK
  - 192 healthy adult contacts of tuberculosis
  - Oral dose of 2.5 mg (100,000 IU) vitamin D or placebo
  - Vitamin D significantly enhanced participants' BCG-lux assay* in vitro compared with placebo (0.57, vs. 0.71, p=0.03) but did not affect antigen-stimulated interferon-gamma secretion
  - Potential role of vitamin D in prevention of tuberculosis infection (or activation of latent infection)

*Note: BCG-lux assay is a functional whole blood assay, which measures the ability of whole blood to restrict luminescence (growth) of recombinant reporter mycobacteria in vitro (luminescence ratio)
Table 3 Multivariate Correlates of Low Vitamin D Status

<table>
<thead>
<tr>
<th>Variable</th>
<th>Risk Ratio (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD8 cells, per 100 cells/µL</td>
<td>1.04 (1.01, 1.07)</td>
<td>0.01</td>
</tr>
<tr>
<td>ESR ≥ 81 mm/hr</td>
<td>1.45 (1.14, 1.83)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Vitamin A levels, per 0.35 µmol/L</td>
<td>0.87 (0.77, 0.98)</td>
<td>0.02</td>
</tr>
<tr>
<td>Vitamin E levels, per 10 µmol/L</td>
<td>1.69 (1.16, 2.45)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

a P values are from binomial regression models

b P values represent overall significance of the curve between the variable and low vitamin D status using the log likelihood ratio test

* Non-linear variables

Table 4 Maternal Maternal Vitamin D Status and Maternal Pregnancy Outcomes in HIV-infected Women in Tanzania

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Low Vitamin D Status</th>
<th>Adequate Vitamin D Status</th>
<th>Univariate RR (95% CI)</th>
<th>Multivariate RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low birth weight (&lt;2500 grams)</td>
<td>10.7 (261)</td>
<td>12.6 (414)</td>
<td>0.85 (0.55, 1.32)</td>
<td>0.84 (0.55, 1.28)</td>
</tr>
<tr>
<td>Preterm birth (&lt;37 weeks)</td>
<td>23.9 (289)</td>
<td>28.8 (469)</td>
<td>0.83 (0.65, 1.07)</td>
<td>0.84 (0.65, 1.07)</td>
</tr>
<tr>
<td>Severe preterm birth (&lt;34 weeks)</td>
<td>9.0 (289)</td>
<td>11.7 (469)</td>
<td>0.77 (0.49, 1.19)</td>
<td>0.77 (0.50, 1.18)</td>
</tr>
<tr>
<td>Small for gestational age</td>
<td>12.6 (261)</td>
<td>10.1 (414)</td>
<td>1.25 (0.81, 1.91)</td>
<td>1.25 (0.82, 1.90)</td>
</tr>
<tr>
<td>Adverse pregnancy outcome (composite of low birth weight, preterm birth, and small for gestational age)</td>
<td>34.3 (289)</td>
<td>37.1 (469)</td>
<td>0.92 (0.76, 1.13)</td>
<td>0.92 (0.76, 1.12)</td>
</tr>
</tbody>
</table>

1 P values are from log-binomial regression models

2 All multivariate models adjusted for multivitamin supplementation, maternal age at baseline, CD4 cell counts at baseline, and HIV disease stage at baseline
Table 3: HIV Infection and Mortality Outcomes up to 6 Weeks Postpartum in Offspring of HIV-Infected Women by Maternal Vitamin D Status

<table>
<thead>
<tr>
<th>Maternal Vitamin D</th>
<th>Low (N=233)</th>
<th>Adequate (N=350)</th>
<th>Univariate RR (95% CI)</th>
<th>Multivariate RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal loss</td>
<td>9.5 (336)</td>
<td>6.4 (519)</td>
<td>1.50 (0.94, 2.39)</td>
<td>1.05 (0.63, 1.74)</td>
</tr>
<tr>
<td>HIV+ at birth</td>
<td>10.7 (265)</td>
<td>6.5 (400)</td>
<td>1.65 (0.97, 2.83)</td>
<td>1.54 (0.90, 2.64)</td>
</tr>
<tr>
<td>HIV+ or dead at birth</td>
<td>21.5 (265)</td>
<td>14.0 (400)</td>
<td>1.54 (1.10, 2.15)</td>
<td>1.49 (1.07, 2.09)</td>
</tr>
<tr>
<td>HIV+ at 6 weeks</td>
<td>27.5 (120)</td>
<td>19.3 (239)</td>
<td>1.43 (0.97, 2.11)</td>
<td>1.50 (1.02, 2.20)</td>
</tr>
</tbody>
</table>

1 All multivariate models adjusted for multivitamin supplementation, maternal age at baseline, CD4 cell counts at baseline, and HIV disease stage at baseline.
2 Adjusted for continuous CD8 cell counts at baseline and primiparity in addition to covariates in footnote 1.

Table 4: HIV Infection and Mortality Outcomes up to 24 Months Postpartum in Offspring of HIV-Infected Women by Maternal Vitamin D Status

<table>
<thead>
<tr>
<th>Maternal Vitamin D</th>
<th>Low (N=233)</th>
<th>Adequate (N=350)</th>
<th>Univariate RR (95% CI)</th>
<th>Multivariate RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV transmission through breastfeeding (among children known to be HIV-uninfected at six weeks of age)</td>
<td>20.7 (87)</td>
<td>12.4 (193)</td>
<td>1.89 (1.03, 3.49)</td>
<td>2.03 (1.08, 3.82)</td>
</tr>
<tr>
<td>HIV transmission through breastfeeding (among children not known to be HIV-infected at six weeks of age)</td>
<td>21.2 (222)</td>
<td>15.8 (392)</td>
<td>1.55 (1.06, 2.26)</td>
<td>1.55 (1.06, 2.27)</td>
</tr>
<tr>
<td>HIV+ at 24 months (Total HIV infection)</td>
<td>35.2 (270)</td>
<td>27.0 (452)</td>
<td>1.49 (1.14, 1.95)</td>
<td>1.46 (1.11, 1.98)</td>
</tr>
<tr>
<td>Death by end of follow-up among live births</td>
<td>38.8 (304)</td>
<td>26.8 (486)</td>
<td>1.64 (1.28, 2.11)</td>
<td>1.61 (1.25, 2.07)</td>
</tr>
<tr>
<td>Total mortality by end of follow-up (including fetal deaths)</td>
<td>44.6 (336)</td>
<td>31.4 (519)</td>
<td>1.61 (1.29, 2.01)</td>
<td>1.58 (1.26, 1.97)</td>
</tr>
</tbody>
</table>

1 P values are from Cox (Proportional Hazards) Regression Models.
2 All multivariate models adjusted for maternal age at baseline, HIV disease stage at baseline, CD4 cell count categories (CDC), and regime received.
3 Additionally adjusted for maternal death while child alive.

Table 2: Vitamin D Status and HIV Disease Progression and Mortality among HIV-infected Women

<table>
<thead>
<tr>
<th>Vitamin D</th>
<th>Low (N=333)</th>
<th>Adequate (N=531)</th>
<th>Univariate Models</th>
<th>Multivariate Models</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progression to $\geq$ stage III</td>
<td>60.1 (333)</td>
<td>61.2 (531)</td>
<td>1.20 (1.00, 1.43)</td>
<td>1.25 (1.05, 1.50)</td>
</tr>
<tr>
<td>Progression to $\geq$ stage III or death from AIDS-related causes</td>
<td>67.8 (338)</td>
<td>66.4 (533)</td>
<td>1.20 (1.02, 1.42)</td>
<td>1.26 (1.06, 1.49)</td>
</tr>
<tr>
<td>Progression to $\geq$ stage III or death from all causes</td>
<td>71.7 (339)</td>
<td>71.7 (533)</td>
<td>1.19 (1.01, 1.40)</td>
<td>1.23 (1.04, 1.45)</td>
</tr>
<tr>
<td>Death from AIDS-related causes</td>
<td>22.5 (347)</td>
<td>22.9 (537)</td>
<td>1.04 (0.78, 1.38)</td>
<td>1.11 (0.83, 1.48)</td>
</tr>
<tr>
<td>Death from all causes</td>
<td>32.9 (347)</td>
<td>32.2 (537)</td>
<td>1.08 (0.85, 1.37)</td>
<td>1.13 (0.89, 1.43)</td>
</tr>
</tbody>
</table>

1 All multivariate models adjusted for maternal age, treatment regimen, CD4 cell counts at baseline, and HIV disease stage at baseline.
2% (N): Percentage of cases (Total number).
3P values obtained from Cox regression models.
Table 4 Vitamin D Status and Anemia Outcomes

<table>
<thead>
<tr>
<th>Status</th>
<th>Univariate models</th>
<th>Multivariate models*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% (N)</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>Severe Anemia (Hb &lt;8.5 g/dL)</td>
<td>38.7 (212)</td>
<td>1.44 (1.08, 1.92)</td>
</tr>
<tr>
<td>Anemia (Hb &lt;11 g/dL)</td>
<td>92.5 (40)</td>
<td>1.18 (0.80, 1.75)</td>
</tr>
<tr>
<td>Hypochromic microcytosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe3</td>
<td>21.9 (256)</td>
<td>2.34 (1.58, 3.45)</td>
</tr>
<tr>
<td>Moderate4</td>
<td>43.6 (234)</td>
<td>1.39 (1.08, 1.80)</td>
</tr>
<tr>
<td>Mild and above5</td>
<td>73.5 (151)</td>
<td>1.39 (1.10, 1.77)</td>
</tr>
<tr>
<td>Macrocytosis</td>
<td>4.5 (269)</td>
<td>0.99 (0.49, 2.02)</td>
</tr>
</tbody>
</table>

1p-values are from Cox Regression Models
2% (N): Percentage of cases (Total number)
3Severe: Hypochromasia ≥2+ and microcytic cells observed
4Moderate and above: Hypochromasia ≥1+ and microcytic cells observed
5Mild and above: Hypochromasia ≥1+

All multivariate models adjusted for maternal age, CD4 cell counts, HIV disease stage at baseline, and regime received.
Table 4. Low Maternal Vitamin D Status and Pediatric Growth Outcomes (n=732)

<table>
<thead>
<tr>
<th>Status</th>
<th>Low Vitamin D</th>
<th>Adequate Vitamin D</th>
<th>Univariate Hazard Ratio (95% CI)</th>
<th>p-value</th>
<th>Multivariate Hazard Ratio (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stunting</td>
<td>160/276</td>
<td>256/456</td>
<td>1.27 (1.04, 1.55)</td>
<td>0.02</td>
<td>1.30 (1.06, 1.59)</td>
<td>0.01</td>
</tr>
<tr>
<td>Underweight</td>
<td>122/276</td>
<td>182/456</td>
<td>1.36 (1.08, 1.72)</td>
<td>0.01</td>
<td>1.38 (1.09, 1.74)</td>
<td>0.01</td>
</tr>
<tr>
<td>Wasting</td>
<td>79/276</td>
<td>115/456</td>
<td>1.34 (1.01, 1.79)</td>
<td>0.04</td>
<td>1.34 (1.01, 1.79)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

All multivariate models were adjusted for maternal age, HIV disease stage at baseline, CD4 cell count at baseline, and maternal vitamin regimen.


Benefits of Vitamin D

- Optimal benefits at serum 25(OH)D levels of 40-60 ng/mL
- Bone Health; Fall and Fracture Prevention
- Cardio-metabolic outcomes
- Cancer
- Mortality
- Infectious Diseases
- Maternal and Child Health

Global Deficiency

Most common medical condition in the world?
What Needs to Be Done

Screen for vitamin D insufficiency

Dose Needed

Target range for serum 25(OH)D levels: at least 30-40 ng/mL

Safety?
Acknowledgments

- Wafaie W. Fawzi
- Christopher P. Duggan
- Ferdinand M. Mugusi
- Said Aboud
- Karim P. Manji
- Julia L. Finkelstein