Debate on Vitamin D

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Overview

- **Background:**
  - Vitamin D metabolism
  - Proposed mechanisms for extra-skeletal benefits

- **Vitamin D in clinical outcomes and limits of available data** *(the discordance between observational studies and RCTs):*
  - Caner
  - Cardiovascular
  - Diabetes
  - CKD

- **Summary**
Vitamin D metabolism

7-dehydrocholesterol → UV light skin → Cholecalciferol (vitamin D3)

Diet/supplements → Ergocalciferol (vitamin D2)

Liver → Calcidiol (25-hydroxyvitamin D)

Kidney → Calcitriol (1,25-dihydroxyvitamin D)

Inactive metabolite (24,25-dihydroxyvitamin D)

↑ Intestinal absorption of calcium
↑ Bone resorption
↓ Renal Ca++ and phosphate excretion
Skeletal action of vitamin D

- Vitamin D resistance
  - DDR-1 = 1-OHase defect
  - DDR-2 = VDR defect
  - DDR-3 = HRBP excess

- Vitamin D or calcium deficiency
  - Decreased Ca^{2+}

- Parathyroid glands
  - Increased PTH

- Normal or decreased Ca^{2+}

- Decreased Ca^{2+} x HPO_4^{2-} product

- Decreased HPO_4^{2-}

- Mineralization defect
  - Rickets/osteomalacia

- Increased FGF23 and/or phosphatonin(s)

- XLH, ADHR, TIO

- Increased HPO_4^{2-}

- Decreased HPO_4^{2-}, 1,25(OH)_2D
  - Urine
  - Blood

- Decreased Ca^{2+} x HPO_4^{2-} product

M.F. Holick, JCI 2006
Vitamin D

• Binds to nuclear VDR, resulting in direct or indirect regulation over a large number of genes:
  – 200-1250 (0.5-5% of total genome) genes have vit D response elements
  – Regulation over cellular proliferation/terminal differentiation, immunity, angiogenesis, insulin production, apoptosis, renin production.

• VDR present in most cells (including endothelial cells, pancreatic islet, neurons, T lymphocytes, cardiomyocytes, vascular smooth muscle and skeletal muscle, and hematopoietic cells)

• The local production of 1,25 (OH)2 D depends on circulating levels of 25 OH D.
Human cells co-expressing the CYP27B1-hydroxylase and vitamin D receptor

<table>
<thead>
<tr>
<th>Macrophage</th>
<th>Enterocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dendritic cell</td>
<td>Decidual stromal cell</td>
</tr>
<tr>
<td>Parathyroid cell</td>
<td>Fetal trophoblast</td>
</tr>
<tr>
<td>Osteoblast</td>
<td>Prostate epithelial cell</td>
</tr>
<tr>
<td>Osteoclast</td>
<td>Vascular endothelial cell</td>
</tr>
<tr>
<td>Keratinocyte</td>
<td>Pancreatic islet cell</td>
</tr>
<tr>
<td>Mammary epithelial cell</td>
<td>Renal tubular cell</td>
</tr>
</tbody>
</table>
Regulation of the extrarenal CYP27B1-hydroxylase

The local production of 1,25 (OH)2 D depends on circulating levels of 25 OH D.

J Steroid Biochem & Molecular Biology (2014)144:22
Map of vitamin D related outcomes

- Autoimmune diseases: 9%
- Cancer outcomes: 21%
- Cardiovascular outcomes: 7%
- Metabolic disorders: 14%
- Neonatal/infant/child related outcomes: 7%
- Pregnancy related outcomes: 7%
- Skeletal outcomes: 19%
- Other outcomes: 12%

BMJ. 2014;348:g2035
Vitamin D and cancer
Vitamin D and immunology

Induction of monocytic differentiation by 1,25-dihydroxyvitamin D3 (human promyelocytic cell line)

Control

1,25 (OH) _2_ D x 48h

PNAS 1983
Antineoplastic effects of Vitamin D

• Inhibition of proliferation and induction of differentiation:
  – 1,25-(OH)2D blocks the progression of cells from the G1 to the S phase of the cell cycle either directly or through the induction of other growth factors.

• Induction of apoptosis:
  – induces apoptosis in a number of tumor models, including carcinomas of the breast, colon, and prostate
  – Mechanism not fully elucidated

• Inhibition of angiogenesis and invasiveness
  – *Effect shown in vitro and in vivo* experimental models
The Health Professional Study: N=51,529 men

25 OH D level available in 1095:
- Determinants of vit D level (sun exposure, skin color, BMI, intake, season, age) quantified through multiple linear regression model.
- The results from the model used to compute a predicted 25(OH)D level for each of 47,800 men in the cohort.
- prospectively examined predicted 25 OH D level in relation to cancer risk with multivariable Cox proportional hazards models.

RR for cancer for an increment of 25 nmol/L in predicted plasma 25-OH D level
Prospective Study of 25 (OH) D3 level and cancer mortality in the US

Relative Risks for cancers according to baseline vitamin D levels in NHANES III Study, 1988-2000

<table>
<thead>
<tr>
<th>Cancer Site</th>
<th>25 (OH) D (nmol/L)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;50</td>
<td>50-&lt;80</td>
<td>80-&lt;100</td>
<td>≥100</td>
<td>P trend</td>
</tr>
<tr>
<td>Lung</td>
<td>1.0</td>
<td>0.78</td>
<td>0.65</td>
<td>1.14</td>
<td>0.41</td>
</tr>
<tr>
<td>Breast</td>
<td>1.0</td>
<td>0.28</td>
<td></td>
<td></td>
<td>0.76</td>
</tr>
<tr>
<td>prostate</td>
<td>1.0</td>
<td>0.91</td>
<td></td>
<td></td>
<td>0.95</td>
</tr>
<tr>
<td>Lymphoma/leukemia</td>
<td>1.0</td>
<td>1.34</td>
<td></td>
<td></td>
<td>0.96</td>
</tr>
<tr>
<td>Colorectal</td>
<td>1.0</td>
<td>0.44</td>
<td>0.28</td>
<td></td>
<td>0.02</td>
</tr>
</tbody>
</table>

Vitamin D therapy does not reduce colorectal cancer risk

A RCT involving 36,282 postmenopausal women from 40 Women's Health Initiative centers (1000 mg of calcium + 400 IU D3)

Calcium & vitamin D
Placebo

HR 1.08, P = 0.51

Cumulative Hazard for Colorectal Cancer

Wactawski-Wende J et al. NEJM 2006;354:684
Colorectal Cancer risk according to the baseline 25-OH D Level a Nested Case-Control Study.

<table>
<thead>
<tr>
<th>Baseline Serum 25-Hydroxyvitamin D</th>
<th>Main-Effect Odds Ratio (95% CI)†</th>
<th>Calcium + Vitamin D</th>
<th>Placebo</th>
<th>Intervention Odds Ratio (95% CI)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥58.4 nmol/liter</td>
<td>1.00</td>
<td>33/48</td>
<td>27/45</td>
<td>1.15 (0.58–2.27)</td>
</tr>
<tr>
<td>42.4–58.3 nmol/liter</td>
<td>1.96 (1.18–3.24)</td>
<td>44/41</td>
<td>34/32</td>
<td>1.12 (0.59–2.12)</td>
</tr>
<tr>
<td>31.0–42.3 nmol/liter</td>
<td>1.95 (1.18–3.24)</td>
<td>35/32</td>
<td>45/41</td>
<td>0.99 (0.51–1.91)</td>
</tr>
<tr>
<td>&lt;31.0 nmol/liter</td>
<td>2.53 (1.49–4.32)</td>
<td>46/39</td>
<td>42/28</td>
<td>0.75 (0.39–1.48)</td>
</tr>
</tbody>
</table>

* To convert values for 25-hydroxyvitamin D to nanograms per milliliter, multiply by 0.401. CI denotes confidence interval. † Odds ratios were derived from a logistic-regression model, conditioned on case–control pairs, estimating the main effect of the serum 25-hydroxyvitamin D level on the risk of invasive colorectal cancer (P for trend = 0.02). ‡ P for interaction = 0.54. The odds ratios were obtained from a logistic-regression model, conditioned on case–control pairs, and estimate the calcium with vitamin D intervention effect on the risk of colorectal cancer, according to serum 25-hydroxyvitamin D levels.

Vitamin D (higher dose) and calcium supplementation reduces cancer risk

RCT in 1179 healthy postmenopausal women in Nebraska (1500 mg Ca/1100 IU of D3)

25 (OH) D levels

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>12 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>72</td>
<td>71</td>
</tr>
<tr>
<td>Ca only</td>
<td>72</td>
<td>71</td>
</tr>
<tr>
<td>Ca + vit D</td>
<td>72</td>
<td>96</td>
</tr>
</tbody>
</table>

The first RCT that raised serum 25(OH)D >80 nmol/L for cancer outcome.

Am J Clin Nutr 2007(85):1586
Summary I: Vitamin D and cancer risk

• In contrast to experimental and epidemiologic data, no clear evidence to show that D3 therapy significantly reduces cancer incidence overall from available RCTs and meta-analyses.
  – Available data favor for possible benefit at higher dose mostly for colorectal CA.
  – Breast and prostate CA with more variable results

• Questionable dose- or level-dependent benefit? (higher dose/D3 level need to be achieved for benefit?)

• Role of VDR agonist/1,25 (OH)2 D for cancer prevention unknown.
Vitamin D and the heart
Mechanisms by which vitamin D deficiency may confer cardiovascular risk

Al Mheid et al. BMJ 2013
Vitamin D deficiency stimulates Renin-Angiotensin System

Vitamin D deficiency

- mRNA (in vitro)
  - protein (VDR-KO)
  - activity (VDR-KO and CYP27B1)

Angiotensin II

renal perfusion

Blood pressure

Extracellular fluid

Systemic Hypertension

Cardiac Hypertrophy (VDR-KO)

Endocr Rev. 2008; 29: 726
Vitamin D regulates renin biosynthesis

Control  VDR KO

Heart (mg/body (g))

<table>
<thead>
<tr>
<th>+/+</th>
<th>-/-</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5</td>
<td>6.0</td>
</tr>
</tbody>
</table>

22% ↑ in diameter

VDR (+/+)

VDR (-/-)

Heart (mg/body (g))

<table>
<thead>
<tr>
<th>untreated</th>
<th>captopril</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>4.5</td>
</tr>
</tbody>
</table>

Captopril reduces cardiac hypertrophy

Am J Physiol Endocrinol Metab.
2005;288:E125
Optimal vitamin D status attenuates the age-associated increase in systolic blood pressure in white Americans: a cross-sectional study

25(OH)D by SBP with the JNC 7 hypertension classifications among adults in NHANES III; 1988–1994

Am J Clin Nutr 2008(87) 136
25(OH)D levels are inversely and independently associated with the risk of developing hypertension: a prospective study

The Nurses’ Health Study 2 (Nested case-control study): N=1484, ages 32-52, no baseline HTN

- Women in the lowest compared with highest quartile of plasma 25(OH)D had an adjusted odds ratio for incident hypertension of 1.66 (P for trend=0.01)

- Vit D deficiency (<30 ng/mL, 66%) with OR of 1.47

Hypertension 2008(52):828
Low-dose D3 therapy does not prevent or improve hypertension

The Women's Health Initiative:
- a RCT of 36,282 post-menopausal women – the largest study
- 1000 mg Ca + 400 IU of D3 daily versus placebo
- Over a median of 7 yrs of follow up, no difference in mean change over time in SBP and DBP between two groups.
Summary II: D3 therapy is not associated with improvement in blood pressure in interventional trials

- The second largest interventional study (N=438) randomized to weekly D3 40,000 IU, 20,000 IU, or placebo: no change in BP in all groups despite increasing D3 levels from <30 to >50 ng/mL.
  - But only 1-yr follow up.
  - Had ongoing antihypertensive therapy.

- Only 2 RCTs performed for primary HTN prevention trial without any use of antihypertensive, but with again, mixed results and limited by very short follow ups (5-8 wks)

- Over 10 interventional studies show mostly no effect of D3 therapy on BP or incident HTN.
Vitamin D therapy in the setting of RCT does not improve mortality, MI, and stroke.

J Clin Endocrinol Metab. 2011;96:1931

RR and 95% CI

Favors vitamin D  Favors control
Vitamin D and the kidney
Postulated mechanisms for the role of vitamin D in CKD progression

- 25 (OH) D3 deficiency
- 1,25 (OH)2 D
- VDR activation
- Pro-inflammation
- Insulin resistance

Chronic kidney disease

Renin production

angiotensin II

HTN, LVH

Glomerular hypertrophy, proteinuria, fibrosis

Progression of CKD

VDR agonist
Increased prevalence of albuminuria with decreasing 25 OH D levels in NAHNES III (N=15,068)

![Bar chart showing prevalence of albuminuria with decreasing 25 OH D levels.](chart.png)

- Prevalence of albuminuria (%)
  - 3.5-17.6: 15.8 (Macroalbuminuria: 2.0, Microalbuminuria: 13.8)
  - 17.7-24.1: 13.7 (Macroalbuminuria: 1.7, Microalbuminuria: 12.0)
  - 24.2-32.0: 11.5 (Macroalbuminuria: 1.2, Microalbuminuria: 10.3)
  - 32.0-97.6: 8.9 (Macroalbuminuria: 0.8, Microalbuminuria: 8.1)

- Statistical significance: p < 0.001
Selective VDR activation with paricalcitol lowers albuminuria in patients with type 2 diabetes (VITAL study): a randomised controlled trial (24 wks)

Lancet 2010 (376) 1543
VDR agonist reduces proteinuria

<table>
<thead>
<tr>
<th>Study drug</th>
<th>Number of studies</th>
<th>Number of patients (vitamin D/control)</th>
<th>Mean difference (95% CI)</th>
<th>Mean difference</th>
<th>p for subgroup differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>paricalcitol</td>
<td>3</td>
<td>264 / 171</td>
<td>16% (5 to 28%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>calcitriol</td>
<td>2</td>
<td>72 / 69</td>
<td>31% (14 to 48%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paricalcitol dose</td>
<td>2</td>
<td>123 / 118</td>
<td>16% (3 to 28%)</td>
<td></td>
<td>0.86</td>
</tr>
<tr>
<td>1 ug/day</td>
<td>2</td>
<td>141 / 141</td>
<td>17% (1 to 34%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 ug/day</td>
<td>2</td>
<td>141 / 141</td>
<td>17% (1 to 34%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient population</td>
<td>2</td>
<td>230 / 133</td>
<td>20% (4 to 35%)</td>
<td></td>
<td>0.69</td>
</tr>
<tr>
<td>diabetic nephropathy only</td>
<td>3</td>
<td>106 / 107</td>
<td>24% (8 to 40%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>not (only) diabetic nephropathy</td>
<td>3</td>
<td>106 / 107</td>
<td>24% (8 to 40%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of follow-up</td>
<td>2</td>
<td>77 / 75</td>
<td>24% (11 to 38%)</td>
<td></td>
<td>0.46</td>
</tr>
<tr>
<td>&lt;24 weeks</td>
<td>3</td>
<td>259 / 165</td>
<td>17% (3 to 31%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 24 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study size</td>
<td>3</td>
<td>57 / 54</td>
<td>24% (8 to 41%)</td>
<td></td>
<td>0.61</td>
</tr>
<tr>
<td>number of participants &lt;80</td>
<td>3</td>
<td>279 / 186</td>
<td>19% (7 to 31%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>number of participants ≥80</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Vitamin D therapy does not improve cardiac structure in CKD**

**The PRIMO Study: A RCT in CKD patients (eGFR 15-60) with mild to moderate LVH and normal EF**

<table>
<thead>
<tr>
<th>Change in MRI measures from baseline to 48 wks</th>
<th>Placebo (n=91)</th>
<th>Paricalcitol (n=88)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV mass</td>
<td>-0.07 (-0.6 to 0.4)</td>
<td>0.34 (-0.1 to 0.8)</td>
<td>0.15</td>
</tr>
<tr>
<td>LV EF (%)</td>
<td>-0.54 (-2.1 to 0.1)</td>
<td>0.62 (-0.9 to 2.1)</td>
<td>0.18</td>
</tr>
<tr>
<td>Thoracoabdominal aortic plaque volume (mL)</td>
<td>-0.03 (-0.03 to -0.02)</td>
<td>-0.02 (-0.03 to -0.02)</td>
<td>0.09</td>
</tr>
</tbody>
</table>

>50% with diabetes and >30% with diabetic nephropathy

*JAMA 2012;307:674*
Vitamin D and diabetes
Observational studies suggest protective effect of D3 on diabetic risk

25(OH)D > 25 ng/ml: a 43% lower risk of developing type 2 diabetes compared to 25(OH)D < 14 ng/ml

Obesity and insufficient 25(OH)D interact to synergistically influence the risk of insulin resistance

**NHANES 2001-2006 (N=12,900)**

**OR for type 2 diabetes in adults (≥20 yrs)**

<table>
<thead>
<tr>
<th>BMI category</th>
<th>25 (OH) D level (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;20</td>
</tr>
<tr>
<td>normal</td>
<td>1.49</td>
</tr>
<tr>
<td>overweight</td>
<td>2.89</td>
</tr>
<tr>
<td>obese</td>
<td>6.78</td>
</tr>
</tbody>
</table>

Diabetes Care. 2012; 35: 2048
Calcium + vitamin D3 therapy does not reduce the risk of incident diabetes

The Women's Health Initiative:
- A RCT of 33951 post-menopausal women
- D3 400 IU + 1000 mg Ca or placebo
- Median follow up of 7 yrs
- The dose too low?

Diabetes Care. 2008; 31: 701
RCT data of vitamin D and glycemic outcomes remain inconclusive

- 11 RCTs on effects of D2 or D3 on glycemia.
- Too heterogeneous for proper meta-analysis:
  - Duration 6wks to 9 yrs
  - Dose 400 to 8600 IU/d to large infrequent pulse doses
- In RCTs, vitamin D therapy had:
  - No effect in participants with normal glycemia
  - Possible beneficial effects (reduced HOMA-R) among patients with glucose intolerance or insulin resistance at baseline.
Severe vitamin D deficiency independently predicts all-cause mortality in type 1 diabetes

All-cause mortality in type 1 diabetes: HR 2.7

A prospective observational study (N=220) in incident type 1 diabetic patients:
- A median f/u of 26 yrs
- Severe vitamin D deficiency at baseline did not predict the development of these microvascular complications.

Diabetes Care. 2011;34:1081
Very low vit D levels (<10\textsuperscript{th} percentile) independently predict mortality in type 2 diabetes

\textbf{A longitudinal observational study (N=289) in type 2 diabetes:}

- A median 15- yr follow up
- 60\% with normo-, 25\% with micro-, 15\% with macroalbuminuria.
- Mortality association independent of cardiac risk factors and renal function.
- Severe vitamin D deficiency at baseline did not predict progression to micro- or macroalbuminuria.

\textit{Diabetes Care} 2010; 33(10): 2238
Summary I

• Preclinical, cross-sectional, and observational epidemiologic studies have suggested strong association between vitamin D deficiency and increased risk of cancer, autoimmune diseases, diabetes, HTN/CV diseases, and overall mortality.

• Randomized controlled studies have yielded much more variable results, in part due to significant differences in study design, dose, and duration.
Summary II:

• “Despite a few hundred systemic reviews and meta-analyses, highly convincing evidence of a clear role of vitamin D does not exist for any outcome, but association with a selection of outcomes are probable.”

• The lack of concordance between observational studies and RCTs suggests that vitamin D is more likely to be a correlate marker of overall health and not causally involved in disease.

   – Theodoratou et al. BMJ 2014
Observational study vs. RCT

**Coronary Heart Disease**

- **HR**: 1.29
- HRT vs. Placebo

**Stroke**

- **HR**: 1.41
- HRT vs. Placebo

*JAMA. 2002;288:321*
### Summary of benefits of vitamin D concentration or supplementation

<table>
<thead>
<tr>
<th>Probable</th>
<th>Suggestive</th>
</tr>
</thead>
</table>
| • Decreases dental caries in children  
• Increases birth weight  
• Increases PTH in CKD | • **Decreases:** colorectal cancer, non-vertebral fx, HTN, CVD prevalence, CVA, metabolic syndrome prevalence, and type 2 & gestational diabetes, overall mortality in older adults  
• **Increases:** BMD in femoral neck, muscle strength, head circumference at birth |

Summary III: who should be screened in primary care?

- Caucasian and Asian women just before and any time after menopause, especially if they smoke or are thin.
- Women and older men with a family history of osteoporosis.
- Individuals who have had a hip, wrist, spine, or other fracture after age 50.
- Chronic steroid use.
Summary IV: Vitamin D therapy

- **Suggested target serum 25(OH)D levels:**
  - 20 and 40 ng/mL (50 to 100 nmol/L)
  - 30 and 50 ng/mL (75 to 125 nmol/L)

- **Ergocalciferol vs. cholecalciferol:**

<table>
<thead>
<tr>
<th></th>
<th>D2</th>
<th>D3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source</td>
<td>Plant-derived</td>
<td>Sun exposure, fish</td>
</tr>
<tr>
<td>Potency (Δ in affinity to DBP or 25-hydroxylase)</td>
<td>&gt;2-3x D2</td>
<td></td>
</tr>
<tr>
<td>Half life</td>
<td>1 d</td>
<td>12-30 d</td>
</tr>
</tbody>
</table>

- The dose and duration of supplementation remains unclear and need to be monitored for *the risk of hypercalcemia and nephrolithiasis*. 