Die Bedeutung von Vitamin D – ein Update

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Chapters

1. What are "Normal Levels" of 25-(OH)-Vitamin D₃?

2. Serum Levels of 25-(OH)-Vitamin D for optimal Bone Health

3. Cardiorespiratory Fitness and 25-(OH)-Vitamin D Levels

4. Lung Function, Seasonal Infections and Serum 25-(OH)-Vitamin D

5. Staphylococcus aureus and Vitamin D

6. Achieving Optimal Vitamin D Status

7. Genetic Factors for Serum 25-(OH)-Vitamin D Levels

8. Apolipoprotein E, Vitamin D: Better Bones, earlier Death?
1. What are "Normal Levels" of 25-(OH)-Vitamin D₃?

• Modern Humans left Africa 100,000 to 50,000 years ago ... as "Hunters and Gatherers"

• **Current Human Genes** are virtually identical to those of our ancestors 50,000 years ago . . .

• Genetic evolution continued slowly (pigmentation of hair, eyes, skin, lactase retention beyond infancy, adaptive defenses against malaria . . .)

• The **Paleolithic Diet** contained very little calcium, no milk beyond infancy, but high amounts of protein from game

1. What are "Normal Levels" of 25-(OH)-Vitamin D₃?

Franchthi Cave, Peloponnes, Greece

*J. Hum. Evol.* 60, 618-636 (2011)
1. **What are "Normal Levels" of 25-(OH)-Vitamin D₃?**

Modern Humans left Africa 100,000 to 50,000 years ago ... as "Hunters and Gatherers"

**Current Human Genes** are virtually identical to those of our ancestors 50,000 years ago . . .

Genetic evolution continued slowly (pigmentation of hair, eyes, skin, lactase retention beyond infancy, adaptive defenses against malaria . . .)

The **Paleolithic Diet** contained very little calcium, no milk beyond infancy, but high amounts of **Meat from Game**

Our ancestors were highly exposed to the **Sun** . . .

1. What are "Normal Levels" of 25-(OH)-Vitamin D?

1.1 Caucasians

Highly sun-exposed cohorts have 25-(OH)-Vitamin D₃ levels of 50 – 150 nmol/l

Mean Level ≈ 90 – 100 nmol/l

1. What are "Normal Levels" of 25-(OH)-Vitamin D$_3$?

1.2 Afro-Caribbeans close to the Equator

Tobago Island: 11° N
1. What are "Normal Levels" of 25-(OH)-Vitamin D₃?

1.2 Afro-Caribbeans close to the Equator

- 424 healthy Afro-Caribbean men (mean age 72.1 years) with all 4 Grandparents of African Ethnicity
- Mean 25-(OH) -Vitamin-D Level: 90 nmol/l

Despite of very high Melanin content in the Skin and old Age, the level is as high as 100.000 to 50.000 years ago

*Ethn. Dis. 21, 79-84 (2011)*
1. What are "Normal Levels" of 25-(OH)-Vitamin $D_3$?

Mean Levels of 25-(OH)-D$_3$ of around 100 nmol/l – acquired by the Sun – are normal with respect to our Genetic Make-up – finished and optimized 50,000 years ago

Such Levels almost never exceeded 160 nmol/l and were never lower than 50 nmol/l

Distinction between deficiency and insufficiency in Vitamin D Status does not appear useful nor necessary!

Vitamin D deficiency is defined by levels below 25 nmol/l!
2. Serum levels of 25-(OH)-Vitamin D for optimal Bone Health

2.1. Histomorphometric Analysis

- Transiliac Bone Specimens from 675 Individuals
- Autopsies in the Department of Legal Medicine (Cancer, Renal Diseases, Hospitalization, Primary Hyperparathyreoidism excluded)
- Serum Test for 25-(OH)-Vitamin D
- 2000 Sections stained for mineralized bone matrix and non-mineralized Osteoid
- Histomorphometric Analysis and Correlation with Serum 25-(OH)-Vitamin D

High Bone Mass  Low Bone Mass  Normal Bone but high Osteoid

Bone  Osteoid

2. Serum levels of 25-(OH)-Vitamin D for optimal Bone Health

2.1. Histomorphometric Analysis

Levels above 75 nmol/l are optimal for Bone Health with any Age or Sex

2. Serum levels of 25-(OH)-Vitamin D for optimal Bone Health

2.2. Correlation between PTH and Serum 25-(OH)-Vitamin D Levels

- Serum 25-(OH)-Vitamin D and PTH levels were measured in 14,681 participants aged ≥6 years (NHANES 2003-2006)

- 25-(OH)-Vitamin D thresholds are calculated for maximum PTH suppression

- Results were stratified by age, sex, race/ethnicity
2. Serum levels of 25-(OH)-Vitamin D for optimal Bone Health

2.2. Correlation between PTH and Serum 25-(OH)-Vitamin D Levels

Optimal PTH Suppression is observed from 40 ng/ml (≈100 nmol/l) and higher. This is valid for all Ages, Males and Females and independent from Race/Ethnicity.

### 2. Serum levels of 25-(OH)-Vitamin D for optimal Bone Health

#### 2.3. Risk of stress fractures

Stress fractures are frequently seen among military recruits

<table>
<thead>
<tr>
<th>Military Unit</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States Marine Corps</td>
<td>1%</td>
</tr>
<tr>
<td>Finnish Army Units</td>
<td>64%</td>
</tr>
<tr>
<td>Israeli Defense Forces</td>
<td>12%</td>
</tr>
</tbody>
</table>

**Observational Study**

2591 Israeli Soldiers (2001)<sup>1</sup>

<table>
<thead>
<tr>
<th></th>
<th>Stress Fracture</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTH [pg/ml]</td>
<td>28.7</td>
<td>25.7</td>
</tr>
<tr>
<td>25-(OH)-Vitamin D [nmol/l]</td>
<td>63.1</td>
<td>87</td>
</tr>
</tbody>
</table>

**Prospective Observational Study**

800 healthy Finnish Soldiers (2006)<sup>2</sup>

<table>
<thead>
<tr>
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<th>Stress Fracture</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-(OH)-Vitamin D [nmol/l]</td>
<td>64.3</td>
<td>76.2</td>
</tr>
<tr>
<td>Muscle Strength</td>
<td>7</td>
<td>9</td>
</tr>
</tbody>
</table>

2. Serum levels of 25-(OH)-Vitamin D for optimal Bone Health

2.3. Risk of stress fractures

- 600 cases and 600 controls matched for age, race/ethnicity, length of service, within ±30 days of the date of diagnosis of the fracture cases (tibia and fibula)

- Mean age 19.5 years

2. Serum levels of 25-(OH)-Vitamin D for optimal Bone Health

2.3. Risk of stress fractures

Case-Control Study

<table>
<thead>
<tr>
<th>Median Pre-diagnostic Serum 25-(OH) -D concentration (nMol/l)</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>0.79</td>
</tr>
<tr>
<td>47</td>
<td>0.74</td>
</tr>
<tr>
<td>59</td>
<td>0.60</td>
</tr>
<tr>
<td>72</td>
<td>0.51</td>
</tr>
<tr>
<td>95</td>
<td></td>
</tr>
</tbody>
</table>

Odds ratio for incident stress fracture

2. Serum Levels of 25-(OH)-Vitamin D for optimal Bone Health

2.3. Risk of stress fractures

<table>
<thead>
<tr>
<th></th>
<th>Vitamin D + Calcium</th>
<th>Placebo</th>
<th>Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stress Fractures (total)</td>
<td>226</td>
<td>270</td>
<td>– 21%</td>
</tr>
<tr>
<td>Stress Fractures (severe)</td>
<td>11</td>
<td>27</td>
<td>– 50%</td>
</tr>
</tbody>
</table>

Important Finding: there was a highly significant correlation between physical fitness and risk of fracture in the Placebo group – but not in the supplemented Vitamin D group. Vitamin D benefits were the greater the lower the physical fitness was!

\(^3\)J. Bone Miner. Res. 23, 741-749 (2008)
3. Cardiorespiratory Fitness and 25-(OH)-Vitamin D Levels

STOMP Study

• 200 healthy individuals (40 ±14 years), BMI = 25 ±5.1 (m/f ≈1:1)

• Aerobic Cardiorespiratory Fitness tested via Balke Treadmill Test to determine $\text{O}_2\text{max}$ [ml*kg$^{-1}$*min$^{-1}$]

• Daily Physical Activity Levels at Baseline were recorded: Moderate to Vigorous Physical Activity, MVPA

• Mean Maximal Oxygen Uptake was 34 ±10.3 ml*kg$^{-1}$*min$^{-1}$

• Mean MVPA was 37 ±19 hours per week

• Mean Serum 25-(OH)-Vitamin D was 34 ng/ml

Am. J. Cardiol. 107, 1246-1249 (2011)
3. Cardiorespiratory Fitness and 25-(OH)-Vitamin D Levels

Cross-Sectional Study

Am. J. Cardiol. 107, 1246-1249 (2011)
3. Cardiorespiratory Fitness and 25-(OH)-Vitamin D Levels

Cross-Sectional Study

STOMP Study: Results

- Maximal Oxygen Uptake is directly correlated to 25-(OH)-D Level
- The relationship is significant after adjusting for Age, Gender, BMI and MVPA
- There was a significant interaction between MVPA and 25-(OH)-D Levels

*Am. J. Cardiol.* 107, 1246-1249 (2011)
3. Cardiorespiratory Fitness and 25-(OH)-Vitamin D Levels

The effect of 25-(OH)-D Level on Cardiorespiratory Fitness depends on Training
The less "Training", the more pronounced is the Effect of higher Levels!

Am. J. Cardiol. 107, 1246-1249 (2011)
4. Lung Function, Seasonal Infections and Serum 25-(OH)-Vitamin D

Cross-Sectional Study

- Nationwide 1958 British Cohort (n=6789)
- **Forced Expiratory Volume in 1 Second** (FEV$_1$) and **Forced Vital Capacity** (FVC) were measured
- Respiratory Infections are self-reported

**Results**

4. Lung Function, Seasonal Infections and Serum 25-(OH)-Vitamin D

Cross-Sectional Study

Infections

Respiratory infection (%)

Winter  | Spring  | Summer  | Autumn
--- | --- | --- | ---
Green (50–75) | Green 50–75 | Green 50–75 | Green 50–75
Light Gray (75–100) | Light Gray 75–100 | Light Gray 75–100 | Light Gray 75–100
Dark Green (>100) | Dark Green >100 | Dark Green >100 | Dark Green >100

Season

25-(OH)-Vitamin D [nmol/l]

4. Lung Function, Seasonal Infections and Serum 25-(OH)-Vitamin D

**Lung function depends on 25-(OH)-Vitamin D Level**

**FEV$_1$:** Each 10 nmol/l increase of 25-(OH)-Vitamin D is associated with 8 ml increase

**FVC:** Each 10 nmol/l increase of 25-(OH)-Vitamin D is associated with 13 ml higher volume

**Important Finding**

- Individuals who **did not engage in** vigorous exercise had a much larger increase in **FEV$_1$:** 24 ml per 10 nmol/l 25-(OH)-Vitamin D increase than active participants (10 ml)

- Less active people benefit from higher levels!
5. *Staphylococcus aureus* and Vitamin D

- About 20% of healthy adults are persistent nasal carriers of **Methicillin**-sensitive *S. aureus*
- About 1% of healthy adults are persistent nasal carriers of **Methicillin-resistant** *S. aureus*: **MRSA**
- Smoking is the only identified protective Factor
- Nasal carriage is a major risk factor for infections with *S. aureus*
- Levels of Serum **25-(OH)- Vitamin D** < 20 ng/ml = 50 nM are associated with increased risk (OR = 2.04) of **Methicillin-resistant** *S. aureus* (NHANES)

5. *Staphylococcus aureus* and Vitamin D

**The Tromsø Staph and Skin Study**

- Nasal Swabs (twice, interval: weeks)
- Incubation for 48 hours in Chrom ID *S. aureus* Agar
- Colonization = positive in first swab
- Carrier state = positive in two samples

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5. Staphylococcus aureus and Vitamin D

The Tromsø Staph and Skin study

In Non-Smokers and only in Males, Colonization and Carriage of Staphylococcus aureus are a function of 25-(OH)-Vitamin D

Serum Levels of 50-100 nmol/l or higher protect from S. aureus Colonization and Carriage

6. Achieving optimal Vitamin D Status

6.1. UV-B from Sun or Sunbeds

• Skin-derived (UV-B) **Vitamin D\textsubscript{3}** has a Bioavailability of 1.0
• Skin-derived (UV-B) **Vitamin D\textsubscript{3}** is **always** converted into **25-(OH)-Vitamin D\textsubscript{3}**
6. Achieving optimal Vitamin D Status

6.1. Sunbeds
Ten human volunteers treated with increasing exposure (1.0 MED) with a commercially available sunbed (UV-A 12 mW/cm², UV-B 0.48 mW/cm²) for a total of 4 weeks.

All of the participants (100%) increased their 25-(OH)-D₃ Levels from 12 to 40 nmol/l.

6. Achieving optimal Vitamin D Status

6.1. UV-B from Sun or Sunbeds

• Skin-derived (UV-B) **Vitamin D₃** has a Bioavailability of 1.0
• Skin-derived (UV-B) **Vitamin D₃** is **always** converted into **25-(OH)-Vitamin D₃**
• Increase in **25-(OH)-Vitamin D₃** is determined by starting level and/or ratio of:
  
  $24,25$-Dihydroxyvitamin D₃ / **25-(OH)-Vitamin D₃**

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**The most reliable source:**

Sun (2 hours each day in Bikini) or Sunbeds

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[Gerontology 57, 350-353 (2011); EAS Meeting 2011, Abstract 1479]
6. Achieving optimal Vitamin D Status

6.2. Is Sunshine dangerous?

• The answer is: No!
• A recent case-control study of the entire Danish population (7.5 million) identified 130,673 cases of non-melanoma skin cancer (mainly basalioma), 333,558 cases of myocardial infarction, 130,915 cases of hip fracture and 1,688,830 deaths.
• Non-melanoma skin cancer patients had a highly significant (p < 10^{-20}) lower risk of
  • Myocardial Infarction – 34%
  • Hip Fracture – 32%
  • Total Mortality – 6%

• Total Mortality Reduction is identical to RCTs with Vitamin D!

*Gerontology* 57, 350-353 (2011); EAS Meeting 2011, Abstract 1479
6. Achieving optimal Vitamin D Status

6.1. Oral Vitamin D₃ as a DRUG in Pharmacological Doses (>1,000 I.U. / day)

- **Systemic Bioavailability**: 0.6 to 1.0 if given with long-Chain Fatty Acids, Milk or a full Meal for healthy Persons

- **Vitamin D₃** travels as a „Blind Passenger“ in Chylomicrons in the Lymph with long-Chain Fatty Acids and Cholesterol bound to Lipoproteins

- „Half-time“ to Maximum of **25-(OH)-Vitamin D₃** at daily Doses: about 2 Months. It takes up to 8 Months until a Steady-State Level of **25-(OH)-Vitamin D₃** is achieved in a Population!
6. Achieving optimal Vitamin D Status

6.1. Oral Vitamin D₃ as a DRUG in Pharmacological Doses (>1.000 I.U. / day)

• The Fate of 70-90% of Oral Vitamin D₃ is unknown!

• 70 to 90% are never converted into 25-(OH)-Vitamin-D₃!

• There are many "Non-Responders" – despite complete "Systemic Absorption"
Pharmacokinetics of Oral Vitamin D$_3$

50% are Non-Responders

16 healthy participants (74 years, mean level: 30 ng/ml 25-(OH)-Vitamin D) received 1.600 I.U. Vitamin D$_3$ daily for 12 months.
Levels of 25-(OH)-Vitamin D$_3$ were measured monthly.

7 out of 16 did not increase or even decreased their level!

6. Achieving optimal Vitamin D Status

6.1. Oral Vitamin $D_3$ as a DRUG in Pharmacological Doses (>1.000 I.U. / day)

- The Fate of 70-90% of Oral Vitamin $D_3$ is unknown!
- It is never converted into 25-(OH)-$D_3$!
- There are many "Non-Responders" – despite complete "Systemic Absorption"
- Increase (if any) is determined by previous level and/or 24,25 dihydroxyvitamin $D_3$ to 25-(OH)-Vitamin D ratio: the lower the ratio, the higher the response

Oral Vitamin $D_3$ in pharmacological Doses is an unreliable Source for increasing Vitamin $D_3$ Status
6.2 Oral Vitamin D$_3$ in Food (<200 I.U. / day)

- **Vitamin D$_3$**
- **NPC1L1**
- **Ezetimibe**
- **MTP**
- **Chylomicrons**
- **Lymphatic System**
- **Remnant**
- **ABCA1**
- **ABCG5**
- **ABCG8**
- **ACAT2?**
- **Portal Vein**
- **HDL**
- **LDLR / LRP**
- **SR-BI**

**Diagram Notes**
- **Second Order (Saturable) Process**
- **Bile Acid (BA)**
- **Phospholipids (PL)**

Paleolithic Diet supplied 25-(OH)-D₃ and Vitamin D₃ in Meat

J. Dairy Sci. 93, 2025-2029 (2010)
6. Achieving Optimal Vitamin D Status

6.3. 25-(OH)-Vitamin D₃

The most efficient and reliable Vitamin D Source

- Bioavailability: 0.6 to 1.0
- Transport in the Portal Vein bound to Vitamin D Binding Protein (VDBP)
- Half-life in Serum/Plasma: 10–12 days
- Distribution Volume (Vd, l/kg): 0.12–0.20
- Increase in 25-(OH)-Vitamin D₃ is immediate (2-3 days) and can be calculated for any Individual exactly by a formula:

\[
327.5 \times \text{ng/kg/day} = \text{Increase in nmol/l}
\]

Changes of 25-(OH)-Vitamin D₃ Levels in Cows

Serum
190 nmol/l

Meat
25 µg/kg

Serum
no increase above 10 nmol/l

Meat
0.0 – 0.2 µg/kg

25 µg 25-(OH)-Vitamin D₃ equals 5.000 – 10.000 I.U. Vitamin D₃
6. Achieving Optimal Vitamin D Status

6.3. 25-(OH)-Vitamin D₃

The best Source for 25-(OH)-Vitamin D₃ was (is) Beef (e.g. Steaks from Cattle raised on Pasture – like in Tyrol)

Beef contains up to 25 ng/g or more of 25-(OH)-Vitamin D₃

3kg Steak per Month will increase 25-(OH)-Vitamin D₃ Levels by 12 nmol/l!

Meat Eaters have about 20 nmol/l higher 25-(OH)-Vitamin D Levels than Vegans!

7. Genetic Factors for Serum 25-(OH)-Vitamin D Levels

7.1. Genome-wide Association Studies

Genome-wide Association Studies prove strong influence of Polymorphisms in **3 Enzymes** and in **VDBP** on the Serum Level

- DHCR-7
- Serum Level
- 25-(OH)-Vitamin D₃
- CYP 2R1, CYP 24A1
- Vitamin D Binding Protein

8. Apolipoprotein E, Vitamin D: Better Bones, earlier Death?

- Apolipoprotein E (a component of secreted VLDL) is important in transport and catabolism of cholesterol and triglycerides for chylomicrons and remnants

- There is a common polymorphism Apo ε 3, Apo ε 2, Apo ε 4

- In Europe, the most ancient allele, Apo ε 4, shows a North–South gradient

8. Apolipoprotein E, Vitamin D: Better Bones, earlier Death?

![Graph showing allele frequency vs. latitude north]

- APOE 3
- APOE 4
- APOE 2

8. Apolipoprotein E, Vitamin D: Better Bones, earlier Death?

- Apolipoprotein E (a component of secreted VLDL) is important in transport and catabolism of cholesterol and triglycerides for chylomicrons and remnants.

- There is a common polymorphism: **Apo ε 3, Apo ε 2, Apo ε 4**

- In Europe, the most ancient allele, **Apo ε 4**, shows a North–South gradient.

- **Apo ε 4** carriers are higher absorbers of cholesterol and have higher serum cholesterol levels.

# 8. Apolipoprotein E, Vitamin D: Better Bones, earlier Death?

## 8.1. Population Studies

<table>
<thead>
<tr>
<th>Serum Levels</th>
<th>APO ε 4</th>
<th>no APO ε 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL Cholesterol</td>
<td>[mg/dl]</td>
<td>150</td>
</tr>
<tr>
<td>HDL Cholesterol</td>
<td>[mg/dl]</td>
<td>68</td>
</tr>
<tr>
<td>25-(OH)-Vitamin D₃</td>
<td>[nM]</td>
<td>49</td>
</tr>
<tr>
<td>PTH</td>
<td>[pg/ml]</td>
<td>60</td>
</tr>
<tr>
<td>Calcium</td>
<td>[mmol/l]</td>
<td>2.29</td>
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</tbody>
</table>
# 8. Apolipoprotein E, Vitamin D: Better Bones, earlier Death?

## 8.2. Knock-in Mouse Models

<table>
<thead>
<tr>
<th>Serum Levels</th>
<th>APO ε 4</th>
<th>Wild-Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-(OH) Vitamin-D$_3$ [nM]</td>
<td>71</td>
<td>24</td>
</tr>
<tr>
<td>Hepatic Bile Acids [nmol/g]</td>
<td>140</td>
<td>98</td>
</tr>
<tr>
<td>Femoral Calcium [mg/g]</td>
<td>151</td>
<td>133</td>
</tr>
<tr>
<td>Urinary Calcium [mmol/l]</td>
<td>0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Calcium Resorption [%]</td>
<td>45</td>
<td>31</td>
</tr>
</tbody>
</table>

7. Genetic Factors for Serum 25-(OH)-Vitamin D Levels

Genome-wide Association Studies prove strong influence of Polymorphisms in **3 Enzymes** and in **VDBP** on the Serum Level

- **CYP 2R1, CYP 24A1**
- **Vitamin D Binding Protein**

Lancet **376**, 180-188 (2010); Diabetes **60**, 1629-1631 (2011)
Genetic Factors for Serum 25-(OH)-Vitamin D Levels

Genome-wide Association Studies prove strong influence of Polymorphisms in **3 Enzymes** and in **VDBP** on the Serum Level

**DHCR-7**

**Vitamin D Binding Protein**

**Serum Level 25-(OH)-Vitamin D$_3$**

**CYP 2R1, CYP 24A1**

**APOE ε4**

*Lancet* 376, 180-188 (2010); *Diabetes* 60, 1629-1631 (2011)