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Serum 25-hydroxyvitamin D status and anaerobic performance in female collegiate basketball players

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Serum 25-Hydroxyvitamin D Status and Anaerobic Performance in Female Collegiate Basketball Players

Primary Investigator: Anna Krieger
Co-Investigators: Amy Olson, PhD, RD, LD and Mani Campos, PhD
Vitamin D and Athletes

- Optimal serum 25(OH)D concentration is at least 75 nmol/L\(^1\)

- **Mean 25(OH)D level for U.S. population\(^2\):** 56 nmol/L
  
  - *Do all ages/populations demonstrate low vitamin D statuses?*

- **Study in urban Boston hospital\(^3\):**
  
  - 42% of adolescents examined had vitamin D deficiency

- **Deficiency rates in athletes:**
  
  - Gymnasts (83%)\(^4\)
  
  - Collegiate athletes (63%)\(^5\)
  
  - Basketball players (94%)\(^4\)

*Does this matter?*
Personal Study Purpose

Three-Fold

• *Examine relationship between vitamin D status and anaerobic performance*

• *Determine if the temporary deficiency that occurs during the late fall and winter months is associated with decreased anaerobic performance*

• *Examine the efficacy of 2000 IU vitamin D₃ daily supplementation to maintain and/or improve vitamin D status in female young adults*
Vitamin D: The Basics

**Classical Actions**
- Intestinal Ca\(^{2+}\) absorption
- Bone metabolism
- Parathyroid function

**Non-Classical Actions**
- Immune function/disease
- Heart/vascular function
- Pregnancy/lactation
- Obesity
- Cancer
- Muscle function
- Cognitive function

**Figure 1.** Vitamin D\(_3\) structure (1930s, Windaus)
Vitamin D and Athletes

• Low levels of vitamin D in athletes → decreased muscle strength and increased risk of bone and muscle injuries\(^9\)

**Table 1. Comparison of vitamin D sufficiency rates throughout year**

<table>
<thead>
<tr>
<th>Population</th>
<th>Latitude</th>
<th>Summer period</th>
<th>Winter period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polish professional soccer players(^9)</td>
<td>51 ° N</td>
<td>50%</td>
<td>16.7%</td>
</tr>
<tr>
<td>College athletes(^10)</td>
<td>41.3 ° N</td>
<td>75.6%</td>
<td>15.20%</td>
</tr>
<tr>
<td>Spanish soccer players(^11)</td>
<td>37 ° N</td>
<td>93%</td>
<td>36%</td>
</tr>
</tbody>
</table>

St. Cloud

45.5 ° N

\(\leftarrow 37 ° N\)
Vitamin D and Athletic Performance

- Higher serum 25(OH)D concentrations are associated with greater muscle strength and athletic performance in some studies, but not all:
  - **Post-menarchal girls**: positive relationship between vitamin D and jump velocity, jump height, power, and force
  - **Healthy men and women**: Vitamin D was significantly associated with arm and leg muscle strength when controlling for age and gender
  - **English professional soccer players**: significant change in 10 m sprint times and vertical jump, but no significant change in 30 m sprint times or Illinois agility run
  - **Club-level athletes**: increases in serum 25(OH)D had no significant effect on the physical anaerobic tests
PERSONAL STUDY
Who were the subjects?

- Study approved by IRB of CSB/SJU
- Received support from coach and athletic director

- 17 varsity female collegiate basketball players volunteered and provided informed consent

- 3 athletes withdrew due to either external injuries (n=2) or an unwillingness to comply with the rules of the study (n=1)

Therefore, 14 subjects completed the study

Table 2. Descriptive characteristics at baseline (mean ± SD)

<table>
<thead>
<tr>
<th>Supplement group</th>
<th>Age (yr)</th>
<th>Weight (kg)</th>
<th>Serum 25(OH)D (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (n=7)</td>
<td>20.3 ± 1.4</td>
<td>72.2 ± 4.4</td>
<td>67.9 ± 24.2</td>
</tr>
<tr>
<td>2000 IU vitamin D₃ (n=7)</td>
<td>18.7 ± 1.1</td>
<td>70.4 ± 9.3</td>
<td>66.9 ± 26.5</td>
</tr>
</tbody>
</table>
Research Design

• Double-blind, placebo-controlled study
• Participants were randomly assigned to their respective supplement group

Baseline (i.e. October)
- Health questionnaire
- Blood collection
- Anaerobic performance tests

Supplementation Period
- Consumed 1 supplement/day for 60 days

Final (i.e. December)
- Health questionnaire
- Blood collection and analysis (ELISA)
- Anaerobic performance tests

100 IU vitamin E (placebo) (n=7)
2000 IU vitamin D₃ (n=7)

* Statistical analysis performed using SPSS and paired t-tests
Anaerobic Tests

• Identical protocols were followed during baseline and final testing sessions

SPEED/AGILITY

VERTICAL JUMP

Figure 2. T drill agility test

Figure 3. Just Jump electronic jump mat

• 2 measurements/test, taken 5 minutes apart → best result used for analysis
Assessment of Serum-Hydroxyvitamin D Status

- Baseline and final resting finger capillaries were collected from each participant
- Serum 25[OH]D quantification was analyzed using an ALPCO 25[OH]D ELISA assay
- Vitamin D status was defined in accordance with the Endocrine Society guidelines

Table 3. Endocrine Society vitamin D concentration classifications

<table>
<thead>
<tr>
<th>Serum 25(OH)D (nmol/L)</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 50</td>
<td>Deficient</td>
</tr>
<tr>
<td>50-75</td>
<td>Insufficient</td>
</tr>
<tr>
<td>75-125</td>
<td>Optimal</td>
</tr>
</tbody>
</table>
What were the baseline and final serum total 25(OH)D concentrations?

Table 4. Serum vitamin D₃ status changes over 60 day supplementation period (mean ±SD)

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=7)</th>
<th>2000 IU vitamin D₃ (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D₃ (nmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-supplementation</td>
<td>66.9 ± 26.5</td>
<td>67.9 ± 24.2</td>
</tr>
<tr>
<td>Post-supplementation</td>
<td>56.7 ± 26.5</td>
<td>79.0 ± 18.2*</td>
</tr>
<tr>
<td>Vitamin D Sufficient (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-supplementation</td>
<td>42.9</td>
<td>14.3</td>
</tr>
<tr>
<td>Post-supplementation</td>
<td>42.9</td>
<td>57.1*</td>
</tr>
</tbody>
</table>

* p < 0.05
What were the baseline and final serum total 25[OH]D concentrations?

* p < 0.05

Figure 4. Changes in serum 25(OH)D₃ (nmol/L) following 60-day supplementation

Overall: 79% deficient
Did these post-supplementation changes in vitamin D status affect anaerobic performance?

**Table 4.** Anaerobic performance changes over 60 day supplementation period (mean ±SD)

<table>
<thead>
<tr>
<th>Test</th>
<th>Placebo (n=7)</th>
<th>2000 IU vitamin D$_3$ (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T Drill Agility Test (s)</strong></td>
<td>11.3 ± 0.7</td>
<td>11.6 ± 1.1</td>
</tr>
<tr>
<td>Pre-supplementation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-supplementation</td>
<td>11.4 ± 0.3</td>
<td>11.1 ± 0.6</td>
</tr>
<tr>
<td><strong>Vertical Jump (cm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-supplementation</td>
<td>47.3 ± 6.7</td>
<td>47.8 ± 6.6</td>
</tr>
<tr>
<td>Post-supplementation</td>
<td>48.2 ± 6.2</td>
<td>48.8 ± 6.2</td>
</tr>
</tbody>
</table>

There were no changes in any of the performance tests over the 60 day supplementation period.
Why were so many participants vitamin D insufficient/deficient at baseline?

- Causes can be multifactorial:
  - Low UVB exposure
  - Low dietary and supplemental intake of vitamin D
    ✓ Only 14% (n=2) reported taking a daily vitamin D supplement prior to study
    ✓ Health questionnaire revealed low intakes of vitamin D-rich foods (i.e. milk, fatty fish)

**Figure 5.** Questions taken from baseline health questionnaire
**Chronic vs. acute vitamin D deficiency effects: does the temporary dip matter?**

- **Threshold effect:**
  - Lower baseline concentrations result in a greater magnitude of response to vitamin D supplementation\(^{15}\)
- **The “optimal cutoff” of 75 nmol/L may not be enough for enhanced anaerobic effects:**
  - The response curve one tissue to a given extracellular signal (i.e. hormone) differs from another\(^{16}\)
  - Implies that the optimal 25(OH)D concentration for a perceptible physiological response in one tissue may not be optimal for another
  - A higher serum total 25(OH)D concentration may be necessary in skeletal muscle
    - Heaney & Holick proposed the range of **120-225 nmol/L** for skeletal muscle\(^{17}\)
Was compliance a factor?

- Yes
  - Greatest challenge of study

**Table 4. Results of final participant questionnaire**

<table>
<thead>
<tr>
<th>Frequency of supplement use</th>
<th>Percentage of participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-7 days/week</td>
<td>64% (n=9)</td>
</tr>
<tr>
<td>3-5 days/week</td>
<td>29% (n=4)</td>
</tr>
<tr>
<td>1-3 days/week</td>
<td>7% (n=1)</td>
</tr>
</tbody>
</table>
Other limitations to the study?

- Small sample size
- Limited number of anaerobic tests
  - Additional tests
    - Maximum strength tests (e.g. 1-RM measurements)\textsuperscript{12}
    - Short sprints (e.g. 10 m sprint)\textsuperscript{11}
  - Did not monitor changes in training and physical activity
    - Training effects and changes in fitness levels throughout the supplementation period may have affected performance tests
Next steps: future research

• Repeat study with added changes:
  ✓ Incorporate methods to increase compliance
  ✓ Monitor immune health
    o Vitamin D affects immunity, which in turn affects athletes and their performance\(^\text{18}\)

• Need for randomized controlled trials that examine:
  ✓ Optimal vitamin D levels for peak athletic performance
  ✓ Effects of chronic vs. acute vitamin D deficiencies
Take-away messages

• 2000 IU vitamin D₃/daily over a 60-day period increased serum 25(OH)D₃ to optimal levels

✓ The elevated vitamin D status did not improve our chosen measures of anaerobic performance in collegiate female basketball players

May indicate that a chronic deficiency of vitamin D or a more severe deficiency is needed to adversely affect muscle function
References


Baseline Questionnaire

ID Number ______________________________
Date ________________________________
Age ___________________

Are you currently taking any dietary supplements?
[ ] Yes [ ] No

If yes, please provide names of supplements (if known):

____________________________________
____________________________________
____________________________________
If yes, how often do you take the supplements?
[ ] Daily [ ] 3-5 times/week
[ ] 1 time/week [ ] <1 time/week

Are you currently taking a supplement that contains vitamin D?
[ ] Yes [ ] No [ ] I don’t know

If yes, do you know the amount of vitamin D are you taking?
[ ] Yes ______I.U. [ ] I don’t know

Do you wear sunscreen on a daily basis?
[ ] Yes [ ] No

How often do you use a tanning bed?
[ ] 3+ times/week [ ] 1-2 times/week
[ ] 1-3 times/month [ ] Never

How many glasses of milk do you consume per day?
[ ] 3+ glasses [ ] 2-3 glasses [ ] 1 glass
[ ] <1 glass [ ] I don’t drink milk

How often do you eat “fatty fish” (e.g. salmon, tuna, etc.)?
[ ] 3+ times/week [ ] 1-2 times/week
[ ] 1-3 times/month [ ] I don’t eat “fatty fish”
Final Questionnaire

Last 4 Digits of Banner ID______________________________

How often did you take the study’s supplement?
[ ] 5-7 times/week  [ ] 1-3 times/week
[ ] 3-5 times/week  [ ] Never

Did you initially take the supplement, but then stopped?
[ ] Yes  [ ] No

If yes, explain when and why you stopped:
__________________________________________________________________________

_________________________________________________________

Are you currently taking any other vitamins/minerals?
[ ] Yes  [ ] No

If yes, please provide names of vitamins/minerals (if known):
__________________________________________________________________________

__________________________________________________________________________

If yes, how often do you take the vitamins/minerals?
[ ] Daily  [ ] 3-5 times/week
[ ] 1 time/week  [ ] <1 time/week

Are you currently taking an additional supplement (i.e. one not provided by the study) that contains vitamin D?
[ ] Yes  [ ] No  [ ] I don’t know

If yes, do you know the amount of vitamin D you are taking?
[ ] Yes ______I.U.  [ ] I don’t know

Do you wear sunscreen on a daily basis?
[ ] Yes  [ ] No

How often do you use a tanning bed?
[ ] 3+ times/week  [ ] 1-2 times/week
[ ] 1-3 times/month  [ ] Never

Did you travel during the supplementation period?
[ ] Yes  [ ] No

If yes, where? ________________________________

How many glasses of milk do you consume per day?
[ ] 3+ glasses  [ ] 2-3 glasses  [ ] 1 glass
[ ] <1 glass  [ ] I don’t drink milk

How often do you eat “fatty fish” (e.g. salmon, tuna)?
[ ] 3+ times/week  [ ] 1-2 times/week
[ ] 1-3 times/month  [ ] I don’t eat “fatty fish”
ALPCO 25(OH)D ELISA Assay

• Utilized a competitive ELISA technique with a selected monoclonal antibody recognizing 25(OH)D
• Participants’ serum was incubated with a releasing reagent
• Pre-incubated solutions were then transferred to a microplate coated with 25(OH)D and the anti-25(OH)D antibody was added
• During the overnight incubation step, the 25(OH)D in the serum samples and a fixed amount of 25(OH)D bound to the microtiter well competed for the binding of the antibody.
• Then, a peroxidase-conjugated antibody was added to each microplate well → a complex of 25(OH)D-anti-25(OH)D antibody-peroxidase conjugate if formed
• Tetramethylbenzidine (TMB) was used as a peroxidase substrate
• Finally, an acidic stop solution was added to terminate the reaction, whereby the color changes from blue to yellow.
  ✔ The intensity of the yellow color was inversely proportional to the concentration of 25(OH)D
# Vertical jump and agility T-test descriptive data

<table>
<thead>
<tr>
<th>Group</th>
<th>Agility T Test (s)</th>
<th>Vertical Jump (in)</th>
</tr>
</thead>
<tbody>
<tr>
<td>College basketball players (women)</td>
<td>9.0</td>
<td>21</td>
</tr>
<tr>
<td>Competitive college athletes (women)</td>
<td>10.8</td>
<td>16-18.5</td>
</tr>
<tr>
<td>Sedentary college students (women)</td>
<td>13.5</td>
<td>8-14</td>
</tr>
</tbody>
</table>

*The values listed are either means or 50th percentile (medians).*
Role of Vitamin D in Muscle

• Upon activation to 1,25(OH)D, vitamin D-responsive gene expression in muscle is altered

• **These genes affect**:  
  — Muscle protein synthesis  
  — Muscle strength  
  — Muscle size  
  — Reaction time  
  — Balance  
  — Coordination  
  — Endurance

*Fig 1. An increase in the storage form of vitamin D is associated with incremental improved musculoskeletal performance*
Vitamin D Conversion Mechanism

1. 7-dehydrocholesterol in skin
2. Sun exposure
3. Cholecalciferol (D₃)
4. 25-hydroxylase in liver
5. 25-hydroxyvitamin D
6. 1-alpha-hydroxylase in kidney
7. 1,25-dihydroxyvitamin D (1,25-dihydroxycholecalciferol or calcitriol - active)
8. Binding to vitamin D receptors
9. Biological actions
Illinois Agility Test