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 D$_3$ daily supplementation to maintain and/or improve vitamin D status in female young adults
Vitamin D: The Basics

1651

- First scientific description of a vitamin D-deficiency (rickets)\(^6\)

Early 1900’s – Mid-1990’s

- “First Wave” of Vitamin D Awareness\(^7\)

Mid-1990’s - Present

- “Second Wave” of Vitamin D Awareness\(^7\)

Classical Actions\(^6\):
- Intestinal Ca\(^{2+}\) absorption
- Bone metabolism
- Parathyroid function

Non-Classical Actions\(^8\):
- Immune function/disease
- Heart/vascular function
- Pregnancy/lactation
- Obesity
- Cancer
- Muscle function
- Cognitive function

Figure 1. Vitamin D\(_3\) structure (1930s, Windaus)\(^7\)
Vitamin D and Athletes

- Low levels of vitamin D in athletes $\rightarrow$ 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>
Vitamin D and Athletic Performance

• Higher serum 25(OH)D concentrations are associated with greater muscle strength and athletic performance in some\textsuperscript{12,13,14}, but not all studies\textsuperscript{14,15}:
  
  — Post-menarchal girls: positive relationship between vitamin D and jump velocity, jump height, power, and force\textsuperscript{12}

  — Healthy men and women: Vitamin D was significantly associated with arm and leg muscle strength when controlling for age and gender\textsuperscript{13}

  — 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\textsuperscript{14}

  — Club-level athletes: increases in serum 25(OH)D had no significant effect on the physical anaerobic tests\textsuperscript{15}
Who were the subjects?

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

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

- **Two Weeks**
  - **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

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

- 100 IU vitamin E (placebo) (n=7)
- 2000 IU vitamin D\(_3\) (n=7)
**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><strong>79.0 ± 18.2</strong>*</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><strong>57.1</strong>*</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)D$_3$ (nmol/L) following 60-day supplementation
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></th>
<th>Placebo (n=7)</th>
<th>2000 IU vitamin D₃ (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T Drill Agility Test (s)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-supplementation</td>
<td>11.3 ± 0.7</td>
<td>11.6 ± 1.1</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
    o Maximum strength tests (e.g. 1-RM measurements)\textsuperscript{12}
    o Short sprints (e.g. 10 m sprint)\textsuperscript{11}
  o 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\(^{18}\)

• 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 D$_3$/daily over a 60-day period increased serum 25(OH)D$_3$ 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
QUESTIONS?
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 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

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 affect⁴:
  — 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 performance⁴
Vitamin D Conversion Mechanism

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