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

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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]
  - <u>Basketball players </u>(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]

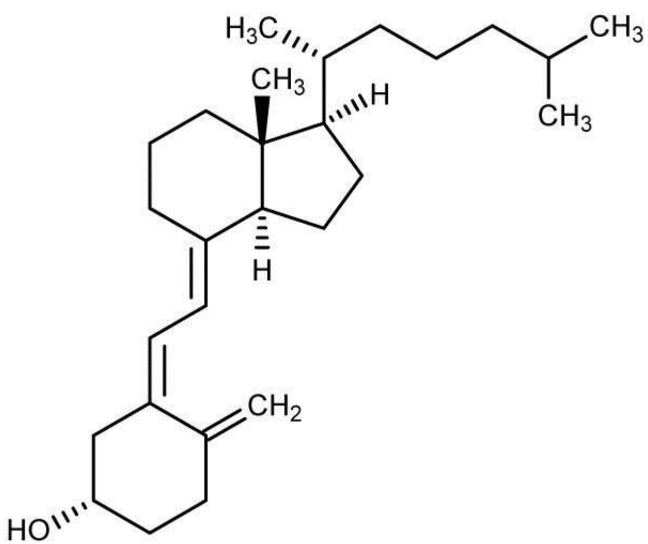


Figure 1. Vitamin D_3 structure (1930s, Windaus)[7]

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

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

Population	Latitude	Summer period	Winter period
Polish professional soccer players[9]	51 ° N	50%	16.7%
College athletes[10]	41.3 ° N	75.6%	15.20%
Spanish soccer players[11]	37 ° N	93%	36%

←37 ° N

Vitamin D and Athletic Performance

- Higher serum 25(OH)D concentrations are associated with greater muscle strength and athletic performance in some[12,13,14], but not all studies[14,15]:

(+)

- — **Post-menarchal girls**: positive relationship between vitamin D and jump velocity, jump height, power, and force[12]
- — **Healthy men and women**: Vitamin D was significantly associated with arm and leg muscle strength when controlling for age and gender[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[14]

- — **Club-level athletes**: increases in serum 25(OH)D had no significant effect on the physical anaerobic tests[15]

PERSONAL STUDY

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)

Supplement group	Age (yr)	Weight (kg)	Serum 25(OH)D (nmol/L)
Placebo (n=7)	20.3 ± 1.4	72.2 ± 4.4	67.9 ± 24.2
2000 IU vitamin D_3 (n=7)	18.7 ± 1.1	70.4 ± 9.3	66.9 ± 26.5

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 D_3
(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

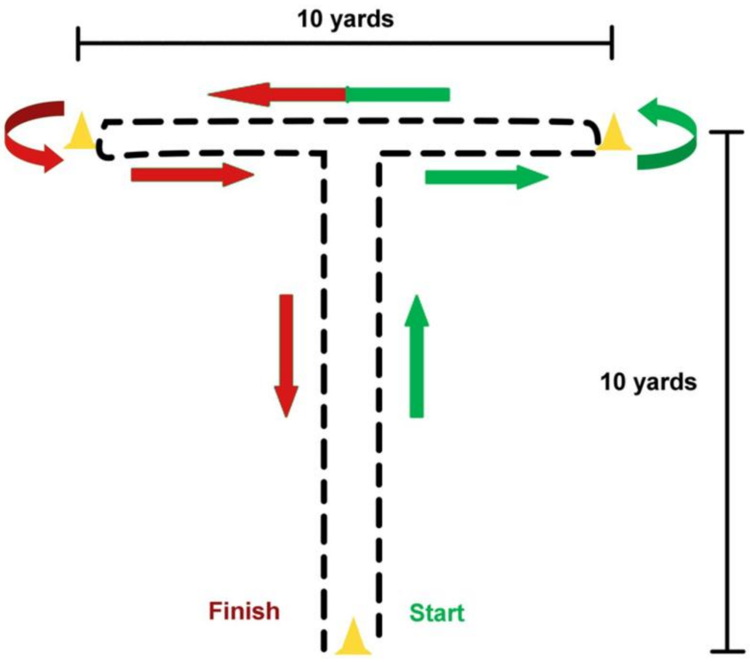


Figure 2. T drill agility test

VERTICAL JUMP

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

Serum 25(OH)D (nmol/L)	Status
< 50	Deficient
50-75	Insufficient
75-125	Optimal

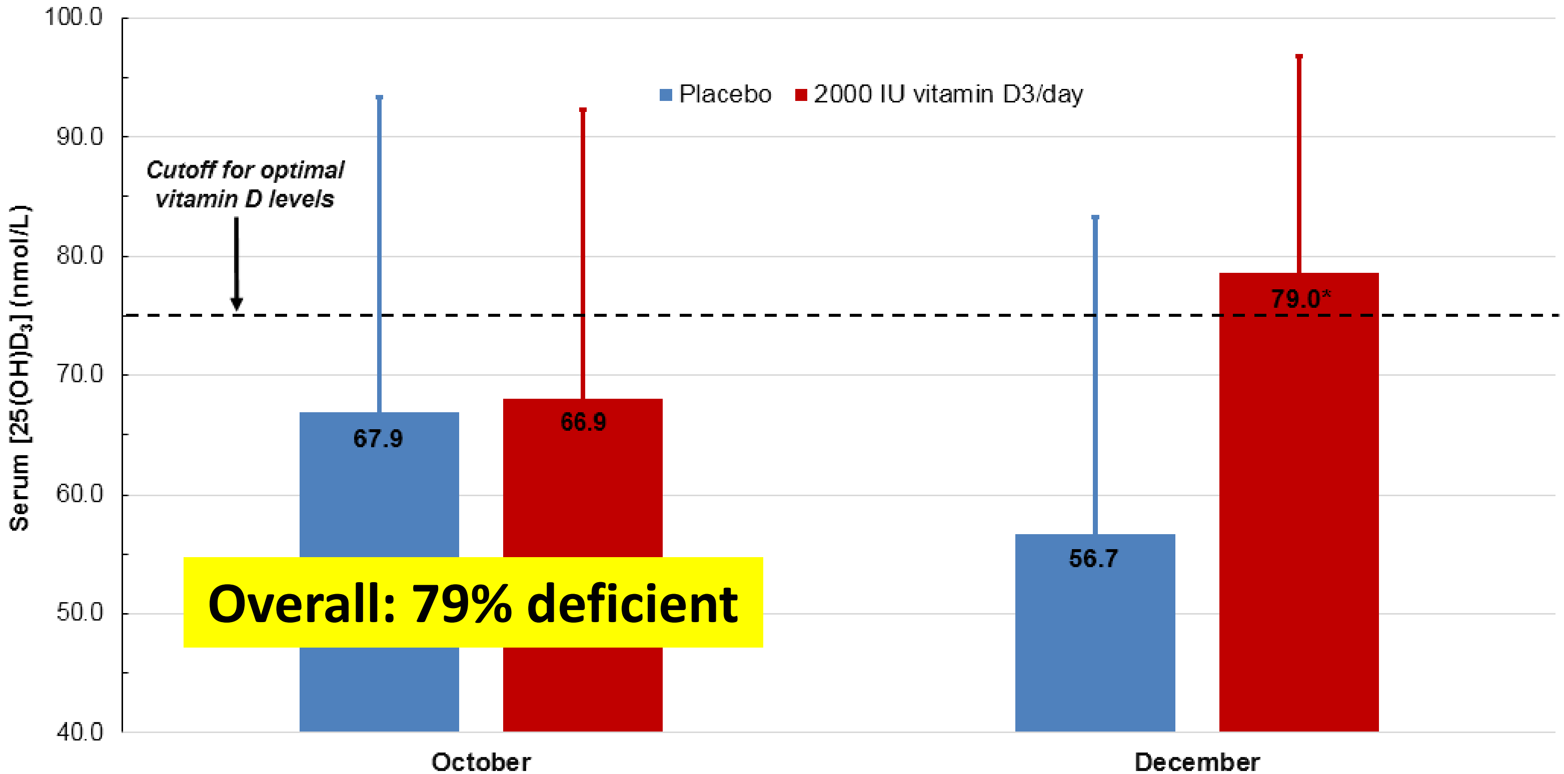
What were the baseline and final serum total 25[OH]D concentrations?

Table 4. Serum vitamin D_3 status changes over 60 day supplementation period (mean ±SD)

	Placebo (n=7)	2000 IU vitamin D_3 (n=7)
25(OH)D_3 (nmol/L)		
Pre-supplementation	66.9 ± 26.5	67.9 ± 24.2
Post-supplementation	56.7 ± 26.5	79.0 ± 18.2*
Vitamin D Sufficient (%)		
Pre-supplementation	42.9	14.3
Post-supplementation	42.9	57.1*

** $p < 0.05$*

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)

	Placebo (n=7)	2000 IU vitamin D_3 (n=7)
T Drill Agility Test (s)		
Pre-supplementation	11.3 ± 0.7	11.6 ± 1.1
Post-supplementation	11.4 ± 0.3	11.1 ± 0.6
Vertical Jump (cm)		
Pre-supplementation	47.3 ± 6.7	47.8 ± 6.6
Post-supplementation	48.2 ± 6.2	48.8 ± 6.2

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)

Are you currently taking a supplement that contains vitamin D?

[ ] Yes [X] No [ ] I don't know

How often do you use a tanning bed?

[ ] 3+ times/week [ ] 1-2 times/week

[ ] 1-3 times/month [X] Never

How many glasses of milk do you consume per day?

[ ] 3+ glasses [ ] 2-3 glasses [ ] 1 glass

[ ] <1 glass [X] 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 [X] I don't eat "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

Frequency of supplement use	Percentage of participants
5-7 days/week	64% (n=9)
3-5 days/week	29% (n=4)
1-3 days/week	7% (n=1)

Other limitations to the study?

- **Small sample size**
- **Limited number of anaerobic tests**
  - ✓ Additional tests
    - o Maximum strength tests (e.g. 1-RM measurements)[12]
    - o Short sprints (e.g. 10 m sprint)[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*
    - 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?

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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

Group	Agility T Test (s)	Vertical Jump (in)
College basketball players (women)	9.0	21
Competitive college athletes (women)	10.8	16-18.5
Sedentary college students (women)	13.5	8-14

*The values listed are either means or 50^{th} 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[4]:**
  - Muscle protein synthesis
  - Muscle strength
  - Muscle size
  - Reaction time
  - Balance
  - Coordination
  - Endurance

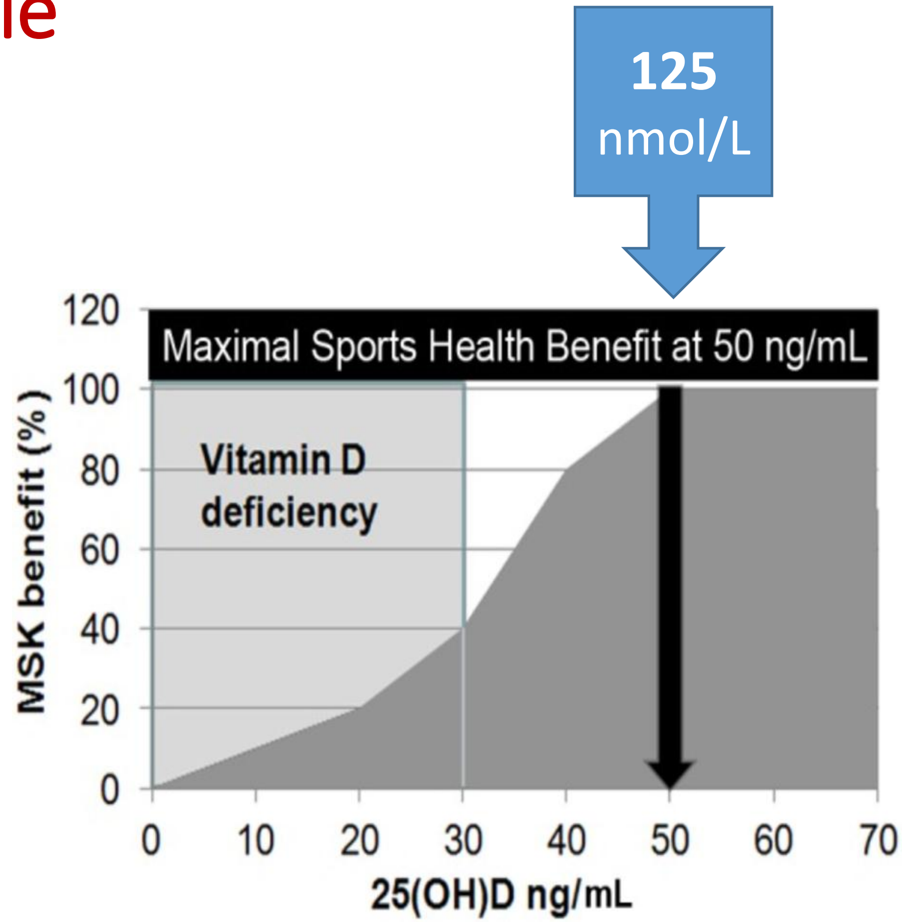
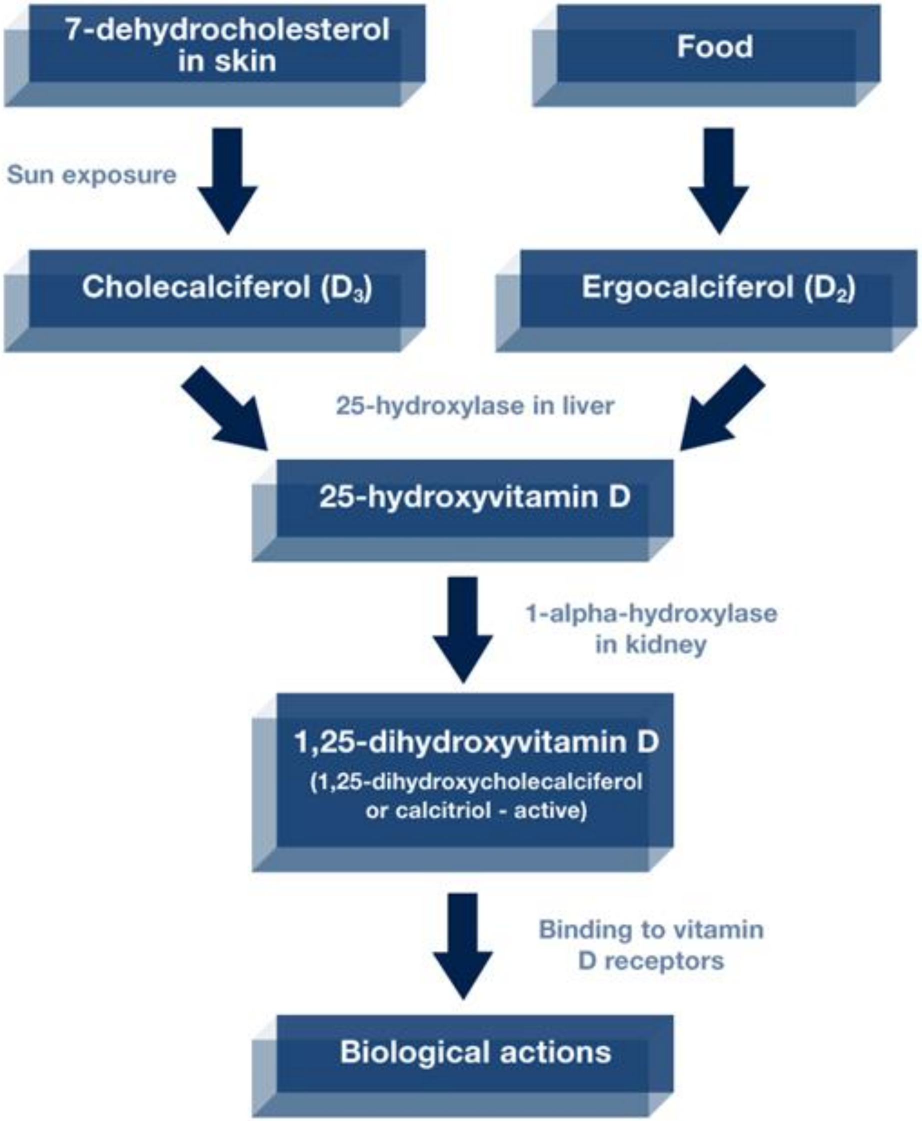


Fig 1. An increase in the storage form of vitamin D is associated with incremental improved musculoskeletal performance[4]

Vitamin D Conversion Mechanism



Illinois Agility Test