Supplemental vitamin D increases serum cytokines in those with initially low 25-hydroxyvitamin D: A randomized, double blind, placebo-controlled study

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ABSTRACT

The purpose of this study was to determine if vitamin D status before supplementation influences the cytokine response after supplemental vitamin D. Forty-six reportedly healthy adults (mean(SD); age, 32(7) y; body mass index (BMI), 25.3(4.5) kg/m^2; serum 25-hydroxyvitamin D (25(OH)D), 34.8(12.2) ng/mL) were randomly assigned (double blind) to one of three groups: (1) placebo ($n = 15$), or supplemental vitamin D (cholecalciferol) at (2) 4000 ($n = 14$) or (3) 8000 IU ($n = 17$). Supplements were taken daily for 35 days. Fasting blood samples were obtained before (Baseline, Bsl) and 35-days after (35-d) supplementation. Serum 25(OH)D, 1,25-dihydroxyvitamin D (1,25(OH)D), cytokines, and intact parathyroid hormone with calcium were measured in each blood sample. Supplemental vitamin D increased serum 25(OH)D (4000 IU, ≈29%; 8000 IU, ≈57%) and 1,25(OH)D (4000 IU, ≈12%; 8000 IU, ≈38%) without altering intact parathyroid hormone or calcium. The vitamin D metabolite increases in the supplemental vitamin D groups ($n = 31$) were dependent on initial levels as serum 25(OH)D ($r = -0.63$, $p < 0.05$) and 1,25(OH)D ($r = -0.45$, $p < 0.05$) at Bsl correlated with their increases after supplementation. Supplemental vitamin D increased interferon (IFN)-γ and interleukin (IL)-10 in subjects that were vitamin D insufficient (serum 25(OH)D < 29 ng/mL) compared to sufficient (serum 25(OH)D ⩾ 30 ng/mL) at Bsl. We conclude that supplemental vitamin D increase a pro- and anti-inflammatory cytokine in those with initially low serum 25(OH)D.

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1. Introduction

Cytokines are instrumental in orchestrating immune responses, host defenses, and intra- and inter-cellular signaling. The identification of the vitamin D receptor on immune cells [1,2] and the increase in circulating 1,25-dihydroxyvitamin D (1,25(OH)D) concentrations in patients with sarcoidosis [3,4] triggered the regulatory interest of vitamin D on cytokines. Previously, in young adults, low vitamin D (serum 25-hydroxyvitamin D (25(OH)D) ⩽ 32 ng/mL) associated with an increase in tumor necrosis factor (TNF)-α, interferon (IFN)-γ, interleukin (IL)-1β, and IL-2 [5]. Consistent with that finding, the seasonal increase

in serum 25(OH)D and 1,25(OH)D concentrations during summer associated with a down-regulation in cytokine production [6]. These data suggest that low serum 25(OH)D and 1,25(OH)D associate with a cytokine increase. Reports also indicate that supplemental vitamin D decreases pro-inflammatory (e.g., TNF-α and IL-6) and increases anti-inflammatory (e.g., IL-10) cytokines [7–9], although contrasting results exist [10–12]. A variety of factors could contribute to the discrepancies between studies, such as the selected patient or subject cohort or the dose of supplemental vitamin D studied. Surprisingly, it is unknown if the cytokine response to supplemental vitamin D is altered by the pre-supplemental vitamin D status, such as vitamin D insufficiency compared to sufficiency.

Vitamin D deficiency or insufficiency hinders muscle strength and physical performance, which can be detrimental to athletic performance in young adults and to activities of daily living in

elderly [13–23]. Although supplemental vitamin D increases serum 25(OH)D, results regarding the influence of supplementation on muscle strength and physical performance are inconsistent [24–30]. In a previous investigation, supplemental vitamin D (4000 IU/d of cholecalciferol) enhanced strength recovery following (Post to 24-h) an exercise protocol that induced immediate and persistent deficits in muscle strength [31]. Despite that provocative finding, the sum of the data could suggest otherwise as more subjects tended to complete the exercise protocol, and peak isometric force and peak power output tended to decrease to a greater extent immediately after exercise in the vitamin D group [31]. These findings imply that supplemental vitamin D could increase the amount of work performed during exercise, and consequentially, exacerbate fatigue (i.e., immediate deficit in peak isometric force or power output) and enhance recovery after exercise.

Unraveling the physiological influence of vitamin D on cytokines and muscle work are paramount to understanding the immune/inflammatory events that govern host defenses and the endocrine role of vitamin D on muscular performance. Based on the aforementioned gaps in our knowledge, the purpose of this study was twofold. First, we sought to identify if vitamin D status prior to supplementation influences the cytokine response after supplemental vitamin D. Second, we examined the influence of supplemental vitamin D on work and work fatigue during fatiguing exercise. We hypothesized that: (1) vitamin D insufficiency before supplementation associates with a cytokine decrease after supplemental vitamin D, and (2) supplemental vitamin D increases work and work fatigue during fatiguing exercise.

2. Materials and methods

The Urban Central Region Institutional Review Board at Intermountain Healthcare (Salt Lake City, UT, USA) approved this study. Subjects were informed of and provided written and verbal consent to the experimental protocol and procedures. Reportedly healthy and modestly active (i.e., 30 min of continuous physical activity at least 3 times per week) adults were recruited to participate in this study. Potential subjects were excluded from participation if: they were taking a dietary supplement, using tobacco, using prescribed or recommended medication, reported a known history of any disease or condition requiring medical attention, suffered a leg injury during the previous year that required the use of crutches, planning on increasing or decreasing the amount of time spent in the sun or tanning bed, traveling south of 37°N in latitude during study participation, or morbidly obese (body mass index (BMI) > 40 kg/m^2). Data was collected between September 2012 and May 2013 in Salt Lake City, UT (40°N latitude).

2.1. Study design

This study consisted of a randomized, double-blind, placebo controlled experimental design. Subjects were randomly assigned to one of three groups: (1) placebo or supplemental vitamin D (cholecalciferol) at (2) 4000 IU or (3) 8000 IU. Supplements were taken daily for 35 days. USANA Health Sciences (Inc., Salt Lake City, UT, USA) donated and provided a quality control analysis of the supplements. Randomization was stratified by gender and permutated into blocks of six.

During participation, subjects were asked to keep their diet consistent with their regular eating habits during the previous year and to refrain from the use of dietary supplements. Subjects were also instructed to refrain from physical activity and using aspirin, ibuprofen, naproxen sodium, acetaminophen, or other anti-inflammatory agents 72 h prior to a blood draw. Fasting blood samples were obtained and single-leg strength testing was performed prior

to (Baseline; Bsl) and 35 days after supplementation (35-d). An isokinetic exercise protocol was performed after the single-leg strength testing at 35-d. Immediately after the isokinetic protocol, each subject performed another peak knee extension isometric contraction (Post-Ex; see below).

2.2. Blood sample handling

Fasting blood samples were obtained from the antecubital vein into four 6.0 cc red top serum Vacutainer tubes and one 4.5 cc green-top Vacutainer tube (PST gel and lithium heparin 76 units). Plasma was separated by centrifugation (VWR International, Clinical 50 Centrifuge, Radnor, PA, USA) at 1400g for 15 min within 20 min of sample collection. Following separation, plasma samples were sent to ARUP Laboratories (Salt Lake City, UT, USA) for clinical chemistries (see below). After coagulation, serum was separated by centrifugation (VWR International, Clinical 50 Centrifuge, Radnor, PA, USA) at 1100g for 10 min, and then aliquoted into several small micro-centrifuge tubes. Aliquoted serum samples were stored at −80 °C (Revco Freezer, GC Laboratory Equipment, Asheville, NC, USA) until analysis (see below).

2.3. Analytical procedures

Serum 25(OH)D concentrations (ng/mL) were measured in duplicate (coefficient of variation (CV) = 6.65%) in each blood sample, as described [10]. In brief, analytes were separated on an Agilent high performance-liquid chromatography system (series 6460, Model G6460A, Santa Clara, CA, USA) and detected on an Agilent tandem mass spectrometer (Series 6410, Model G6410B, Santa Clara, CA, USA) using atmospheric pressure chemical ionization detection (350 °C gas temperature, 400 °C vaporizer). The 25(OH)D$_3$, deuterated 25(OH)D$_3$ internal standard, and 25(OH)D$_2$ precursor ions were 383.3, 386.3, and 395.4, respectively. The 25(OH)D$_3$, deuterated 25(OH)D$_3$, and 25(OH)D$_2$ product ions were 365.3, 368.3, and 208.9, respectively. Serum 25(OH)D$_2$ and 25(OH)D$_3$ concentrations were determined relative to authentic standards and corrected for recovery of the 25(OH)D$_3$ internal standard. Serum 25(OH)D$_2$ (limit of detection = 2.0 ng/mL) was not detected in any of the subjects. Therefore, serum 25(OH)D total concentrations are referred to as serum 25(OH)D concentrations hereafter. Serum 1,25(OH)D concentrations were determined using a quantitative radioimmunoassay (ARUP Laboratories, Salt Lake City, UT, USA).

Cholesterol (mg/dL), triglycerides (mg/dL), intact parathyroid hormone (iPTH; pg/mL) with calcium (mg/dL), and a metabolic panel were measured in plasma samples (ARUP Laboratories, Salt Lake City, UT, USA).

The multiplex technology of Luminex (MAGPix; Austin, TX, USA) was used to analyze serum cytokine concentrations (pg/mL) with high sensitivity (EMD Millipore, Billerica, MA, USA; catalog #: HSCYMAG60SPMX13).

Knee extension peak isometric torque ($N * m$) testing was performed on a Biodex S4 (Shirley, NY, USA). Subjects were seated in an upright (90° hip flexion) position with the chair and dynamometer adjusted to align the lateral condyle of the femur to the center of the dynamometer shaft. The dynamometer lever attachment was adjusted and strapped above the malleoli. Subjects were stabilized to the chair with shoulder, waist, and thigh straps. Subjects performed several submaximal isometric contractions to become familiar with the testing protocol and procedure prior to the maximal effort isometric contractions. Thereafter, knee extension isometric contractions were performed in triplicate (CV = 5.79%) with the reportedly dominant leg. Each contraction was 5 s in duration and separated by 1 min of rest. Subjects were verbally instructed and strongly encouraged to exert maximal

force against the lever. Peak torque was defined as the highest torque produced at each testing session.

2.4. Isokinetic exercise protocol

After isometric testing at Post, each subject performed fatiguing exercise. During this test, subjects performed 50 isokinetic (concentric knee extension and flexion contractions at 120°/s) contractions with the dominant leg. Starting from a flexed position (90° of knee flexion), subjects performed several submaximal contractions to become familiar with the testing protocol and procedure. Thereafter, subjects performed repetitive, maximal effort knee extension-flexion contraction cycles. Subjects were instructed and verbally encouraged to perform each contraction with maximal effort through a full range of motion (90° of knee flexion to full extension). Immediately following the completion of the test, another peak isometric contraction was performed using the same procedures described above.

2.5. Statistical analyses

Data were checked for normality prior to statistical analyses with a Shapiro–Wilk Test. In order to achieve normality, rank (change from Bsl to 35-d (Δ) in 1,25(OH)D, cytokines) or log (cholesterol, triglycerides, potassium, blood urea nitrogen, glucose, aspartate aminotransferase, alanine aminotransferase, bilirubin, extension peak torque, extension last third work, extension average peak torque, and flexion first third work) transformations were performed. Statistical significance of subject characteristics and muscular endurance data between groups were assessed with separate one-way analysis of variance (ANOVA) tests. Group associations with gender and baseline vitamin D status were assessed using separate Goodman–Kruskal's Gamma tests. Statistical significance of data were assessed using separate repeated measures ANOVA and followed by post hoc *t*-tests on multiple pairwise comparisons when appropriate. Statistical significance in changes from Bsl to 35-d (i.e., Δ) were assessed with a one-way ANOVA followed by a Bonferroni post hoc test on multiple pairwise comparisons when appropriate. A Pearson Product moment linear correlation was performed to examine the association between variables. Significance was set at $p < 0.05$. All statistical analyses were performed with SYSTAT (version 13.1, Chicago, IL, USA).

3. Results

Subject characteristics (Table 1) were not significantly different between groups. Vitamin D status classification (i.e., deficient,

Table 1
Subject characteristics upon enrollment.

| | Supplement | | |
	placebo	4000 IU	8000 IU
n (males:females)	15 (8:7)	14 (8:6)	17 (10:7)
Age (y)	34 (5)	32 (7)	29 (7)
Height (cm)	171 (12)	169 (8)	170 (13)
Body mass (kg)	75.9 (17.7)	76.9 (20.7)	69.9 (17.7)
BMI (kg/m^2)	25.8 (4.3)	26.5 (6.0)	23.9 (2.6)
Pre vitamin D status			
Deficient (*n*)	1	2	2
Insufficient (*n*)	3	2	4
Sufficient (*n*)	11	10	11

Data presented as mean (SD) unless noted otherwise.
Deficient, serum 25(OH)D $\leqslant$ 20 ng/mL.
Insufficient, serum 25(OH)D 20–29 ng/mL.
Sufficient, serum 25(OH)D $\geqslant$ 30 ng/mL.

insufficient, or sufficient) was not significantly different between groups and the majority (~70%) of the subjects were vitamin D sufficient before supplementation. Clinical chemistries (including iPTH and calcium) were not significantly different between groups before or after supplemental vitamin D (Table 2).

It should be noted that one subject (female, serum 25(OH)D was 46.4 ng/mL at Bsl and 69.0 ng/mL at 35-d) in the 8000 IU group had a plasma calcium concentration (10.6 mg/dL) above the clinical reference range (8.2–10.2 mg/dL) at 35-d. The subjects' iPTH concentration was within clinical reference range (15–65 pg/mL) at Bsl (59 pg/mL) and 35-d (40 pg/mL). Regarding iPTH, three subjects displayed a concentration greater than 65 pg/mL upon enrollment. After supplementation, one subject (male, 4000 IU group; serum 25(OH)D was 40.4 ng/mL at Bsl and 44.9 ng/mL at 35-d) displayed an iPTH concentration of 71 pg/mL.

3.1. Serum 25(OH)D and 1,25(OH)D concentrations

Following supplementation, serum 25(OH)D concentrations were significantly increased in the 4000 ($\approx$29%) and 8000 IU groups ($\approx$57%) (Fig. 1A). Surprisingly, serum 25(OH)D concentrations at 35-d were not significantly different between the 4000 and 8000 IU groups. This unexpected finding could be the result of greater (~9%), albeit not statistically significant, serum 25(OH)D concentrations before supplementation in the 4000 IU group and a smaller change in serum 25(OH)D after supplementation. Supporting this speculation, the Δ in serum 25(OH)D following supplemental vitamin D inversely correlated ($n = 31$ (4000 and 8000 IU groups only), $r = -0.63$, $p < 0.05$, data not shown) with serum 25(OH)D concentrations prior to supplementation and was significantly increased in the 8000 compared to the 4000 IU group following supplementation (Fig. 1B).

Before supplementation, serum 1,25(OH)D concentrations were not significantly different between groups (Fig. 1C). Following supplemental vitamin D at 4000 and 8000 IU, serum 1,25(OH)D concentrations were significantly increased ($\approx$12% and $\approx$38%, respectively). Despite a trend for increasing concentrations in the highest supplemental vitamin D group, 1,25(OH)D concentrations at 35-d were not significantly different between the 4000 and 8000 IU groups. The Δ in serum 1,25(OH)D was not significantly different between vitamin D groups despite a significant increase in the 8000 IU compared to the placebo group (Fig. 1D).

In the supplemental vitamin D groups, Bsl serum 25(OH)D concentrations correlated with those of 1,25(OH)D (Fig. 2A), and Bsl serum 1,25(OH)D concentrations inversely correlated with the Δ in serum 1,25(OH)D (Fig. 2B). The Δ in serum 25(OH)D correlated with the Δ in serum 1,25(OH)D (Fig. 2C).

3.2. Cytokines

Serum cytokine concentrations were not significantly different between groups prior to or following supplementation (Table 3). In a previous study, we found a pro-inflammatory cytokine increase in subjects with an insufficient serum 25(OH)D concentration [5]. Therefore, we examined the influence of supplemental vitamin D status at Bsl on serum cytokine changes after supplemental vitamin D. Subjects categorized as vitamin D deficient (serum 25(OH)D < 20 ng/mL) and insufficient (serum 25(OH)D 20–29 ng/mL) before supplementation were pooled into one group due to few subjects in each group respectively (see Table 1). The changes in serum IFN-γ and IL-10 were significantly increased in subjects classified as vitamin D insufficient (serum 25(OH)D < 29 ng/mL, $n = 10$) compared to sufficient (serum 25(OH)D $\geqslant$ 30 ng/mL, $n = 21$) before supplementation (Fig. 3). Although changes from Bsl were not correlated, IFN-γ and IL-10 significantly correlated at Bsl and 35-d (Table 4).

Table 2
Circulating clinical chemistries.

|	Supplement					
	placebo		4000 IU		8000 IU	
	Bsl	35-d	Bsl	35-d	Bsl	35-d
Cholesterol (mg/dL)	178 (37)	187 (33)	162 (34)	163 (9)	171 (47)	174 (42)
Triglycerides (mg/dL)	105 (54)	116 (59)	94 (48)	117 (58)	97 (34)	106 (33)
iPTH (pg/mL)	43.9 (16.1)	33.9 (9.9)	40.9 (10.5)	37.8 (11.0)	37.5 (15.7)	30.1 (12.1)
Calcium (mg/dL)	9.27 (0.30)	9.34 (0.24)	9.34 (0.22)	9.47 (0.31)	9.44 (0.30)	9.59 (0.37)
Sodium (mmol/L)	150 (10)	150 (11)	148 (10)	147 (10)	147 (10)	147 (8)
Potassium (mmol/L)	4.21 (0.38)	4.19 (0.28)	4.14 (0.26)	4.06 (0.25)	4.12 (0.40)	4.10 (0.38)
Chloride (mmol/L)	108 (8)	108 (8)	106 (7)	105 (6)	106 (8)	105 (5)
CO_2 (mmol/L)	20.7 (2.3)	20.8 (2.5)	20.7 (2.2)	20.1 (2.8)	21.2 (2.9)	21.3 (1.9)
Alkaline phosphatase (U/L)	65.0 (17.9)	65.6 (17.3)	65.9 (16.9)	66.5 (21.1)	66.8 (17.5)	68.9 (19.4)
Alanine aminotransferase (U/L)	19.6 (12.0)	21.3 (17.9)	17.0 (7.8)	16.9 (11.3)	15.1 (8.7)	15.8 (9.0)
Aspartate aminotransferase (U/L)	24.9 (7.1)	26.5 (12.6)	22.1 (7.5)	23.6 (9.3)	21.2 (7.2)	21.2 (7.3)
Glucose (mg/dL)	95.6 (11.3)	94.7 (12.7)	97.2 (16.0)	98.4 (20.2)	92.2 (9.7)	91.3 (11.3)
Blood urea nitrogen (mg/dL)	14.0 (3.0)	14.8 (3.5)	14.0 (4.2)	14.4 (3.3)	15.0 (4.3)	14.2 (3.9)
Creatinine (mg/dL)	0.96 (0.12)	0.96 (0.17)	1.03 (0.25)	1.02 (0.23)	0.97 (0.21)	0.98 (0.18)
Total protein (g/dL)	7.86 (0.88)	7.93 (0.93)	7.69 (0.56)	7.75 (0.76)	7.87 (0.66)	7.99 (0.83)
Albumin (g/dL)	4.91 (0.54)	4.86 (0.63)	4.79 (0.43)	4.74 (0.72)	4.75 (0.46)	4.63 (0.91)
Total bilirubin (mg/dL)	0.50 (0.24)	0.47 (0.28)	0.49 (0.38)	0.51 (0.24)	0.75 (0.55)	0.64 (0.560)

Data presented as mean (SD).
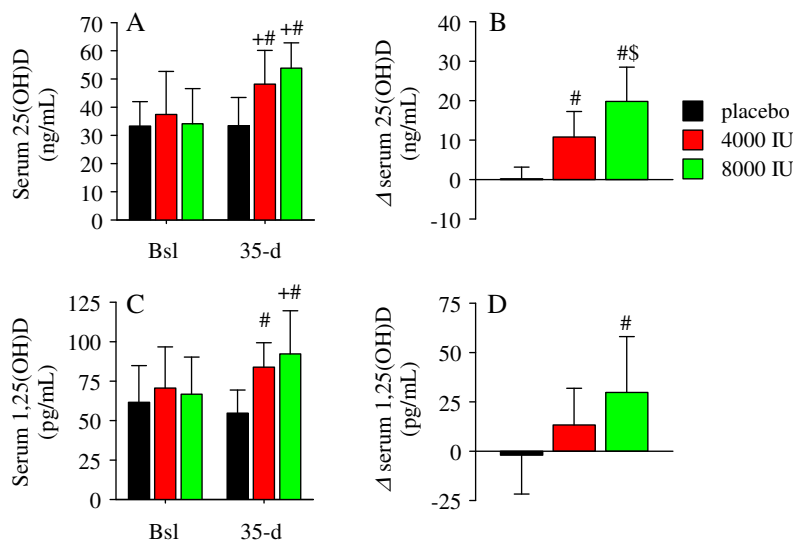
Fig. 1. Serum 25(OH)D and 1,25(OH)D concentrations and concentration changes from Bsl to 35-d (Δ). (A) Serum 25(OH)D concentrations were significantly (treatment × time interaction, $p < 0.05$) increased following supplemental vitamin D at 4000 and 8000 IU. (B) The Δ in serum 25(OH)D concentrations were significantly ($p < 0.05$) different between groups. (C) Serum 1,25(OH)D concentrations were significantly (treatment × time interaction, $p < 0.05$) increased following supplemental vitamin D at 4000 and 8000 IU. (D) The change from Bsl to 35-d (Δ) in serum 1,25(OH)D concentrations was significantly ($p < 0.05$) increased in the 8000 IU group. [+]$p < 0.05$ vs. Bsl; [#]$p < 0.05$ vs. corresponding placebo; [$]$p < 0.05$ vs. corresponding 4000 IU. Data presented as mean (SD).
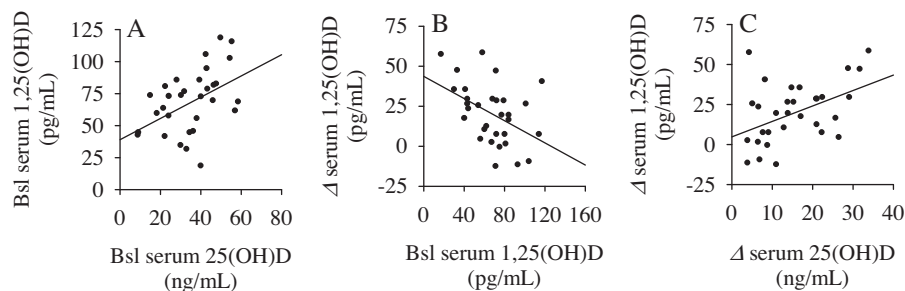
Fig. 2. Correlation between serum 25(OH)D and 1,25(OH)D concentrations and concentration changes from Bsl (Δ). (A) Bsl serum 25(OH)D concentrations significantly ($n = 31$, $r = 0.47$, $p < 0.05$) correlated with Bsl serum 1,25(OH)D concentrations. (B) Bsl serum 1,25(OH)D concentrations significantly ($n = 31$, $r = -0.45$, $p < 0.05$) correlated with the Δ in serum 1,25(OH)D concentrations. (C) The Δ in serum 25(OH)D correlated ($n = 31$, $r = 0.46$, $p < 0.05$) with the Δ in serum 1,25(OH)D concentrations.

Table 3
Serum cytokine concentrations, pg/mL.

	Supplement					
	placebo		4000 IU		8000 IU	
	Bsl	35-d	Bsl	35-d	Bsl	35-d
GM-CSF	3.19 ± 1.61	2.46 ± 1.11	0.97 ± 1.31	0.98 ± 1.10	3.17 ± 4.07	2.04 ± 2.88
IFN-γ	15.8 ± 4.18	15.3 ± 3.99	6.04 ± 4.98	7.20 ± 4.66	9.21 ± 13.7	9.50 ± 10.1
TNF-α	7.51 ± 1.09	7.22 ± 0.97	5.73 ± 0.66	5.83 ± 0.56	7.59 ± 0.48	6.47 ± 0.52
IL-1β	1.18 ± 0.34	1.19 ± 0.31	0.49 ± 0.20	0.63 ± 0.16	0.72 ± 0.43	0.88 ± 0.32
IL-2	2.32 ± 0.68	2.48 ± 0.73	1.58 ± 0.86	0.91 ± 0.72	2.10 ± 0.97	2.29 ± 0.86
IL-4	16.1 ± 16.2	14.1 ± 15.8	10.2 ± 17.3	8.67 ± 19.0	10.2 ± 10.1	7.76 ± 12.4
IL-5	0.27 ± 0.31	0.20 ± 0.26	0.15 ± 0.10	0.13 ± 0.08	0.26 ± 0.29	0.27 ± 0.22
IL-6	1.56 ± 0.27	1.52 ± 0.45	0.98 ± 0.40	1.01 ± 0.27	1.39 ± 0.36	1.32 ± 0.32
IL-7	6.88 ± 1.31	8.90 ± 1.07	5.67 ± 1.86	5.78 ± 1.77	5.97 ± 1.06	6.19 ± 0.80
IL-8	7.25 ± 0.78	8.77 ± 0.96	6.38 ± 0.90	6.38 ± 0.71	7.24 ± 0.95	7.48 ± 0.84
IL-10	15.3 ± 5.89	15.2 ± 5.19	11.0 ± 1.96	14.2 ± 1.49	12.7 ± 5.30	11.8 ± 4.68
IL-12	3.24 ± 1.68	2.95 ± 1.12	1.34 ± 2.74	1.70 ± 2.05	2.74 ± 2.44	2.95 ± 2.11
IL-13	9.37 ± 6.78	8.64 ± 6.80	5.59 ± 2.38	4.82 ± 2.42	10.8 ± 7.71	12.2 ± 7.51

Data presented as median ± SEM.
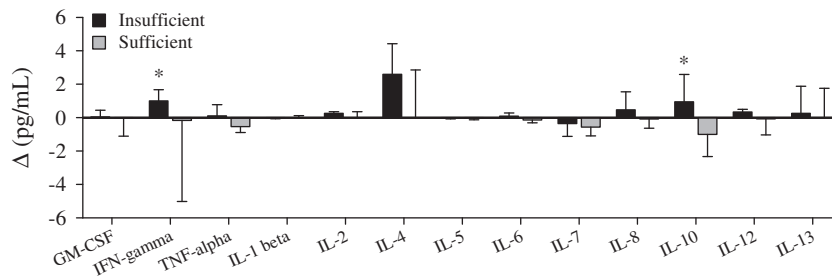
Fig. 3. Serum cytokine concentration (pg/mL) changes from Bsl to 35-d (Δ). *p < 0.05 vs. sufficient. Data presented as median ± SEM.

Table 4
IFN-γ and IL-10 Spearman Rank coefficients.

		IFN-γ		
		Bsl	35-d	Δ
IL-10	Bsl	0.37*		
	35-d	0.36	0.45*	
	Δ	0.10	0.23	0.18

Δ, change from Bsl to 35-d.
* p < 0.05.
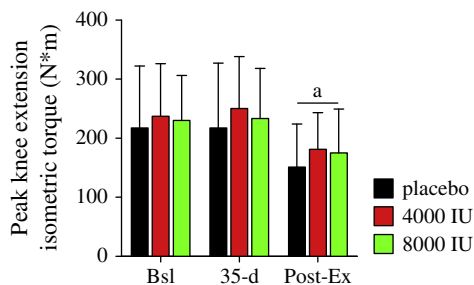
Fig. 4. Peak-concentric knee extensor isometric torque (N * m). Torque was significantly decreased at Post-Ex ([a]p < 0.05 vs. Bsl and 35-d). Differences between groups (placebo vs. 4000 IU vs. 8000 IU) were not significant. Data presented as mean (SD).

3.3. Peak knee extensor isometric torque and isokinetic data

Supplemental vitamin D increased serum 25(OH)D and 1,25(OH)D concentrations without altering peak knee extensor isometric torque (Fig. 4). Similarly, the deficit (placebo ≈30.4%, 4000 IU ≈ 25.7%, and 8000 IU ≈ 24.4%) in peak isometric torque

Table 5
Isokinetic protocol results.

	Supplement		
	placebo	4000 IU	8000 IU
Concentric knee extension			
Peak torque (N * m)	132 (58)	145 (46)	136 (45)
Avg. peak torque (N * m)	78.5 (29.5)	86.0 (22.3)	84.3 (30.5)
Time to peak torque (ms)	359 (75)	309 (73)	330 (74)
Total work (J)	4375 (1722)	4615 (1400)	455 (1443)
First third work (J)	2148 (877)	2288 (752)	2208 (763)
Last third work (J)	880 (366)	900 (244)	909 (284)
Work fatigue (%)	58.3 (10.9)	59.3 (9.1)	55.9 (15.4)
Concentric knee flexion			
Peak torque (N * m)	84.6 (29.6)	92.7 (35.4)	83.7 (27.7)
Avg. peak torque (N * m)	57.3 (17.6)	61.7 (15.2)	56.9 (18.0)
Time to peak torque (ms)	304 (73)	258 (88.0)	278 (102)
Total work (J)	3419 (1311)	3521 (1020)	3270 (1108)
First third work (J)	1604 (654)	1666 (511)	1519 (563)
Last third work (J)	768 (298)	754 (246)	709 (243)
Work fatigue (%)	51.4 (8.2)	54.1 (9.9)	51.7 (10.5)

Data presented as mean (SD).
Avg., average.

at Post-Ex was not significantly different following supplementation. Work and work fatigue were not significantly different between groups (Table 5). Isometric torque and isokinetic results were similar when stratified by initial vitamin D status (data not shown).

4. Discussion

Low vitamin D contributes to a number of chronic conditions, such as osteomalacia, osteoporosis, type 2 diabetes, cardiovascular disease, and infectious disorders [32]. In this investigation,

supplemental vitamin D increased serum 25(OH)D and 1,25(OH)D concentrations in healthy adults. The vitamin D metabolite increases were dependent on initial levels, such that those with low serum 25(OH)D or 1,25(OH)D before displayed the greatest increases after supplementation. In subjects with insufficient serum 25(OH)D at Bsl, supplemental vitamin D increased IFN-γ and IL-10. However, muscular work and work fatigue were not significantly different with supplemental vitamin D.

Similar to others [31,33], serum 25(OH)D before inversely correlated with the change in serum 25(OH)D concentrations after supplemental vitamin D. The changes in 25(OH)D also correlated with those of 1,25(OH)D, suggesting that a disruption in the metabolite used to define vitamin D status associates with a disturbance in the hormonal form of vitamin D, and that those with low serum 25(OH)D before display the greatest increase in serum 25(OH)D and 1,25(OH)D after supplemental vitamin D. Since low vitamin D increases pro-inflammatory cytokines [34,35] and vitamin D treatment (i.e., with 25(OH)D or 1,25(OH)D) inhibits pro-inflammatory and promotes anti-inflammatory cytokine production in cell culture [34–38], we speculated that a greater increase in serum 25(OH)D and 1,25(OH)D with supplemental vitamin D in those with initially low vitamin D levels would associate with a decrease in pro-inflammatory and an increase in anti-inflammatory cytokines. Following stratification to initial vitamin D levels, supplemental vitamin D increased IFN-γ and IL-10 in those with an insufficient compared to a sufficient serum 25(OH)D concentration at baseline. This finding demonstrates that supplemental vitamin D increases a pro- and anti-inflammatory cytokine in subjects with initially low vitamin D.

A recent study in endurance athletes demonstrated that vitamin D insufficiency associated with a decrease in IFN-γ while 1,25(OH)D inhibited IFN-γ production by peripheral blood mononuclear cells [39]. This paradox suggests a pro-inflammatory cytokine decrease with low 25(OH)D and following 1,25(OH)D exposure, thereby underscoring the complexity of investigating the influence of vitamin D on cytokines and extrapolating in vitro results to in vivo conditions. The interpretation of cytokines in vivo is further convoluted when considering the antagonizing relationship between cytokines. The antagonizing regulation between cytokines is classically illustrated by IFN-γ inhibiting IL-10 production [40] and IL-10 inhibiting IFN-γ production [41,42]. With this relationship in mind, we investigated the correlation between IFN-γ and IL-10 prior to and following supplemental vitamin D. Although IFN-γ and IL-10 correlated at Bsl and 35-d, the changes in IFN-γ and IL-10 from Bsl to 35-d were not significantly correlated (see Table 4). Based on these results, we propose that the antagonizing regulation between IFN-γ and IL-10 was not a significant mediator of their changes following supplemental vitamin D, but the IFN-γ and IL-10 correlations at Bsl and 35-d identify the importance of their mutual association with and without supplemental vitamin D. Future research should consider the mutual regulation between cytokines when investigating the role of vitamin D on cytokines in vivo.

Work and fatigue were not modulated by supplemental vitamin D despite previous trends for vitamin D sufficient [43] and vitamin D treated adults [31] to perform more repetitions during and to display greater deficits in peak isometric force and peak power output immediately after exercise. Although it is beyond the scope of this study, several potential explanations could account for the inability of supplemental vitamin D to alter work or work fatigue. First, most subjects ($\sim$67%) were vitamin D sufficient prior to vitamin D supplementation. In a seminal review, Cannell and colleagues [22] proposed that vitamin D deficiency before supplementation could be necessary for strength and functional improvements after supplementation. More recently, Owens and colleagues [24] expressed a similar sentiment after conducting a study in subjects with low serum 25(OH)D concentrations ($\approx$16(7) ng/mL) at baseline and prior to vitamin D supplementation. Recent work support these claims by identifying an increase in energy metabolites (i.e., phosphocreatine to inorganic phosphate ratio) and a decrease in metabolite recovery (i.e., half time recovery ($\tau_{1/2}$) of phosphocreatine) following supplemental vitamin D in healthy adults with severe vitamin D deficiency (serum 25(OH)D $\leqslant$ 6 ng/mL) [44,45]. Thus, severe vitamin D deficiency could be an underlying determinant for muscle strength or physical function improvements with supplemental vitamin D in healthy adults.

Another potential explanation for the inability of supplemental vitamin D to alter work or work fatigue is that this study was conducted in healthy, young adults. In healthy adults, muscle strength was not significantly different despite contrasting serum 25(OH)D with [10] or without [5] supplemental vitamin D. However, a serum 25(OH)D concentration $\geqslant$ 30 ng/mL improved strength recovery following anterior cruciate ligament surgery in otherwise healthy adults [46], and supplemental vitamin D (4000 IU/d of cholecalciferol) increased serum 25(OH)D ($\approx$47 ng/mL) and enhanced strength recovery following exercise-induced muscle damage in young adults [31]. Based on the evidence, muscle performance at rest or in the absence of injury may not be impaired with a low serum 25(OH)D concentration (e.g., >6 but <29 ng/mL), but maintaining or achieving a sufficient serum 25(OH)D concentration (i.e., $\geqslant$30 ng/mL) could be beneficial on the physical rehabilitation from a muscular or ligamentous injury in physically active and otherwise healthy adults.

In addition to those above, there are other limitations to this study worth discussing. The mechanism contributing to a high rate of vitamin D sufficiency in this cohort before supplementation is unknown. It is also unknown if the increase in serum 25(OH)D and 1,25(OH)D with supplemental vitamin D associated with alterations in the vitamin D binding protein, which influences the bioavailability of vitamin D. Albumin, however, is a vitamin D binding protein and it was not significantly different between groups prior to or following supplementation. Finally, results were obtained in the absence of an inflammatory response and it is unknown if these results translate to inflammatory conditions.

In summary, supplemental vitamin D increased serum 25(OH)D and 1,25(OH)D concentrations in healthy adults. The vitamin D metabolite increases were dependent on initial levels, such that those with low serum 25(OH)D or 1,25(OH)D before display the greatest increases after vitamin D supplementation. Supplemental vitamin D increased IFN-γ and IL-10 in subjects with insufficient serum 25(OH)D at baseline. The deviations of vitamin D metabolites and cytokines were apparent without an overt alteration in muscular work or work fatigue. Based on these results, we conclude that supplemental vitamin D could increase pro- and anti-inflammatory cytokine levels in those with initially low serum 25(OH)D. These findings extend our knowledge pertaining to the significance of initial vitamin D levels on the cytokine response to supplemental vitamin D.

Acknowledgement

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