Soluble TNF Receptors are Modulated by Vitamin D Status but not by Acute Perturbations in 25-Hydroxyvitamin D Following A Bolus of Supplemental Vitamin D

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Abstract

The first objective of this study was to identify if soluble tumor necrosis factor-receptor 1 (sTNFr1) and -receptor 2 (sTNFr2) are modulated by vitamin D status (insufficient *vs.* sufficient). The second objective was to reveal if soluble TNF receptors fluctuate with serum 25-hydroxyvitamin D (25(OH)D) concentrations following a bolus of supplemental vitamin D. Reportedly healthy male adults were randomly (double-blind) assigned to a placebo (n=15) or vitamin D (100,000 IU of cholecalciferol; n=14) supplement. Supplements were taken as a bolus immediately after and on the same day as providing the first blood sample (baseline (Bsl)). Fasting blood samples were also obtained at 1-, 3-, 7-, and 24-d after the bolus. Serum 25(OH)D, 1,25-dihyroxyvitamin D (1,25(OH)D), tumor necrosis factor (TNF)-α, sTNFr1, and sTNFr2 were measured in each blood sample. At Bsl, subjects were classified as vitamin D insufficient (serum 25(OH)D <29 ng/mL; n=12) or sufficient (serum 25(OH)D ≥ 30 ng/mL; n=17). Compared to insufficiency, vitamin D sufficiency associated with an increase sTNFr1 (p<0.05) and a decrease in sTNFr2 (p<0.05) without a significant difference in serum TNF-α prior to supplementation. Following supplementation, serum 25(OH)D and 1,25(OH)D transiently increased (both p<0.05) in the absence of robust deviations in TNF-α, sTNFr1, and sTNFr2. We conclude that vitamin D sufficiency differentially regulates sTNFr1 and sTNFr2, but conversely, neither soluble receptor fluctuate following a bolus of supplemental vitamin D that mediates an acute perturbation in serum 25(OH)D and 1,25(OH)D.

Keywords: TNF-α; Soluble TNF receptors; Vitamin D3

Introduction

Tumor necrosis factor (TNF)-α is a quintessential pro-inflammatory cytokine produced from immune and non-immune cells in response to diverse stimuli. Low levels of TNF-α expression is important for tissue remodeling and host defense responses, but excessive production over activates inflammatory responses and leads to tissue damage. Intracellular signals regulating the biological events governed by TNF-α are mediated through a 55 kDa TNF receptor 1 (TNFr1) and a 75 kDa TNF receptor 2 (TNFr2); both of which, are type I transmembrane glycoproteins and members of the TNF receptor family.

TNFr1 and TNFr2 are susceptible to proteolytic cleavage and alternative mRNA splicing. As a result, their soluble forms (sTNFr1 and sTNFr2) are commonly found in the circulation [1] and offer a more stable measure of long-term exposure to TNF-α [2,3]. Circulating soluble TNF receptor concentrations increase with disease [4] and could antagonize TNF-α, and subsequently, ameliorate TNF-α induced activities [2,5-7]. Conversely, soluble TNF receptors could preserve TNF-α activity, prolong the systemic half-life, and serve as a reservoir for TNF-α [2,6,8,9]. These empirical results establish the physiological importance of soluble TNF receptors to govern the inflammatory-driven properties induced by TNF-α, which provocatively, are modulated by vitamin D.

Vitamin D regulates inflammatory cytokines and is routinely assessed by serum 25-hydroxyvitamin D (25(OH)D) concentrations. Despite discrepancies [10-12], an increase in circulating 25(OH)D generally associates with a decrease in circulating TNF-α and TNF-α production [13-19]. Likewise, in isolated immune cells from patients with various diseases, 25(OH)D and 1,25-dihydroxyvitamin D (1,25(OH)D), the most biological active vitamin D metabolite, dose dependently inhibited TNF-α production [20-28]. Vitamin D also neutralizes pro-inflammatory events derived from TNF-α [29-31], collectively demonstrating its ability to regulate TNF-α levels and its biological properties. Few studies, however, report the influence of 25(OH)D on soluble TNF receptors. From sparse data, results suggest that vitamin D (i.e., 1,25(OH)D) suppressed the biological properties of TNF-α by facilitating the ectodomain shedding of TNFr1, and as a by-product, increased sTNFr1 in the culture medium [32]. In elderly, increases in sTNFr1 and sTNFr2 associated with an increase in 25(OH)D and a decrease in 1,25(OH)D [33]. Unfortunately, results are inconsistent as data from elderly men and women also demonstrate a decrease in sTNFr1 and sTNFr2 with an increase in serum 25(OH)D above a vitamin D-deficient demarcation (≥ 21.3 ng/mL) [34]. The inconsistency in the sparse literature is compounded by the void in our knowledge regarding the ability of supplemental vitamin D to modulate soluble TNF receptors concomitantly with serum 25(OH)D.

Based on the aforementioned inconsistencies and gap in our knowledge, the purpose of this investigation was two-fold. The first objective was to identify if sTNFr1 and sTNFr2 are modulated by

vitamin D status prior to vitamin D supplementation. As the second objective, we sought to identify if soluble TNF receptors fluctuate following a large bolus of supplemental vitamin D that induces an abrupt increase in serum 25(OH)D and 1,25(OH)D. We hypothesized that vitamin D sufficiency and an increase in serum 25(OH)D following a bolus of supplemental vitamin D associate with an increase sTNFr1 and sTNFr2 concentrations. Addressing this hypothesis is important because it will advance our knowledge regarding the capacity of vitamin D to regulate soluble cytokine receptors that are instrumental to pro-inflammatory biology and immune system signaling.

Materials and Methods

Subjects

Reportedly healthy and modestly active (i.e., 30 min of continuous physical activity at least 3 times per week) males between 18 and 50 years of age were recruited to participate in this study (n=29, age, 33 (8) y; height, 178 (7) cm; body mass, 84.6 (11.8) kg; body mass index (BMI), 26.6 (3.3) kg/m^2). Potential subjects were excluded from participation if they had a known history of metabolic bone disease, any skeletal muscle pathologies, cardiac or peripheral cardiovascular system abnormalities, clotting disorders, coronary artery disease, peripheral vascular disease, stroke, cancer, high cholesterol or triglycerides, high blood pressure, hypercalcemia or parathyroid dysfunction, iron deficiency within the past year, or impaired liver or kidney function. Potential subjects were also excluded from study participation if they had a known family history or diagnosis of chronic granulomatous disease, taking digoxin or antiarrhymic medications, warfarin, anti-coagulants, cholesterol lowering medication, diagnosed with diabetes mellitus, taking a daily dietary supplement, treated for vitamin D deficiency during the past year, using corticosteroid medication, 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 (BMI >40 kg/m^2). The 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 study protocol and procedures.

Study design and protocol

This study consisted of a randomized, double-blind, placebo controlled design. Subjects were randomly assigned to a placebo (PL; n=15) or vitamin D (VD; cholecalciferol at 100,000 IU; n=14) supplement group. Supplements were taken as a bolus immediately after and on the same day as providing the first blood sample (i.e., Baseline; Bsl). Tablets were taken under the supervision of the investigators. USANA Health Sciences Inc. (Salt Lake City, UT USA) donated and provided a quality control analysis of the supplements. Placebo and vitamin D tablets were identical in appearance and randomization was permutated into blocks of four.

During participation, subjects were asked to keep their dietary habits consistent with their regular routine 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 hours prior to a blood draw. Fasting blood samples were obtained prior to (Bsl) and 1-, 3-, 7-, and 24-d after ingesting the bolus.

Blood sample handling

Fasting blood samples were obtained from the antecubital vein. Plasma was separated by centrifugation (VWR International, Clinical 50 Centrifuge, Radnor, PA USA) at 1100 g for 15 min within 20 min of sample collection. Following separation, plasma samples were sent to ARUP Laboratories (Salt Lake City, UT USA) for the determination of parathyroid hormone (PTH), intact with calcium (see below). After coagulation, serum was separated by centrifugation (VWR International, Clinical 50 Centrifuge) at 1100 g 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 later analyses.

Analytical procedures

Serum vitamin D metabolites: Serum 25(OH)D concentrations were measured in duplicate (coefficient of variation = 5.29%), as previously described [35]. In brief, analytes were separated on an Agilent high performance-liquid chromatography system (series 6460, Model G6460A, Santa Clara, CA USA) and detected on 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)D3, deuterated 25(OH)D3 internal standard, and 25(OH)D2 precursor ions were 383.3, 386.3, and 395.4, respectively. The 25(OH)D3, deuterated 25(OH)D3, and 25(OH)D2 product ions were 365.3, 368.3, and 208.9, respectively. Serum 25(OH)D2 and 25(OH)D3 concentrations were corrected for recovery of the 25(OH)D3 internal standard. Serum 25(OH)D2 (limit of detection=2.0 ng/mL) was not detected in any of the samples. Therefore, serum 25(OH)D total concentrations are referred to as serum 25(OH)D concentrations hereafter. National Institute of Standards and Technology standards were measured in parallel to study samples to confirm the accuracy of the analytical procedure. Subjects were classified as vitamin D deficient, insufficient, or sufficient if they had a serum 25(OH)D concentration ≤ 20, between 21-29, or ≥ 30 ng/mL, respectively [36]. Serum 1,25(OH)D concentrations were determined using a quantitative radioimmunoassay (ARUP Laboratories) and VDBP concentrations (EMD Millipore, Billerica, MA USA) were determined using Luminex technology (MAGPix; Austin, TX USA).

Serum TNF-α, sTNFr1, and sTNFr2 concentrations

The multiplex technology of Luminex was used to analyze serum TNF-α (EMD Millipore) and soluble TNF-receptor (EMD Millipore) concentrations with high-sensitivity in The Physiology Research Laboratory at The Orthopedic Specialty Hospital (Murray, UT USA).

Plasma parathyroid hormone, intact with calcium

Plasma PTH, intact with calcium concentrations were measured using a quantitative electrochemiluminescent immunoassay at ARUP Laboratories.

Statistical analyses

Data were checked for normality prior to statistical analyses with a Shapiro-Wilk Test. Statistical significance of subject characteristics and data between vitamin D status groups (Insufficient and Sufficient) and supplements (Placebo and Vitamin D) at Bsl were assessed with either a Mann-Whitney U test or a t-test depending on the Shapiro-Wilk Test

p-value. Statistical significance of data (concentration and concentration changes) between supplement groups (PL and VD) were assessed with separate Friedman two-way analysis of variance tests and followed by multiple pairwise comparisons when appropriate. A Spearman Rank Correlation was performed to examine the association between variables. Significance was set at $p < 0.05$ and all statistical analyses were performed with SYSTAT (version 13.1, Chicago, IL USA). Data presented as median (interquartile range) unless otherwise noted.

Results

Vitamin D status group comparisons prior to supplementation

The number of subjects classified as vitamin D deficient, insufficient, or sufficient were two (6.9%), 15 (51.7%), and 12 (41.4%), respectively. Due to the small sample size in the vitamin D deficient group, we subsequently combined the deficient and insufficient groups (n=17, Insufficient) for the vitamin D status comparisons prior to supplementation. Circulating concentrations of 1,25(OH)D, VDBP, PTH, and calcium were not significantly different between the vitamin D Insufficient (serum 25(OH)D <29 ng/mL) and Sufficient groups (serum 25(OH)D ≥ 30 ng/mL; Table 1).

Serum TNF-α concentrations were not significantly different between the vitamin D Insufficient and Sufficient groups (Table 1). Conversely, serum sTNFr1 was significantly ($p < 0.05$) increased and sTNFr2 was significantly ($p < 0.05$) decreased in the vitamin D Sufficient compared to the Insufficient group (Figures 1A and 1B).

Subject characteristics and serum 25(OH)D concentrations

Subject characteristics, vitamin D status classification (deficient, insufficient, and sufficient; Table 2), plasma PTH, calcium (Table 3), and serum 25(OH)D concentrations (Figure 2A) were not significantly different between the PL and VD groups prior to supplementation. Following supplementation, serum 25(OH)D concentrations were significantly ($p < 0.05$) increased in the VD group. Serum 25(OH)D concentrations displayed a transient peaked at 3- (~31%) and 7-d (~36%), and despite a gradual decrease thereafter, remained elevated at 24-d (~21%) compared to Bsl in the VD group. Importantly, all subjects with initial vitamin D insufficiency achieved vitamin D sufficiency at 3- or 7-d in the vitamin D group, and no subjects in the VD group achieved a serum 25(OH)D concentration deemed toxic (i.e., serum 25(OH)D) ≥ 100 ng/mL) [36] despite a substantial number of subjects possessing a sufficient concentration prior to supplementation. Serum 25(OH)D concentrations progressively decreased in the PL group at 7- (~3%) and 24-d (~6%).

	Insufficient	Sufficient
n	17	12
Age (y)	31 (5)	38 (17)
Height (cm)	179 (11)	177 (8)
Body mass (kg)	88.2 (17.9)	84.2 (15.0)
BMI (kg/m^2)	25.8 (4.1)	26.0 (2.1)
Serum 25(OH)D (ng/mL)	23.5 (5.9)	33.3 (8.5)*
Serum 1,25(OH)D (pg/mL)	55.0 (18.0)	61.0 (13.5)
Serum VDBP (pg/mL)	28.6 (13.3)	34.5 (19.1)
Plasma PTH (pg/mL)	39.0 (13.3)	39.5 (18.5)
Plasma calcium (mg/DL)	9.20 (0.30)	9.45 (0.40)
Serum TNF-α (pg/mL)	7.03 (4.84)	7.52 (4.50)
Data presented as median (interquartile range) Insufficient, serum 25(OH)D <29 ng/mL Sufficient, serum 25(OH)D ≥ 30 ng/mL *p<0.05 *vs.* Insufficient		

Table 1: Vitamin D Insufficient and Sufficient subject characteristics at Bsl.

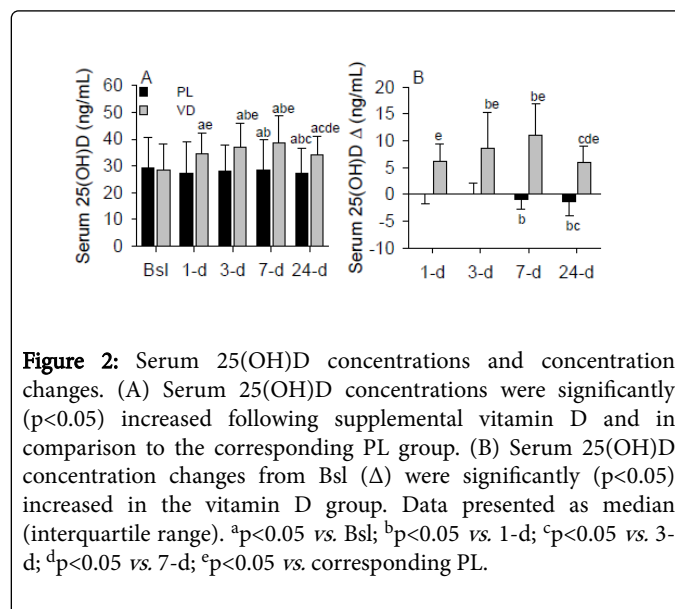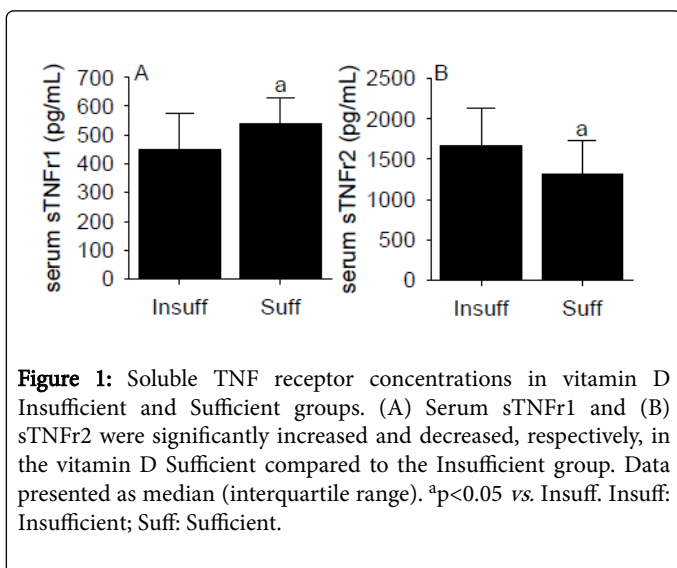As expected, serum 25(OH)D concentration changes from Bsl (Δ) were significantly ($p < 0.05$) increased in the VD group (Figure 2B). Specifically, serum 25(OH)D Δ were significantly (both $p < 0.05$) increased at 3- and 7-d compared to 1- and 24-d. Also, serum

25(OH)D Δ were significantly ($p < 0.05$) increased in the VD compared to the PL group. In the PL group, Δ were significantly decreased at 7- and 24-d (both $p < 0.05$).

	Placebo	Vitamin D
n	15	14
Age (y)	33 (8)	29 (19)
Height (cm)	179 (8)	175 (10)
Body mass (kg)	85.1 (11.5)	85.0 (23.3)
BMI (kg/m^2)	25.6 (2.5)	26.3 (7.7)
Vitamin D status Deficient (n) Insufficient (n) Sufficient (n)	1 8 6	1 7 6
Data presented as median (interquartile range)		

Table 2: Subject characteristics and vitamin D status prior to supplementation.

Figure 1: Soluble TNF receptor concentrations in vitamin D Insufficient and Sufficient groups. (A) Serum sTNFr1 and (B) sTNFr2 were significantly increased and decreased, respectively, in the vitamin D Sufficient compared to the Insufficient group. Data presented as median (interquartile range). [a]p<0.05 *vs.* Insuff. Insuff: Insufficient; Suff: Sufficient.

Figure 2: Serum 25(OH)D concentrations and concentration changes. (A) Serum 25(OH)D concentrations were significantly (p<0.05) increased following supplemental vitamin D and in comparison to the corresponding PL group. (B) Serum 25(OH)D concentration changes from Bsl (Δ) were significantly (p<0.05) increased in the vitamin D group. Data presented as median (interquartile range). [a]p<0.05 *vs.* Bsl; [b]p<0.05 *vs.* 1-d; [c]p<0.05 *vs.* 3-d; [d]p<0.05 *vs.* 7-d; [e]p<0.05 *vs.* corresponding PL.

	Bsl	1-d	3-d	7-d	24-d
Plasma PTH (pg/mL)					
PL	39.0 (11.8)	32.0 (10.0)	34.0 (12.0)	35.0 (12.5)	34.0 (9.9)
VD	38.0 (19.0)	34.0 (17.0)	26.5 (15.0)	32.5 (17.0)	30.0 (15.0)
Plasma calcium (mg/dL)					
PL	9.20 (0.30)	9.40 (0.45)	9.30 (0.20)	9.20 (0.28)	9.30 (0.48)
VD	9.45 (0.50)	9.35 (0.60)	9.45 (0.30)	9.40 (0.50)	9.60 (0.60)
Data presented as median (interquartile range)					

Table 3: Plasma PTH and calcium prior to and following supplementation.

Serum 1,25(OH)D and VDBP concentrations

Following supplementation, serum 1,25(OH)D concentrations were significantly (p<0.05) increased at 3- and 7-d compared to Bsl in the VD group (Figure 3A). At 7-d, serum 1,25(OH)D concentrations were significantly (p<0.05) increased in the VD group compared to 24-d and to the corresponding 7-d concentration in the PL group. Serum

1,25(OH)D Δ were significantly (p<0.05) increased at 7-d in the VD group and at 3-, 7-, and 24-d in the VD compared to the PL group (Figure 3B). Serum 1,25(OH)D concentrations were not significantly different in the PL group.

The VDBP carries 85-90% of the total circulating 25(OH)D [37], and importantly, maintains and stabilizes serum 25(OH)D concentrations during variable vitamin D availability [38]. Although 25(OH)D and 1,25(OH)D are two of the major vitamin D metabolites with significant biological activity, VDBP also regulates the biologic effects of vitamin D as evident by its role in altering neutrophil recruitment [39]. Therefore, we examined the VDBP response to supplemental vitamin D. Despite significant alterations in serum 25(OH)D and 1,25(OH)D, serum VDBP concentrations were not significantly modulated by a large bolus of vitamin D (Figures 3C and 3D). However, a non-significant increase in the VDBP was apparent in the VD group at 3- and 7-d. This finding could assist with generating new hypotheses and determining appropriate sample sizes for future studies examining the role of supplemental vitamin D to modulate the VDBP.

Vitamin D metabolite correlations in the VD group

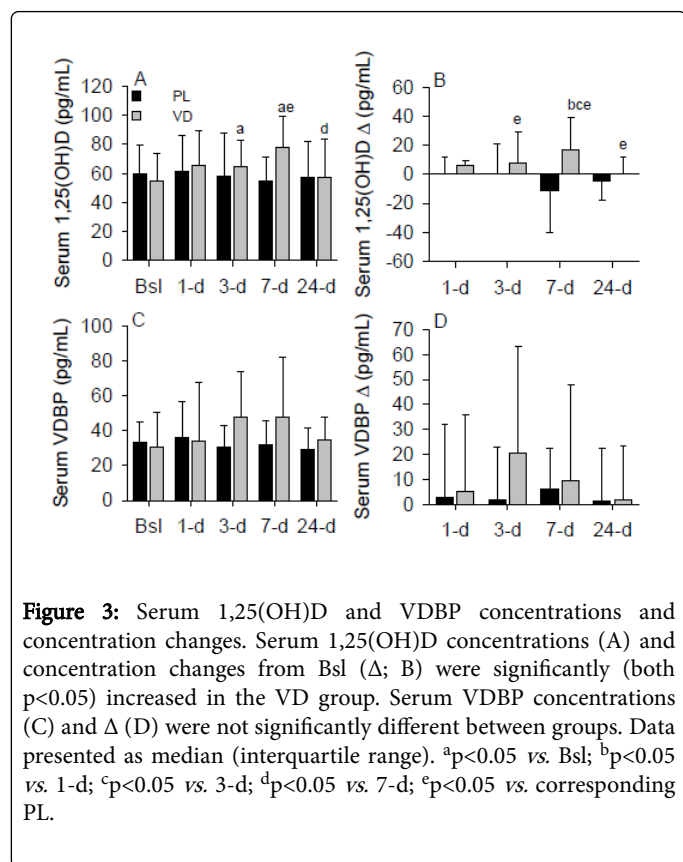As demonstrated by our lab [40] and others [41], Bsl serum 25(OH)D inversely correlated (p<0.05) with the change in serum 25(OH)D following supplementation (Table 4). Extending those findings, we found that serum 25(OH)D and VDBP at Bsl positively and negatively correlated, respectively, with the Δ in VDBP at 7-d. The inverse association between Bsl and concentration changes in VDBP following supplemental vitamin D extends previous work [42] by demonstrating that the VDBP response to supplemental vitamin D is blunted with an increase in Bsl concentrations.

Serum cytokine and soluble cytokine receptor concentrations

Despite numerous investigations illuminating the impact of vitamin D on serum TNF-α and the data from this study suggesting that vitamin D status alters soluble TNF receptors, we were unable to detect a difference in serum TNF-α, sTNFr1, or sTNFr2 concentrations between the PL and VD groups (Figures 4A-4F). In addition, vitamin D status prior to supplementation did not significantly modulate serum sTNFr1 and sTNFr2 concentrations following the bolus of supplemental vitamin D (data not shown).

	Bsl		
	25(OH)D	**1,25(OH)D**	**VDBP**
25(OH)D 1-d Δ	-0.19	0.32	-0.02
25(OH)D 3-d Δ	-0.26	0.06	0.28
25(OH)D 7-d Δ	-0.26	0.43	-0.11
25(OH)D 24-d Δ	-0.61*	0.21	-0.13
1,25(OH)D 1-d Δ	0.04	-0.06	0.11
1,25(OH)D 3-d Δ	0.15	-0.24	0.18
1,25(OH)D 7-d Δ	0.07	-0.16	0.30
1,25(OH)D 24-d Δ	0.06	-0.10	0.20
VDBP 1-d Δ	0.41	0.05	-0.12
VDBP 3-d Δ	0.38	-0.24	-0.49
VDBP 7-d Δ	0.56*	-0.22	-0.68*
VDBP 24-d Δ	0.11	-0.18	-0.45
n=14; *p<0.05			

Table 4: Vitamin D metabolite Spearman Rank correlation coefficients (ρ) in the VD group only.

Figure 3: Serum 1,25(OH)D and VDBP concentrations and concentration changes. Serum 1,25(OH)D concentrations (A) and concentration changes from Bsl (Δ; B) were significantly (both p<0.05) increased in the VD group. Serum VDBP concentrations (C) and Δ (D) were not significantly different between groups. Data presented as median (interquartile range). [a]p<0.05 *vs.* Bsl; [b]p<0.05 *vs.* 1-d; [c]p<0.05 *vs.* 3-d; [d]p<0.05 *vs.* 7-d; [e]p<0.05 *vs.* corresponding PL.

Figure 4: Serum TNF-α and soluble TNF receptor concentrations and concentration changes. Serum TNF-α, sTNFr1, and sTNFr2 concentrations and concentration changes from Bsl (Δ) were not significantly different between the PL and VD groups (A-F). Data presented as median (interquartile range).

Discussion

In this investigation, we provide the first evidence that vitamin D sufficiency increases sTNFr1 and decreases sTNFr2 without altering serum TNF-α in reportedly healthy, male adults. In contrast, however, soluble TNF receptor deviations are absent following a large bolus of supplemental vitamin D that induces an immediate but transient increase in serum 25(OH)D and 1,25(OH)D. These data suggest that soluble TNF receptors are prone to modulation by vitamin D status but less apt to fluctuations following a large bolus of vitamin D that mediates an abrupt and transient increase in serum 25(OH)D and 1,25(OH)D.

A novel finding of the present investigation was the sTNFr1 increase in reportedly healthy male adults with vitamin D sufficiency. This finding conflicts with previous reports demonstrating an increase in sTNFr1 with various diseases or low vitamin D [4,34,43]. Low vitamin D associates with an increase in inflammation [14], and inflammatory stimuli triggers the proteolysis of the TNF-α receptors by metalloproteinases that subsequently increase the soluble form in the circulation [2,44-46]. Also, *in vitro* and *in vivo* results indicate that inflammatory challenge compromises 25(OH)D levels, which consequentially, increases 1,25(OH)D [47-52]. In vascular smooth muscle cells, 1,25(OH)D exposure mediates the ectodomain shedding of TNFr1 and increases the amount of sTNFr1 found in cell culture medium [32]. Thus, inflammation or an increase in 1,25(OH)D (inflammatory- or non-inflammatory-driven) could contribute to the increase in sTNFr1. However, in the present investigation, neither serum 1,25(OH)D nor TNF-α were significantly different despite contrasting sTNFr1 concentrations between vitamin D status groups.
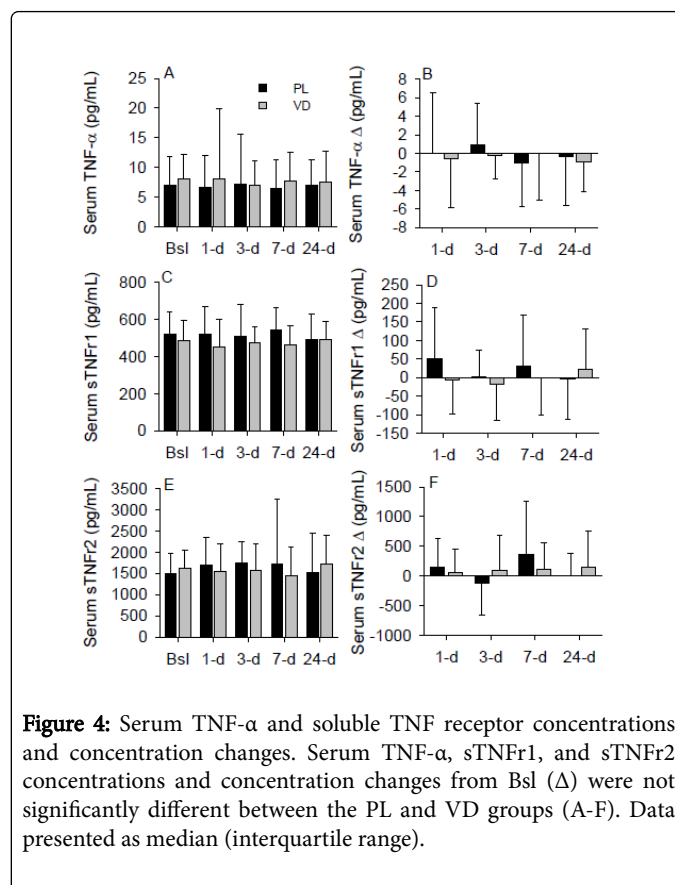
Another unique finding of this study and consistent with elderly data [34], is the decrease in sTNFr2 with vitamin D sufficiency. Proteolysis of the membrane bound receptor decreases the number of receptors on the cell surface and the sensitivity to TNF-α signal transduction [1,53]. Therefore, it is plausible that a decrease in the soluble form could relate to an increase in the membrane-bound TNFr2 and the capacity to augment TNF-α mediated events through the cell-associated receptor. Although it is unknown if the decrease in sTNFr2 associates with an increase in the cell-associated TNFr2 or alters TNF-α mediated events, it is noteworthy that a decrease in sTNFr2 improves survival following hospital admission in children with severe meningococcaemia [54] and reduces the risk of hip fracture in women [55]. Nevertheless, additional studies performed at the cellular and molecular levels are needed to reveal the mechanism(s) underlying the increase in sTNFr1 and decrease in sTNFr2 with vitamin D sufficiency.

Conflicting with the unique findings regarding the divergence in sTNF receptor concentrations with vitamin D status is the inability of supplemental vitamin D to mediate sTNFr1 and sTNFr2 fluctuations concurrently with serum 25(OH)D. One plausible explanation could reside with initial serum 25(OH)D concentrations. The average serum 25(OH)D concentration (28.2 (9.8) ng/mL) prior to supplementation was borderline sufficient. Further supporting the presence of gross vitamin D sufficiency, plasma calcium and parathyroid hormone concentrations were not significantly different between the vitamin D Insufficient and Sufficient groups prior to supplementation. The lack of subjects with low vitamin D could have important ramifications on the ability of supplemental vitamin D to alter soluble cytokine receptors as indirect evidence from our lab suggests that initially low serum

25(OH)D potentiates the ability of supplemental vitamin D to modify circulating cytokine (i.e., interferon-γ and interleukin-10) concentrations [56]. Additionally, this investigation consists of a 24-d protocol in reportedly healthy, male adults following a bolus of supplemental vitamin D. Based on the cytokine receptor differences between vitamin D status groups before supplementation, it is foreseeable that a daily or intermittent (i.e., weekly or monthly) long-term intervention of supplemental vitamin D intended to induce and maintain a sufficient serum 25(OH)D concentration in subjects with initial vitamin D deficiency (i.e., <20 ng/mL and corresponding alterations in parathyroid hormone and calcium levels) could modify soluble TNF receptor concentrations.

Consistent with previous data [40,41,57], there was a significant increase in serum 25(OH)D (from approximately 28.0 to 38.4 (peak at 7-d) ng/mL) following a large bolus of cholecalciferol that inversely correlated with Bsl concentrations. Extending on previous findings, this study provides new results suggesting subjects with high serum 25(OH)D at Bsl possess the greatest change in VDBP concentrations following a 100,000 IU bolus of cholecalciferol. However, despite the trend for increasing concentrations (Figure 2C), there was not a significant increase in serum VDBP following supplemental vitamin D, thereby leaving doubt in the causal relationship of 25(OH)D to mediate an increase in the VDBP. In agreement with this inference, results elsewhere suggest supplemental vitamin D increases serum 25(OH)D without perturbing VDBP concentrations in adults and elderly [42,58]. Those original findings are not universal, however, as data from hip fracture patients suggest an increase in serum VDBP following supplemental vitamin D [59]. Considering the robust inflammatory cascade and limb disuse conditions following the acute trauma of a hip fracture episode, it is plausible that the capacity of 25(OH)D to modulate serum VDBP is condition specific. This assumption, however, requires additional research for later resolve.

In addition to those mentioned above, there are other study limitations worthy of discussion. This study was delimited to reportedly healthy, male adults with unintentionally good vitamin D and corresponding circulating calcium levels. The ability of vitamin D to regulate soluble TNF receptors is calcium dependent [32], and therefore, alterations in circulating calcium with a fluctuating serum 25(OH)D concentration could be a necessity for vitamin D to modulate sTNFr1 and sTNFr2. Also, a single serum 25(OH)D concentration measure was used to identify vitamin D status before supplementation. Serum 25(OH)D concentration measures performed on separate occasions would be ideal to confirm a stable vitamin D status prior to supplementation. Next, it is unclear if the diverging soluble TNF receptor concentrations with vitamin D status moderate biological events mediated by acute or chronic disturbances in TNF-α. These limitations should be taken into consideration when designing future studies pertaining to vitamin D and soluble TNF receptors.

In summary, this study provides the first evidence that vitamin D sufficiency associates with an increase in sTNFr1 and a decrease in sTNFr2 without altering serum TNF-α in reportedly healthy, male adults. However, serum 25(OH)D and 1,25(OH)D concentrations increase while TNF-α and its soluble receptors were not significantly different following a large bolus of supplemental vitamin D. Based on these findings, we conclude that vitamin D sufficiency differentially regulates sTNFr1 and sTNFr2, while neither soluble receptor fluctuate with acute perturbations in serum 25(OH)D following supplemental vitamin D. Additional studies are clearly justified and desired to identify if soluble TNF receptor deviations moderated by vitamin D status or supplementation regulate the pleiotropic properties of TNF-α necessary for optimum host defenses in diverse physiological and pathophysiological conditions.

Acknowledgements

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