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Research paper

# Vitamin D deficiency associates with $\gamma$ -tocopherol and quadriceps weakness but not inflammatory cytokines in subjects with knee osteoarthritis



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## ABSTRACT

Knee osteoarthritis (OA) is a degenerative joint condition and a leading cause of physical disability in the United States. Quadriceps weakness and inflammatory cytokines contribute to the pathogenesis of knee OA, and both of which, increase with vitamin D deficiency. Other micronutrients, such as vitamins C and E and  $\beta$ -carotene, modulate inflammatory cytokines and decrease during inflammation. The purpose of this study was to test the hypothesis that vitamin D deficiency associates with quadriceps weakness, an increase in serum cytokines, and a decrease in circulating micronutrients in subjects with knee OA. Subjects (age,  $48 \pm 1$  y; serum 25(OH)D,  $25.8 \pm 1.1$  ng/mL) with knee OA were categorized as vitamin D deficient ( $n=17$ ; serum 25(OH)D  $\leq 20$  ng/mL), insufficient ( $n=21$ ; serum 25(OH)D 20–29 ng/mL), or sufficient ( $n=18$ ; serum 25(OH)D  $\geq 30$  ng/mL). Single-leg strength (concentric knee extension–flexion contraction cycles at 60 °/s) and blood cytokine, carotene ( $\alpha$  and  $\beta$ ), ascorbic acid, and tocopherol ( $\alpha$  and  $\gamma$ ) concentrations were measured. Quadriceps peak torque, average power, total work, and deceleration were significantly (all  $p < 0.05$ ) impaired with vitamin D deficiency. Serum  $\gamma$ -tocopherol concentrations were significantly ( $p < 0.05$ ) increased with vitamin D deficiency. In the vitamin D sufficient group,  $\gamma$ -tocopherol inversely correlated ( $r = -0.47$ ,  $p < 0.05$ ) with TNF- $\alpha$ , suggesting a pro-inflammatory increase with a  $\gamma$ -tocopherol decrease despite a sufficient serum 25(OH)D concentration. We conclude that vitamin D deficiency is detrimental to quadriceps function, and in subjects with vitamin D sufficiency,  $\gamma$ -tocopherol could have an important anti-inflammatory role in a pathophysiological condition mediated by inflammation.

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## Introduction

Knee osteoarthritis (OA) is a degenerative joint condition and a leading cause of physical disability in the United States, especially with advancing age. With the aging population growing, there is an increasing demand to improve the quality life and daily function in people suffering with knee OA. A compelling body of literature is revealing an increase in cartilage loss and knee OA with vitamin D deficiency (i.e., serum 25-hydroxyvitamin D (25(OH)D)  $< 20$  ng/mL) [1,2]. This is an area of uncertainty, however, as conflicting reports suggest that vitamin D deficiency does not associate with knee OA [3–7]. Despite this ambiguity, vitamin D is an attractive therapeutic alternative because it modulates the

mechanical (i.e., quadriceps weakness) [8] and biochemical (i.e., inflammatory cytokines) [9] factors believed to contribute to the pathogenesis of knee OA.

Quadricep weakness is an early symptom of OA that precedes and mediates cartilage loss by minimizing the shock absorption across the knee joint [10,11]. In vitamin D deficient elderly, physical function is impaired and quadriceps weakness is apparent [12–17]. The culprit(s) contributing to these impairments are unknown in humans, but evidence suggests that decrements in phosphate metabolism, calcium handling and transport, or cytoskeletal protein expression could be the underlying mechanisms mediating muscular weakness with low vitamin D [18,19]. The influence of vitamin D on skeletal muscle function is illustrated further by reports demonstrating that supplemental vitamin D (i.e., with 25(OH)D or ergocalciferol) attenuates the age-related decrease or improves lower extremity function and muscle strength in elderly [20,21]. In contrast, the impact of vitamin D

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on physical function or muscle strength in elderly is questioned as conflicting reports exist [22,23]. Consistent with data suggesting that vitamin D is not influential on physical function in elderly, results in subjects with knee OA imply that an increase in serum 25(OH)D does not attenuate the decline in physical function [5,24]. However, it is unknown if vitamin D deficiency associates with quadriceps weakness in subjects with knee OA.

Although caution is recommended in its interpretation as an exclusive indicator of knee OA [25], an increase in serum C-reactive protein (CRP) is apparent in subjects with knee OA [26]. Additionally, serum CRP and inflammatory cytokines (e.g., interleukin (IL)-6) predict cartilage loss [27,28] and knee OA [29]. These systemic findings reflect the local production of pro-inflammatory cytokines, such as tumor necrosis factor (TNF)- $\alpha$  and IL-1 $\beta$ , that induce cartilage catabolism and knee OA [30,31]. In isolated immune cells, vitamin D (1,25-dihydroxyvitamin D) or increase in serum 25(OH)D decreases pro-inflammatory cytokine production [32,33]. The cytokine-modulating property of vitamin D is related to its interaction with the cell surface or nuclear vitamin D receptors (VDR). The nuclear VDR heterodimerizes with the retinoic X receptor (RXR) upon vitamin D (1,25-dihydroxyvitamin D) binding, forming a complex that attaches to a vitamin D response element that regulates cytokine gene expression *see* [34] *for review*. In addition to the VDR–RXR complex, vitamin D regulates cytokine expression by modulating the expression and activity of nuclear factor- $\kappa$ B (NF- $\kappa$ B) and nuclear factor of activated T cells *see* [34] *for review*. Upon binding to the cell surface receptor, vitamin D induces rapid and non-genomic cytokine responses through mitogen-activated protein kinase, protein kinase C, cGMP, and phosphoinositide metabolism signaling pathways, [35]. Consistent with its cytokine-modulating influence in isolated cell studies, low serum 25(OH)D concentrations associate with an increase in pro-inflammatory cytokines across a range of physiological and pathophysiological conditions in humans [36–39]. There is, however, paucity in the data regarding the influence of vitamin D deficiency on inflammatory cytokines in subjects with knee OA.

It is also unknown if vitamin D deficiency is accompanied by a decrease in other micronutrients that modulate inflammatory cytokines. Vitamins C and E ( $\alpha$ - or  $\gamma$ -tocopherol alone or in combination) are potent dietary antioxidants that decrease cytokine (such as IL-6 and TNF- $\alpha$ ) levels in the blood [40] and inhibit cytokine production from isolated immune cells [41–45]. Conversely,  $\beta$ -carotene increases circulating TNF- $\alpha$  concentrations [46] and production from monocytes [47,48]. In addition to their cytokine modulating property, an emerging hypothesis is that inflammation confounds the interpretation of circulating micronutrient levels [49,50]. Support of this hypothesis is provided by data suggesting vitamin E and  $\beta$ -carotene decrease in the circulation after a hip fracture and during inflammation [51]. Reports from our lab [52–54] and others [55,56] also suggest a circulating decrease in vitamins C (ascorbic acid) and D (25(OH)D) during the acute inflammatory phase following minor and major orthopedic surgery in humans.

Analyzing quadricep strength and inflammatory cytokines in subjects with knee OA and with contrasting serum 25(OH)D concentrations could identify the role of vitamin D status on mechanical and biochemical mediators of this degenerative joint condition. Inflammation contributes to this degenerative joint, and if inflammation decreases micronutrients in the blood, it is foreseeable that vitamins C and E and carotenoids decrease with vitamin D deficiency. Thus, the purpose of this study was to identify quadricep strength, inflammatory cytokines, and micronutrients in subjects with knee OA and with contrasting serum 25(OH)D concentrations. We hypothesized that vitamin D deficiency associates with quadriceps weakness, an increase in serum cytokine, and a decrease in circulating micronutrient in subjects with knee OA.

## Material and methods

To address the proposed hypothesis, potential subjects who were modestly active (i.e., minimum level of 30 min of continuous activity at least 3 times per week) were recruited from the practice of orthopedic surgeons (RHT, GLR, and NGM) at The Orthopedic Specialty Hospital (Murray, UT USA). Subjects were initially screened for unilateral knee symptoms of knee OA, such as self-reported knee pain and muscular (i.e., quadriceps or hamstring) weakness. During screening, subjects were excluded from participation if: they were taking a daily dietary supplement during the previous year, currently using anti-inflammatory medications or other prescribed or recommended medications, suffered a lower leg injury during the previous year that required the use of crutches, tobacco use, or under physician guided treatment for known disease. We limited our study population to 60 years of age or younger as the association between serum 25(OH)D concentrations and knee OA is apparent in this demographic [2]. Subjects were informed of and provided written and verbal consent to the experimental protocol and procedures. The Urban Central Region Institutional Review Board at Intermountain Healthcare (Salt Lake City, UT USA) approved this study. Data was collected between December 2011 and October 2012 in Murray, UT (latitude 40°N).

## Study protocol

Following screening and enrollment, each subject reported to the Physiology Research Laboratory at The Orthopedic Specialty Hospital. At this visit, we obtained fasting blood samples and administered the pain (0–20; 0, no pain; 20, extreme pain) and physical function (0–68; 0, no physical impairment; 68, extreme physical impairment) subsections of the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) questionnaire [57]. We also completed single-leg strength testing and X-ray imaging. Subjects were excluded from analysis if: (1) the WOMAC pain score was < 2 on any of the five questions in its subsection, (2) there was no muscular weakness (i.e., deficit in peak isokinetic knee-extension or flexion strength) in the symptomatic leg, or (3) a Kellgren-Lawrence grade < 2 was scored in the symptomatic knee (Fig. 1). Subjects were instructed to refrain from using non-steroidal anti-inflammatory drugs (NSAIDs), including aspirin, ibuprofen, and naproxen sodium, at least 3 days prior to data collection.

### Single-leg strength testing

Peak isokinetic strength was performed unilaterally on a Biodex S4 (Shirley, NY USA). Testing was performed on the leg with reportedly no symptoms of OA first. The rationale for performing the non-symptomatic knee first was to allow subjects to experience the testing protocol on a healthy knee prior to testing the symptomatic knee. Several practice repetitions were performed on each leg prior to testing for warm-up and to become familiar with the testing protocol and procedure. Once comfortable with the testing protocol and procedure, each subject performed 6 concentric knee extension–flexion contractions at 60° per second through a full range of motion (90° of knee flexion to full extension). Subjects were instructed and verbally encouraged to perform each contraction with maximal effort during the isokinetic testing.

### Radiography

X-ray images were taken of each knee in the anterior-posterior view at 45° of knee flexion. Radiographs of the knee joint space

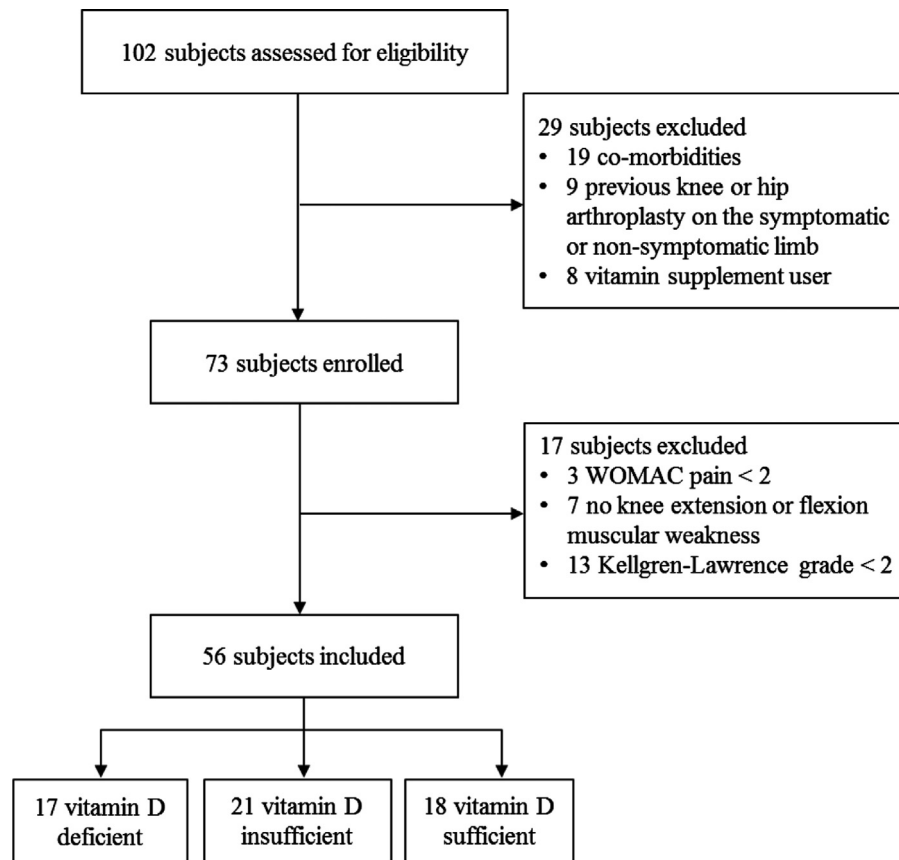


Fig. 1. Eligibility screening, study enrollment, and subject inclusion flow chart.

narrowing (mm) between the symptomatic and non-symptomatic knee were viewed and analyzed using ImageJ software from the National Institutes of Health. Severity of knee OA was classified according to the scoring criteria established by Kellgren and Lawrence [58]. The Kellgren–Lawrence [58] grading was as the following: 0, no osteophytes or joint-space narrowing; 1, questionable osteophyte indicating possible OA; 2, definite osteophyte, no joint-narrowing indicating mild OA; 3,  $\leq 50\%$  joint-space narrowing indicating moderate OA; and 4,  $> 50\%$  joint-space narrowing indicating severe OA.

### Analytical blood chemistry procedures

#### Circulating vitamins

Serum 25(OH)D concentrations (ng/mL) were determined using a chemiluminescent immunoassay (ARUP Laboratories, Salt Lake City, UT USA). Patients were classified as vitamin D deficient, insufficient, or sufficient if they had a serum 25(OH)D concentration  $\leq 20$ , between 21 and 29, or  $\geq 30$  ng/mL, respectively. Serum 25(OH)D concentrations were not known during data collection.

The heparinized plasma samples mixed with oxalic acid were analyzed for ascorbic acid concentrations (mg/dL) using spectrophotometry (ARUP Laboratories). Serum tocopherol ( $\alpha$  and  $\gamma$ ) concentrations (mg/L) were analyzed with high performance liquid chromatography (HPLC; ARUP Laboratories). Serum  $\alpha$ - and  $\beta$ -carotenes, lutein, and zeaxanthin concentrations ( $\mu\text{g/L}$ ) were quantitated using HPLC (ARUP Laboratories).

#### Serum cytokine and CRP concentrations

The multiplex technology of Luminex (MAGPix; Austin, TX USA) was used to analyze inflammatory cytokine concentrations (pg/mL) with high-sensitivity. Serum high-sensitivity CRP concentrations (mg/L) were quantitated using immunoturbidimetry assay (ARUP Laboratories).

#### Clinical chemistries

Serum rheumatoid factor concentrations (IU/mL) were quantitated using an immunoturbidimetry assay and uric acid concentrations (mg/dL) were determined using a quantitative spectrophotometry assay (ARUP Laboratories). Serum parathyroid hormone intact (iPTH; pg/mL) with calcium (mg/dL) concentrations were measured using an electrochemiluminescent immunoassay (ARUP Laboratories).

### Power calculation and statistical analysis

No previous study has investigated the role of vitamin D status on quadriceps strength in subjects with knee OA. Based on pilot data from our lab, the power analysis indicated that in order to detect a 6% difference in peak torque between vitamin D status groups with a statistical power of 80% at the two-sided  $\alpha=0.05$  level, at least 17 subjects were needed per group. Considering the stringent inclusion criteria of radiographic evidence, pain, and muscular weakness, and since the aforementioned symptoms can occur independently from each other [59–65], we estimated screening 102 subjects to achieve the targeted accrual in each group ( $n=17/\text{group}$ ).

Data were checked for normality with a Shapiro–Wilk test prior to statistical analysis. In order to achieve normality, rank (GM-CSF, IFN- $\gamma$ , IL-5, IL-6, IL-7, IL-8, TNF- $\alpha$ , CRP,  $\alpha$ -carotene,  $\beta$ -carotene, lutein, and zeaxanthin) or log (IL-12, IL-13, IL-1 $\beta$ , IL-2, and IL-4) transformations were performed prior to statistical analyses. Statistical significance of subject characteristics (age, height, body mass, BMI, serum 25(OH)D, rheumatoid factor, iPTH, and calcium) were assessed with a one-analysis of variance (ANOVA) followed by a Tukey's post-hoc test when appropriate. Statistical significance of the cytokine, micronutrient, and WOMAC data between vitamin D status groups were assessed with a one-way (vitamin D status) analysis of covariance (ANCOVA) followed by a Bonferroni post-hoc on multiple pairwise comparisons when appropriate. Statistical significance of leg strength data was assessed with a two-way (vitamin D status and leg) ANCOVA followed by a Bonferroni post-hoc test on multiple pairwise comparisons when appropriate. Due to the sparse fitting (i.e., frequency < 5) in some of the cells, the association between vitamin D status and the Kellgren–Lawrence grade were assessed with a Goodman–Kruskal's gamma test. Relationships between variables were examined with a Pearson Product Moment Linear correlation. Gender, age, Kellgren–Lawrence grade, and body mass index (BMI) served as a statistical covariates. Body mass was not included as a covariate due to multicollinearity with BMI. Significance was set at  $p < 0.05$ . All statistical analyses were performed with SYSTAT (version 13.1, Chicago, IL USA). Data presented as mean  $\pm$  SEM unless otherwise noted.

## Results

### Subject characteristics

Following eligibility screening, 73 subjects were enrolled and assessed for study inclusion (Fig. 1). Of the 73 enrolled, 56 subjects (age,  $48 \pm 1$  y; height,  $168 \pm 1$  cm; body mass,  $94.1 \pm 2.8$  kg; BMI,  $33.0 \pm 0.9$  kg/m<sup>2</sup>; serum 25(OH)D,  $25.8 \pm 1.1$  ng/mL) met the subject inclusion criteria.

Age, height, plasma rheumatoid factor, plasma calcium, and WOMAC pain and physical function scores were not significantly different between vitamin D groups (Table 1). However, serum 25(OH)D concentrations inversely correlated with pain ( $n=56$ ,  $r=-0.27$ ,  $p<0.05$ ) and physical function ( $n=56$ ,  $r=-0.28$ ,  $p<0.05$ ) (Fig. 2A and B), suggesting decreases in self-reported knee pain and physical function with an increase in serum 25(OH)D concentrations. Body mass, BMI, plasma iPTH (Table 1), and plasma uric acid (Fig. 3A) were significantly (all  $p < 0.05$ ) increased in the vitamin D deficient group. Approximately 9% of the subjects displayed a Kellgren–Lawrence grade of 2, while 68% and 23% of the subjects had a grade 3 or 4 score, respectively (Table 1). Importantly, the Kellgren–Lawrence scores were not significantly different between groups, suggesting that the severity of knee osteoarthritis was similar between groups.

### Circulating chemistries

Despite trends, circulating  $\alpha$ -carotene,  $\beta$ -carotene, lutein, zeaxanthin, ascorbic acid, and  $\alpha$ -tocopherol were not significantly different between groups after adjusting for BMI and other covariates (Table 2). In contrast, serum  $\gamma$ -tocopherol concentrations were significantly ( $p < 0.05$ ) increased ( $\sim 94\%$  vs. vitamin D sufficient and  $\sim 13\%$  vs. vitamin D insufficient) in the vitamin D deficient group (Fig. 3B). In the vitamin D sufficient group, serum  $\gamma$ -tocopherol inversely correlated ( $r = -0.47$ ,  $p < 0.05$ ) with serum TNF- $\alpha$  (Table 3), suggesting a pro-inflammatory increase with a

**Table 1**  
Subject characteristics and clinical chemistries.

	Vitamin D status			p-value
	Deficient	Insufficient	Sufficient	
n (males:females)	17 (9:8)	21 (9:12)	18 (7:11)	
Age (y)	51 $\pm$ 3	48 $\pm$ 2	47 $\pm$ 3	0.536
Height (cm)	167 $\pm$ 2	170 $\pm$ 2	168 $\pm$ 2	0.487
Body mass (kg)	100.6 $\pm$ 5.4	99.7 $\pm$ 4.6	81.4 $\pm$ 3.3 <sup>a,b</sup>	0.006
BMI (kg/m <sup>2</sup> )	36.0 $\pm$ 1.8	34.2 $\pm$ 1.3	28.6 $\pm$ 0.8 <sup>a,b</sup>	0.001
Serum 25(OH)D (ng/ml)	16.3 $\pm$ 0.7	24.7 $\pm$ 0.6 <sup>a</sup>	36.0 $\pm$ 0.8 <sup>a,b</sup>	< 0.001
Rheumatoid factor (IU/ml)	9.18 $\pm$ 0.46	8.95 $\pm$ 0.44	8.83 $\pm$ 0.45	0.599
iPTH (pg/mL)	50.1 $\pm$ 3.8	44.1 $\pm$ 3.6	33.6 $\pm$ 2.2 <sup>a,c</sup>	0.004
Calcium (mg/dL)	9.35 $\pm$ 0.13	9.29 $\pm$ 0.1	9.49 $\pm$ 0.10	0.632
WOMAC pain (0–20)	9.47 $\pm$ 1.03	7.60 $\pm$ 0.63	6.69 $\pm$ 0.82	0.320
WOMAC function (0–68)	30.1 $\pm$ 3.4	24.7 $\pm$ 2.4	19.7 $\pm$ 2.5	0.461
Kellgren–Lawrence grade				0.468
2 (n)	1	4	0	
3 (n)	14	9	15	
4 (n)	2	8	3	

Data presented as mean  $\pm$  SEM unless otherwise noted.

All subjects were Caucasian.

<sup>a</sup>  $p < 0.05$  vs. deficient.

<sup>b</sup>  $p < 0.05$  vs. insufficient.

<sup>c</sup>  $p = 0.07$  vs. insufficient.

$\gamma$ -tocopherol decrease despite a sufficient serum 25(OH)D concentration. Circulating cytokine and CRP concentrations were not significantly different between groups (Table 2).

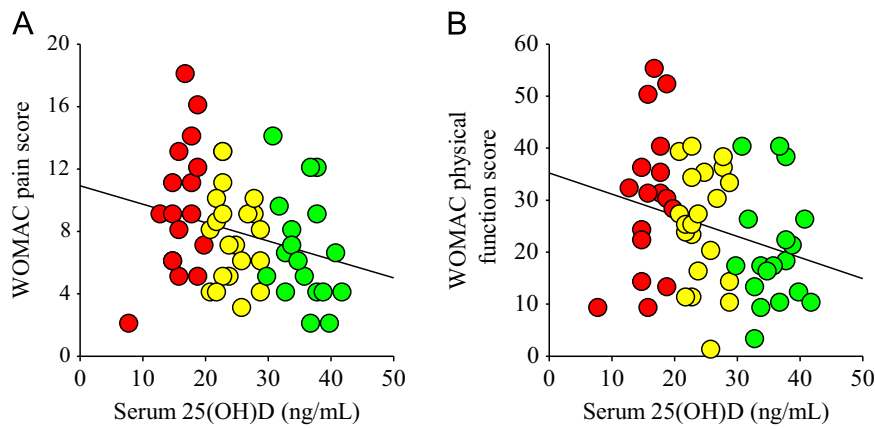
### Isokinetic leg strength data

Concentric-knee extension peak torque, average power, and total work were significantly (all  $p < 0.05$ ) decreased and concentric-knee extension deceleration was significantly ( $p < 0.05$ ) slower in the vitamin D deficient group (Figs. 4A–D). Concentric-knee flexion deceleration was significantly slower in the symptomatic compared to the non-symptomatic leg, but differences between vitamin D groups were not significant (Table 4). Concentric-knee flexion torque, power, work, and acceleration were not significantly different between vitamin D groups.

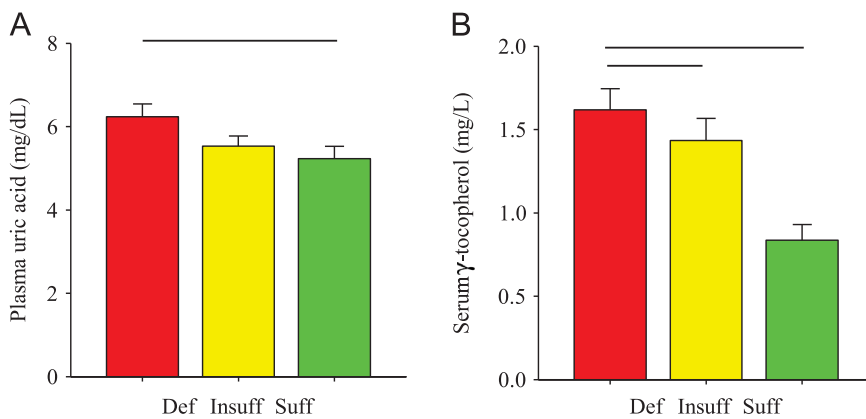
## Discussion

Muscular weakness is a major impairment that hinders the quality of life and activities of daily living in those with and without knee OA. The major finding of the present investigation was that vitamin D deficiency associated with quadriceps weakness, and that an increase in serum 25(OH)D correlated with a decrease in perceived physical dysfunction in subjects with knee OA. The present report also provides original data suggesting that vitamin D deficiency is not associated with an increase in inflammatory cytokines or a decrease in micronutrients that modulate inflammatory cytokines. However, the  $\gamma$ -tocopherol decrease with vitamin D sufficiency correlated with a TNF- $\alpha$  increase in subjects with knee OA, suggesting an anti-inflammatory role of vitamin E in a pathophysiological condition mediated by inflammation and in subjects with sufficient vitamin D.

Quadriceps weakness is a common and early symptom of knee OA that predicts and occurs before radiographic evidence of knee OA and knee pain [10,11]. Although previous studies illustrate the association between low serum 25(OH)D concentrations and muscular weakness across a range of physiological and pathophysiological conditions in humans, this study provides unique data indicating that vitamin D deficiency associates with quadriceps weakness in subjects with knee



**Fig. 2.** Serum 25(OH)D concentrations correlated with pain and physical function. Serum 25(OH)D concentrations (ng/mL) inversely correlated with pain ( $n=56$ ,  $r=-0.27$ ,  $p<0.05$ ) and physical function ( $n=56$ ,  $r=-0.28$ ,  $p<0.05$ ). Vitamin D deficient, red circles; vitamin D insufficient, yellow circles; vitamin D sufficient, green circles.



**Fig. 3.** Plasma uric acid and serum  $\gamma$ -tocopherol concentrations. (A) Plasma uric acid concentrations (mg/dL) were significantly (bar,  $p<0.05$ ) increased in the vitamin D deficient group compared to the vitamin D sufficient group. (B) Serum  $\gamma$ -tocopherol concentrations (mg/L) were significantly (bars,  $p<0.05$ ) increased in the vitamin D deficient group compared to those in the insufficient and sufficient groups. Def, vitamin D deficient (red); Insuff, vitamin D insufficient (yellow); Suff, vitamin D sufficient (green). Data presented as mean  $\pm$  SEM.

**Table 2**  
Circulating vitamin and cytokine concentrations.

	Vitamin D status			<i>p</i> -value
	Deficient	Insufficient	Sufficient	
$\alpha$ -Carotene ( $\mu$ g/L)	47.5 $\pm$ 7.1	55.8 $\pm$ 7.2	87.3 $\pm$ 17.1	0.646
$\beta$ -Carotene ( $\mu$ g/L)	99.9 $\pm$ 10.8	169.1 $\pm$ 28.6	271.0 $\pm$ 49.6	0.122
Lutein ( $\mu$ g/L)	102 $\pm$ 15	119 $\pm$ 14	138 $\pm$ 16	0.832
Zeaxanthin ( $\mu$ g/L)	25.9 $\pm$ 3.6	29.5 $\pm$ 3.0	29.5 $\pm$ 2.8	0.535
Ascorbic acid (mg/dl)	0.89 $\pm$ 0.12	1.20 $\pm$ 0.11	1.13 $\pm$ 0.09	0.176
$\alpha$ -Tocopherol (mg/L)	11.3 $\pm$ 0.8	11.2 $\pm$ 0.6	11.4 $\pm$ 0.7	0.941
GM-CSF (pg/mL)	1.32 $\pm$ 0.34	4.30 $\pm$ 1.75	2.74 $\pm$ 1.00	0.864
IFN- $\gamma$ (pg/mL)	10.4 $\pm$ 2.53	18.9 $\pm$ 4.27	16.3 $\pm$ 6.59	0.216
TNF- $\alpha$ (pg/mL)	5.76 $\pm$ 0.71	5.43 $\pm$ 0.53	5.28 $\pm$ 1.07	0.660
IL-1 $\beta$ (pg/mL)	0.66 $\pm$ 0.22	0.95 $\pm$ 0.30	1.16 $\pm$ 0.45	0.526
IL-2 (pg/mL)	1.31 $\pm$ 0.44	2.20 $\pm$ 0.64	2.54 $\pm$ 0.86	0.423
IL-4 (pg/mL)	16.4 $\pm$ 5.63	30.9 $\pm$ 11.5	25.5 $\pm$ 11.7	0.920
IL-5 (pg/mL)	0.14 $\pm$ 0.05	0.79 $\pm$ 0.27	0.38 $\pm$ 0.16	0.269
IL-6 (pg/mL)	1.52 $\pm$ 0.15	2.83 $\pm$ 0.58	1.50 $\pm$ 0.25	0.185
IL-7 (pg/mL)	6.80 $\pm$ 1.04	7.44 $\pm$ 1.11	8.70 $\pm$ 1.54	0.694
IL-8 (pg/mL)	6.70 $\pm$ 0.53	8.37 $\pm$ 1.15	5.75 $\pm$ 0.57	0.536
IL-10 (pg/mL)	19.8 $\pm$ 3.10	42.1 $\pm$ 16.1	25.9 $\pm$ 4.25	0.249
IL-12 (pg/mL)	1.64 $\pm$ 0.69	7.35 $\pm$ 4.40	5.22 $\pm$ 3.20	0.248
IL-13 (pg/mL)	10.9 $\pm$ 2.95	14.9 $\pm$ 4.13	10.6 $\pm$ 3.74	0.635
CRP (mg/L)	3.38 $\pm$ 0.72	4.61 $\pm$ 1.31	2.32 $\pm$ 0.57	0.365

Data presented as mean  $\pm$  SEM.

OA. Additionally, vitamin D deficiency associated with slower concentric-knee extension deceleration times, which implies a decrement in the eccentric control of the quadriceps with low serum 25

(OH)D concentrations. As proposed elsewhere [8,66], weakness and impaired eccentric control of the quadriceps is detrimental to the structural integrity of the knee joint by minimizing shock absorption. Therefore, altering serum 25(OH)D concentrations could be an attractive therapeutic approach to attenuate quadriceps weakness, which importantly, contributes to knee OA.

Perceived physical function is impaired in elderly with vitamin D deficiency. In knee OA subjects, studies investigating the association between vitamin D and physical function are scarce. Using Lequesne's indices to assess physical function in subjects with knee OA, Al-Jarallah et al. [5] reported that serum 25(OH)D concentrations did not associate with physical function. In an intervention study, supplemental vitamin D (2000 IU/d for 2 y) increased serum 25(OH)D concentrations without improving physical function, such as standing from a chair or a 20-m walk, in subjects with knee OA [24]. Consistent with those results, perceived physical function was not significantly different between patients with contrasting serum 25(OH)D concentrations in the present investigation. However, serum 25(OH)D concentrations inversely correlated with physical dysfunction, suggesting an improvement in physical function with an increase in serum 25(OH)D concentrations. The discrepancy between the group comparisons and correlative findings regarding the association between vitamin D and physical function possibly suggest a narrow range in serum 25(OH)D concentrations or that the demarcation between vitamin D status levels were inappropriately defined to delineate a significant difference in self-reported

physical function. In agreement with the latter postulate, Houston et al. [12] reported that the serum 25(OH)D concentration for improved physical function (28–32 ng/mL) was greater than that for muscular strength (22–28 ng/mL) in elderly.

**Table 3**  
Serum  $\gamma$ -T and cytokine correlation coefficients.

	Vitamin D status			Group $\gamma$ -T
	Deficient $\gamma$ -T	Insufficient $\gamma$ -T	Sufficient $\gamma$ -T	
GM-CSF	0.04	0.13	-0.21	0.01
IFN- $\gamma$	-0.12	0.02	-0.31	-0.04
TNF- $\alpha$	-0.15	0.00	-0.47 <sup>†</sup>	-0.03
IL-1 $\beta$	0.03	0.19	-0.22	0.00
IL-2	-0.07	0.15	-0.15	-0.05
IL-4	-0.18	0.23	-0.21	0.02
IL-5	0.22	-0.14	-0.28	-0.09
IL-6	0.35	-0.06	-0.20	0.08
IL-7	-0.18	0.15	-0.06	-0.04
IL-8	0.10	0.16	-0.25	0.15
IL-10	0.35	0.06	-0.10	0.04
IL-12	0.15	0.23	-0.29	0.06
IL-13	-0.29	0.28	-0.05	0.08

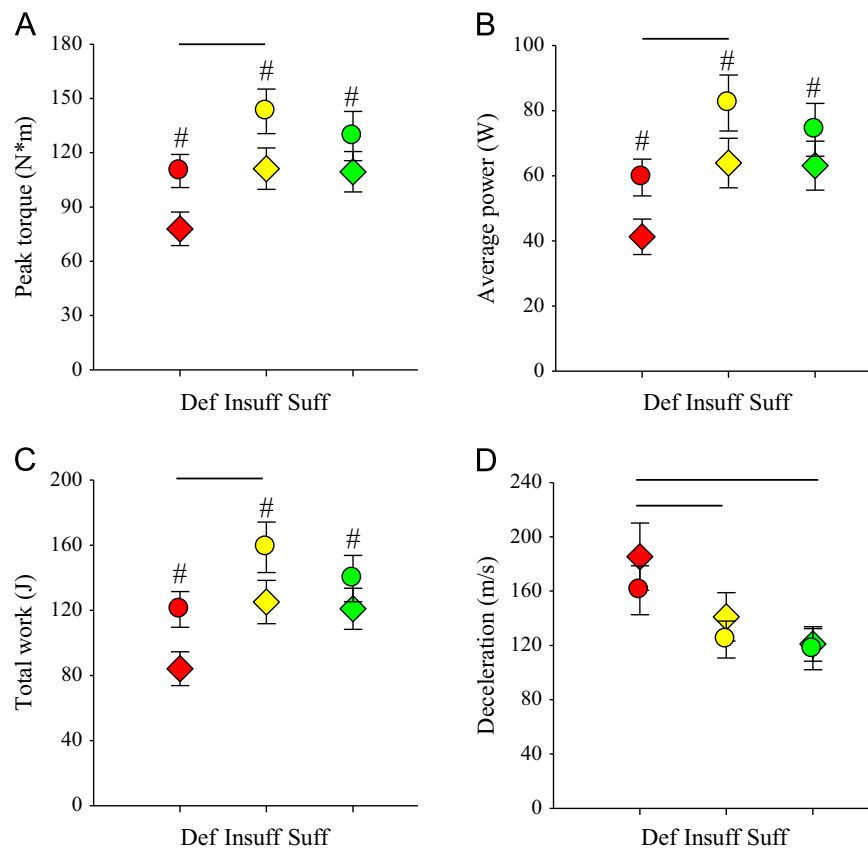
$\gamma$ -T,  $\gamma$ -tocopherol.

Group, all subjects combined.

<sup>†</sup>  $p < 0.05$ .

Despite vitamin D deficiency, circulating cytokine concentrations were similar between vitamin D groups. In a population whose degenerative joint condition is mediated in part by pro-inflammatory cytokines, it appears that low vitamin D does not increase inflammatory cytokines in the blood. This finding, however, conflicts with results showing a circulating increase of pro-inflammatory cytokines in young reportedly healthy adults [36], obese adults [37], chronic heart failure patients [38], and type 2 diabetics [39] with low serum 25(OH)D concentrations. Further, a pro-inflammatory cytokine increase in the blood associates with muscular weakness and impaired physical mobility in elderly [67–70], the development of knee OA [28,71], and muscular weakness in patients with knee OA [9]. Based on those findings, we anticipated vitamin D deficiency to associate with muscular weakness and a cytokine increase in the blood. In contrast, the data here suggest that vitamin D deficiency associates with muscular weakness but not serum cytokines in subjects with knee OA.

Similar to vitamin D, other dietary micronutrients (e.g.,  $\beta$ -carotene, ascorbic acid, and  $\alpha$ - and  $\gamma$ -tocopherol's) modulate inflammatory cytokines. Surprisingly, no study has reported previously the level of other micronutrients concomitantly with serum 25(OH)D when investigating the role of vitamin D status on inflammatory cytokines. Although  $\alpha$ - and  $\beta$ -carotene's, lutein, zeaxanthin, ascorbic acid, and  $\alpha$ -tocopherol were not significantly different between groups, serum  $\gamma$ -tocopherol concentrations were significantly increased with vitamin D deficiency. The explanation for the  $\gamma$ -tocopherol increase with vitamin D deficiency is



**Fig. 4.** Isokinetic-concentric knee extension peak torque, average power, total work, and deceleration in the symptomatic and non-symptomatic legs. (A) Peak torque (Nm) was significantly decreased in the symptomatic leg ( $^{\#}p < 0.05$  vs. non-symptomatic leg) and in the vitamin D deficient group (bar,  $p < 0.05$ ). (B) Average power (W) was significantly decreased in the symptomatic leg ( $^{\#}p < 0.05$  vs. non-symptomatic leg) and in the vitamin D deficient group (bar,  $p < 0.05$ ). (C) Total work (J) was significantly decreased in the symptomatic leg ( $^{\#}p < 0.05$  vs. non-symptomatic leg) and in the vitamin D deficient group (bar,  $p < 0.05$ ). (D) Deceleration (m/s) was significantly slower in the vitamin D deficient group compared to that in the vitamin D insufficient and sufficient groups (bars,  $p < 0.05$ ). Def, vitamin D deficient (red); Insuff, vitamin D insufficient (yellow); Suff, vitamin D sufficient (green); Symptomatic leg (colored diamonds,  $\diamond$ ); Non-symptomatic leg (colored circles,  $\circ$ ). Figure legend provided in 'A'. Data presented as mean  $\pm$  SEM.

**Table 4**  
Symptomatic and non-symptomatic isokinetic leg strength data.

	Vitamin D status			Vitamin D status main effect <i>p</i> -value
	Deficient	Insufficient	Sufficient	
<b>Concentric knee extension (60 °/s)</b>				
Symptomatic acceleration (m/s)	62.4 ± 7.0	56.3 ± 5.7	52.8 ± 4.4	0.071
Non-symptomatic acceleration (m/s)	70.6 ± 9.7	54.3 ± 5.2	43.3 ± 4.6	
Leg main effect <i>p</i> -value	0.835			
<b>Concentric knee flexion (60 °/s)</b>				
Symptomatic peak torque (Nm)	59.4 ± 6.0	78.2 ± 7.9	78.8 ± 9.7	0.127
Non-symptomatic peak torque (Nm)	72.5 ± 4.6	84.7 ± 6.5	83.7 ± 11.7	
Leg main effect <i>p</i> -value	0.224			
Sympomatic average power (W)	35.6 ± 4.2	46.0 ± 5.6	48.6 ± 5.9	0.800
Non-symptomatic average power (W)	87.1 ± 43.8	55.5 ± 4.7	51.7 ± 5.6	
Leg main effect <i>p</i> -value	0.122			
Symptomatic total work (J)	68.6 ± 7.0	102.0 ± 11.5	105.0 ± 16.7	0.097
Non-symptomatic total work (J)	87.3 ± 6.3	105.0 ± 9.0	108.8 ± 19.6	
Leg main effect <i>p</i> -value	0.414			
Symptomatic acceleration (m/s)	85.6 ± 12.2	86.4 ± 14.2	71.1 ± 4.7	0.791
Non-symptomatic acceleration (m/s)	62.4 ± 4.7	58.6 ± 4.3	52.8 ± 3.0	
Leg main effect <i>p</i> -value	0.213			
Symptomatic deceleration (m/s)	175 ± 18	153 ± 14	148 ± 14	0.543
Non-symptomatic deceleration (m/s)	134 ± 18	126 ± 11	119 ± 14	
Leg main effect <i>p</i> -value	0.009			

Data presented as mean ± SEM.

unknown and awaits future resolve, but it is plausible that the serum  $\gamma$ -tocopherol difference between groups is related to disparate dietary habits as  $\gamma$ -tocopherol is the most abundant form of vitamin E in the American diet [72,73].

As mentioned previously, pro-inflammatory cytokines are increased in various pathophysiological conditions with low vitamin D [38,39], and similar to vitamin D,  $\gamma$ -tocopherol is a potent anti-inflammatory micronutrient [74].  $\gamma$ -Tocopherol inhibits cytokine production by decreasing 5-lipoxygenase, cyclooxygenase-2, and NF- $\kappa$ B activation and impairing jun terminal kinase and extracellular signal-regulated kinases 1/2 phosphorylation [44,75–77]. Based on the cytokine modulating properties of both vitamin D and E, it is plausible that contrasting or divergent micronutrient levels (e.g., high vitamin D and low vitamin E) could influence cytokine levels in the blood. Indeed, serum TNF- $\alpha$  inversely correlated with  $\gamma$ -tocopherol in the vitamin D sufficient group with low serum  $\gamma$ -tocopherol (Table 3). This finding indicates that a decrease in  $\gamma$ -tocopherol could increase a pro-inflammatory cytokine despite a sufficient serum 25(OH)D concentration in subjects with knee OA. Given the similar cytokine, carotenoid, ascorbic acid, and  $\alpha$ -tocopherol concentrations between groups, we speculate that the anti-inflammatory property of  $\gamma$ -tocopherol is balancing the serum cytokine concentrations in the vitamin D deficient group compared to those with insufficient and sufficient vitamin D. This speculation, however, warrants future investigation. Results from future studies could advance our knowledge pertaining to the anti-inflammatory role of vitamin E in vitamin D deficient patients suffering from knee OA, a degenerative joint condition mediated in part by pro-inflammatory cytokines, including but not limited to TNF- $\alpha$  [78].

Plasma uric acid increases with the prevalence of knee arthritis [79], and in this study, plasma uric acid concentrations increased in knee OA subjects with vitamin D deficiency. A chronic increase in plasma uric acid is a risk factor for cardiovascular disease, gout, renal failure, and metabolic syndrome. However, an acute increase of uric acid might possess an antioxidant property. Antioxidants prevent the reactive oxygen species-mediated activation of intracellular redox-sensitive signaling pathways that induce cytokine

expression. The antioxidant property of uric acid could explain the similar cytokine concentrations between vitamin D groups. Unfortunately, due to the cross-sectional design of this study, it is unclear if the increase in uric acid with vitamin D deficiency was acute or chronic.

The explanation for an increase in uric acid is lower excretion, increase synthesis, or both. Serum uric acid concentrations also increase with serum PTH concentrations [80,81], and vitamin D deficiency or insufficiency increases PTH concentrations [82]. In the vitamin D deficient group here, the increase in plasma uric acid occurred in parallel with an increase in plasma iPTH. It is plausible, therefore, that the increase in iPTH concentrations with vitamin D deficiency is stimulating an increase in uric acid. However, it is reasonable that other factors are contributing to the uric acid increase with vitamin D deficiency as well.

A limitation of this study is the lack of dietary data that could reveal the impact of micronutrient intake on blood 25(OH)D and other micronutrient concentrations. Along these lines, there were apparent decreases in  $\alpha$ - and  $\beta$ -carotene's, lutein, and ascorbic acid with vitamin D deficiency. These decreases, however, were not significant after adjusting for BMI and other covariates. In addition, it is plausible that the contrasting serum 25(OH)D concentrations could be the product of differences in lifestyle habits. Future studies are encouraged to include dietary, lifestyle, and other circulating micronutrient analyses when investigating the role of vitamin D on inflammatory cytokines and skeletal muscle strength in conditions associated with systemic inflammation. Another limitation of this study includes the selected age range in subjects with modest activity levels. Thus, caution is recommended when extrapolating these findings to other populations. This study also consisted of a cross-sectional design that precludes potential inference on causality. Finally, this was a small sample size study, but in addition to its original findings, these data are valuable in generating new hypotheses.

In summary, muscular weakness and cytokines alter the mechanical stability and inflammatory environment in the knee, respectively, and contribute to the pathogenesis of OA. In the present investigation, vitamin D deficiency associated with

quadriceps dysfunction but not inflammatory cytokines in subjects with knee OA. We propose that the increase in  $\gamma$ -tocopherol with vitamin D deficiency could abrogate a cytokine increase and consequently result in similar cytokine levels between vitamin D groups. In support of this premise, a decrease in  $\gamma$ -tocopherol correlated with an increase in TNF- $\alpha$  in the vitamin D sufficient group. We conclude that vitamin D deficiency associates with quadriceps dysfunction and an increase in  $\gamma$ -tocopherol, and that  $\gamma$ -tocopherol could have an important anti-inflammatory role in subjects with vitamin D sufficiency.

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