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ORIGINAL RESEARCH

Correlation between vitamin D levels and muscle fatigue risk factors based on physical activity in healthy older adults

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Purpose: The purpose of this study was to investigate the relationship of serum vitamin D levels with physical activity, obesity, muscle fatigue biomarkers, and total antioxidant capacity (TAC) in healthy older adults.

Methods: A total of 85 healthy older subjects aged 64–96 years were recruited in this study. Based on estimated energy expenditure scores, the participants were classified into three groups: inactive (n=25), moderate (n=20), and physically active (n=35). Serum 25(OH)D (25-hydroxy vitamin D) levels, metabolic syndrome parameters, TAC activity, muscle fatigue biomarkers (Ca, creatine kinase, lactic acid dehydrogenase, troponin I, hydroxyproline), physical activity, body fatness, and fatigue score (visual analog scale) were estimated using immunoassay techniques and prevalidated questionnaires, respectively.

Results: Physical activity was estimated in 64.6% of the participants. Males showed higher physical activity (42.5%) compared to females (26.25%). Compared to participants with lower activity, significant reduction in body mass index, waist circumference, hips, fasting blood sugar, triglycerides, total cholesterol, HDL-cholesterol, and LDL-cholesterol were observed in moderate and physically active participants. Also, significant increase in the levels of serum 25(OH)D concentrations, calcium, and TAC activity along with reduction in the levels of muscle fatigue biomarkers: creatine kinase, lactic acid dehydrogenase, troponin I, hydroxyproline, and fatigue scores (visual analog scale) were reported in physically active participants compared to those of lower physical activity. In all participants, serum 25(OH)D concentrations correlated positively with Ca, TAC, physical activity scores, and negatively with body mass index, lipid profile, fatigue scores (visual analog scale), and muscle fatigue biomarkers. Stepwise regression analysis showed that serum 25(OH)D concentrations, physical activity, Ca, TAC, and demographic parameters explained approximately 61.4%–85.8% of reduction in both fatigue scores and muscle fatigue biomarkers with substantial improvement in muscle performance in healthy older adults.

Conclusion: The data showed that considerable levels of 25(OH)D concentrations, calcium intake, and lower obesity positively correlated with the improvement in the muscle relief and performance of physically active participants. These results demonstrate that 25(OH)D concentrations and calcium might prevent muscle fatigue by regulation of the biosynthesis of creatine kinase, lactic acid dehydrogenase, troponin I, and hydroxyproline via a proposed antifree radical mechanism reported by higher TAC activity. It was suggested that vitamin D status could be reported as a marker of the improvement of muscle performance, especially in healthy older adults with lower physical activity.

Keywords: exercise, physical fitness, muscle stress, oxidative stress, 25(OH)D concentrations, troponin I, hydroxyproline

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Introduction

Besides its role in the regulation of bones and calcium homeostasis, vitamin D, especially 25(OH)D (serum 25-hydroxy vitamin D), is involved in many biological processes via specific cell receptors.[1,2] Previously, it was reported that exposure to sun for a few times a day provides substantial activation of vitamin D constituents, which promote health performance and strengthen the body biological immune systems against serious diseases.[3–5] Therefore, vitamin D-rich diets should be reported as an alternative source to avoid health problems of vitamin D deficiency in people of all ages, especially older adults.[6,7]

It was reported that delayed physical performance of human bodies was treated and improved with considerable doses of supervised training programs to enhance physical fitness scores. These exercise interventions keep serious cardiovascular and metabolic diseases out of human bodies;[8,9] the most suggested potential role of physical activity (PA) occurs through improvements of the antioxidant defense system.[10] The role of antioxidant against oxidative free radicals proceeds via free radical scavenging activity and acceleration of their decomposition.[11] Total antioxidant capacity (TAC) is an effective estimate of the activity of blood antioxidants, whereas it has been reported to be decreased in many diseases.[12]

There was a potential relation between muscle fatigue and the level of vitamin D, whereas any change in normal ranges of vitamin D faced significant alterations in muscle activity and performance, especially during exercise interventions of different intensities and capacities. The change in vitamin D levels consequently showed apparent effects on physical performance.[13,14] Previously, it was reported that administration of recommended doses of calcium and vitamin D for 3 months produces significant enhancements in the neuromuscular and skeletal muscle functions of elderly population.[15] Most studies reported that muscle power and force in adolescents, especially girls, are linked with vitamin D levels. Whereas, the deficiency in vitamin D produces muscle myopathy that leads to muscle fatigue and reduced motivation to ordinary exercise programs.[16,17] The treatment with suitable doses of vitamin D produces neuromuscular or neuroprotective role via increase in type II muscle fibers, which improve reaction, balance, and performance in muscle.[18,19] Whereas, lower vitamin D levels were shown to correlate positively with muscle fatigue disorders, such as fatty degeneration and muscle function.[20] The reduction in muscle mass and muscle strength was shown to be associated with vitamin D deficiency and physical inability in healthy older adults.[21–23] The occurrence and extent of such PA-induced muscle injury is routinely assessed from increased blood levels of muscle proteins and enzymes, as this provides the simplest way of studying the effects of exercise on muscles.

The proteins usually measured are creatine kinase (CK), hydroxyproline, and troponin I, which along with lactic acid dehydrogenase (LDH) activity generally allow earlier detection of muscle injury, especially muscle soreness following training interventions.[23–29] Thus, these muscles as indicative biomarkers clearly may help in discussing the correlative pivotal role of vitamin D status in the relief of muscle pain.

So, the purpose of this study was to investigate the relationship of serum vitamin D status with PA, obesity, muscle fatigue biomarkers, and TAC in healthy older population.

Materials and methods

Subjects

The experiment was conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki, and was reviewed and approved by the ethical committee of Rehabilitation Research Chair, King Saud University, Riyadh, Kingdom of Saudi Arabia, under file number ID: RRC-2013-015. A total of 85 healthy Saudi subjects (48 males, 37 females) aged 64–96 years were invited to participate in this study. Subjects with chronic conditions, such as asthma, type 1 diabetes mellitus, hypertension, history of cardiac, cancer, kidney or liver disease, use of medications known to affect body weight (such as steroids), and psychiatric conditions, and those taking calcium, vitamin D, or multivitamin supplements were excluded from the study. Prior to the experiment, the risks and benefits of the study were explained, and written informed consent was obtained from each participant after a medical checkup to ensure that they were fit, healthy, and had no physical limitations. All participants were instructed not to change their normal eating habits during the entire period of data collection.

Anthropometric measurements

All demographic parameters, such as weight, height, body mass index (BMI), and body fats, were performed using international standard scale (Digital Pearson Scale, Adam Equipment Ltd., Milton Keynes, UK), and bioelectrical impedance analysis-based body composition analyzer (TBF 105, Tanita Corporation, Tokyo, Japan), respectively. Waist circumference was measured as the minimum circumference between the iliac crest and the rib cage, while hip was measured at the widest part of buttocks.

Assessment of PA

PA scores of all participants were performed by using a prevalidated global PA questionnaire as previously reported;[30,31] the score was calculated according to the intensity of exercise performed in minutes or hours per day for each participant. Physically active subjects were those who participate in vigorous activities ≥3 or ≥7 days of any combination of moderate or vigorous activities, moderately active subjects were those who participate in 30 minutes of moderate-intensity exercise for ≥5 days/week, and low active subjects refers to those with sedentary lifestyle who have no PA during work and transportation. According to the intensity and frequency of PA performed per week, energy expenditure was assessed in the form of metabolic equivalent (MET) of all participants. Consequently, the participants were classified according to energy expenditure into physically inactive (MET minutes/week of ≤500, n=25), moderate PA (MET minutes/week of 500–2,500, n=20), and physically active (≥2,500 MET minutes/week, n=35).

Assessment of VAS

Pain intensity of all participants was generally assessed during each visit on a standard 100 mm visual analog scale (VAS), with 0 mm indicating "no pain" and 100 mm indicating "most severe pain". The VAS has excellent reliability for acute pain[32] as well as well-defined thresholds for meaningful change in pain intensity.[33]

Serum biochemical analysis

Blood samples were collected after an overnight fast (>10 hours) on the same day anthropometric information was gathered. The freshly withdrawn blood was immediately centrifuged at 3,000 rpm for 10 minutes and aliquots of serum samples were separated into dry tubes, and given a coded study identification number. Serum CK activity was assayed without delay and the rest of the serum samples were shipped frozen for later analysis.

Serum calcium, blood glucose, and lipid profile

Commercially available kits from Hoffmann-La Roche Ltd. (Basel, Switzerland) were used to estimate calcium levels in serum by colorimetric assays using Cobas Integra® analyzer. Fasting blood glucose and lipids which included triglycerides, total high-density lipoprotein (HDL)-, and low-density lipoprotein (LDL)-cholesterol were measured routinely using a chemical analyzer (Konelab, Thermo Fisher Scientific, Waltham, MA, USA).

Estimation of serum 25(OH)D level

Enzyme-linked immunosorbent assay was performed to measure vitamin D levels using immunoassay kits (IDS, Boldon Business Park, Boldon, Tyne & Wear,UK). The data were calculated according to the manufacturer's instructions.

CK and LDH activities

Serum CK was measured by a standardized commercially available enzymatic assay (Granutest 15, EMD Millipore, Billerica, MA, USA), and LDH activity was measured by an ultraviolet method provided by Randox Laboratories Ltd (Antrim, UK).

Serum troponin I and hydroxyproline assay

Serum troponin I concentration was measured by enzymatic immunoassay technique using a sandwich human enzyme-linked immunosorbent assay kit (Cat. No.: RLF-EK0128R, BioVendor R&D, LLC, Woodland Hills, California, USA). Serum hydroxyproline was measured using a commercially available colorimetric assay kit (Catalog #K555-100; BioVision Inc., Milpitas, CA, USA). The assay was performed according to the manufacturer's instructions.

Total antioxidant capacity

Serum TAC was measured using colorimetric assay kit (Catalog #K274-100; BioVision Inc.). Serum TAC concentrations were measured at 570 nm and the values were calculated according to the manufacturer's instructions.

Statistical analysis

SPSS package (SPSS 11.5, Chicago, IL, USA) was used for statistical analysis. Frequencies were expressed in percentage (%) and continuous variables were presented as mean ± standard deviation. Analysis of variance was done to compare age-matched groups. Variables were log transformed prior to parametric comparisons. Stepwise linear regression analysis was done to perform the correlation between vitamin D and other studied parameters. *P*-value was significant at $P<0.05$.

Results

A total of 85 healthy older subjects aged 64–96 years were included in this study. Based on the level of PA, subjects were divided into three groups: inactive, moderately active, and active. The demographics and descriptive characteristics of recruited subjects are given in Table 1. The subjects who were physically inactive were ~29.4% in both males and females; while PA was reported in 64.7 of subjects, males showed 42.5% of PA compared to 26.25% of females, respectively.

Table 1 General characteristics of subjects based on level of PA

Parameters	Physically inactive (≤500 METs min/week)	Moderate PA (500–2,500 METs min/week)	Physically active (≥2,500 METs min/week)
N	25	25	35
Male/female	10/15	16/9	22/13
Age (years)	62.7±4.3	63.2±5.6	62.9±3.7
BMI (kg/m^2)	26.1±7.6	23.4±6.3*	18.9±4.3*
Waist (cm)	76.1±27.9	69.7±18.5	64.6±16.5
Hips (cm)	85.9±24.3	85.7±22.3	85.4±21.7
Systolic BP (mmHg)	111.2±9.5	108.9±10.3	109.5±10.5
Diastolic BP (mmHg)	72.5±13.7	72.5±11.3	72.8±8.5
Glucose (mmol/L)	5.7±0.85	5.4±0.65**	4.8±0.75**
TG (mmol/L)	0.85±0.45	0.81±0.65*	0.8±0.41*
Total cholesterol (mmol/L)	4.3±0.56	4.2±0.68*	3.9±0.98*
HDL-cholesterol (mmol/L)	1.5±0.35	1.2±0.30*	1.0±0.28*
LDL-cholesterol (mmol/L)	3.6±0.72	3.4±0.67*	3.0±0.61*

Notes: Values are expressed as mean ± SD; *$P<0.05$, **$P<0.01$. Significance at $P<0.05$.
Abbreviations: BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MET, metabolic equivalent; PA, physical activity; TG, triglyceride; SD, standard deviation.

A significant increase (P=0.05) in BMI, waist circumference, and hips was reported in the physically inactive group compared to moderate and physically active (PA) groups, respectively. Similarly, serum fasting glucose was also highest in the physically inactive group (P=0.01) (Table 1). In subjects with moderate and physically active status, a significant decrease (P=0.05) in lipid profiles (triglycerides, total cholesterol, HDL-, and LDL-cholesterol) was reported compared to the physically inactive group (Table 1). Significant increase in the serum levels of 25(OH)D, calcium, and TAC was greatly reported in subjects with moderate (P=0.01) and physically (P=0.001) active status compared to physically inactive group (Table 2). In all groups, levels of 25(OH) D were less in the group with lesser PA along with TAC and calcium levels. However, in physically active subjects, a significant reduction in the level of muscle fatigue markers (CK, LDH, troponin I, and hydroxyproline) was observed and supported with the reduction in fatigue score (VAS) as shown in moderate (P=0.01) and physically active (P=0.001) groups (Table 2). The data obtained showed positive significant correlations among levels of 25(OH)D, TAC, and serum calcium levels in moderate (P=0.05) and physically active (P=0.01) groups, respectively.

BMI was negatively and significantly associated with 25(OH)D levels in all groups as well as serum markers of muscle fatigue: CK, LDH, troponin I, hydroxyproline, and fatigue score (VAS). This correlation suggested the importance of 25(OH)D levels toward obesity and muscle fatigue in physically inactive and physically active subjects as shown in Table 3. Whereas, the increase of the level of 25(OH)D is attributed with muscle relief and improvement of BMI or obesity as shown in moderate (P=0.01) and physically active (P=0.05) subjects compared to the physically inactive group (Table 3). Stepwise regression analysis showed that

Table 2 Change in muscle fatigue biomarkers, 25(OH) vitamin D, serum calcium, TAC, and mean pain scores (VAS) of subjects based on level of PA (mean ± SD)

Parameters	Physically inactive, N=25 (≤500 METs min/week)	Moderate PA, N=25 (500–2,500 METs min/week)	Physically active, N=35 (≥2,500 METs min/week)
25(OH) vitamin D (nmol/L)	23.8±2.9	48.6±4.8**	62.3±7.61***
sTCa^{2+} (mmol/L)	1.9±0.56	2.50±4**	5.70±4.1***
sf-Ca^{2+} (mmol/L)	1.1±0.7	1.6±0.2**	2.7±0.2***
CK (UI/L)	309.8±7.9	276.3±3.7**	245.1±5.3***
LDH (IU/L)	65.3±11.6	48.5±7.1**	32.8±4.9***
Troponin I (ng/mL)	1.9±0.18	1.16±0.12**	0.82±0.05***
Hydroxyproline (ng/mL)	3.9±0.15	2.65±0.21**	1.75±0.31***
TAC (nmol/µL)	16.5±6.3	22.9±11.7**	38.3±13.8***
VAS score	55.3±20.5	36.7±12.3**	22.4±9.3***

Notes: Values are expressed as mean ± SD; **$P<0.01$, ***$P<0.001$. Significance at $P<0.05$.
Abbreviations: CK, creatine kinase; LDH, lactate dehydrogenase; MET, metabolic equivalent; PA, physical activity; SD, standard deviation; sf-Ca^{2+}, serum-free calcium; sTCa^{2+}, serum total calcium; TAC, total antioxidant capacity; VAS, visual analog scale.

Table 3 Bivariate associations of 25(OH) vitamin D to various parameters based on PA

Parameters	Physically inactive, N=25 (≤500 METs min/week)	Moderate PA, N=25 (500–2,500 METs min/week)	Physically active, N=35 (≥2,500 METs min/week)
BMI (kg/m^2)	−0.28*	−0.2*	−0.18*
WC (cm)	−0.011*	−0.14*	−0.21**
Hips (cm)	−0.07*	−0.25*	−0.59**
Glucose (mmol/L)	−0.014*	−0.27*	−0.31**
TG (mmol/L)	−0.041*	−0.59*	−0.72**
Total cholesterol (mmol/L)	−0.09*	−0.085*	−0.067**
HDL-cholesterol (mmol/L)	−0.011*	−0.017*	−0.021**
LDL-cholesterol (mmol/L)	−0.006*	−0.015*	−0.031**
sTCa^{2+} (mmol/L)	0.042*	0.051*	0.038**
sf-Ca^{2+} (mmol/L)	0.016*	0.035*	0.046**
CK (UI/L)	−0.21*	−0.25*	−0.29**
LDH (IU/L)	−0.34*	−0.51*	−0.37**
Troponin I (ng/mL)	−0.61*	−0.47*	−0.54**
Hydroxyproline (ng/mL)	−0.27*	−0.35*	−0.47**
TAC (nmol/μL)	0.32*	0.41*	0.59**
VAS score	−0.12*	−0.15*	−0.17**
PA score	0.23*	0.52*	0.71**

Note: Data presented as coefficient (R); *Denotes significance at <0.05; **Denotes significance at <0.01.
Abbreviations: BMI, body mass index; CK, creatine kinase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LDH, lactic acid dehydrogenase; MET, metabolic equivalent; PA, physical activity; sf-Ca^{2+}, serum-free calcium; sTCa^{2+}, serum total calcium; TAC, total antioxidant capacity; TG, triglyceride; VAS, visual analog scale; WC, waist circumference.

vitamin D levels, PA, Ca, TAC, and demographic parameters explained approximately 61.4%–85.8% of reduction in both muscle fatigue scores and muscle fatigue biomarkers with substantial improvement in muscle performance in healthy older adults (Table 4).

Discussion

Physically active lifestyle has been associated with various health benefits and its absence can have harmful effects on health and well-being.[34,35] It was suggested that PA has a substantial beneficial effect on vitamin D levels,[36] whereas higher self-reported activity was linked with higher levels of 25(OH)D,[37] and decreased PAs were associated with obesity and low vitamin D levels.[38,39] The best estimate of an individual's vitamin D status is the measurement of circulating vitamin D, 25(OH)D,[40] which provides an indication of vitamin D stores obtained from both ultraviolet light and dietary intake.

In the present study, subjects with lower activity showed significantly lower serum 25(OH)D levels compared to physically active subjects. Whereas, the prevalence of vitamin D deficiency was 29.4% among physically inactive subjects, the data matched with previous researches,[38–42] which reported the prevalence of vitamin D deficiency in healthy and diseased subjects. The data obtained from serum 25(OH)D levels are correlated positively with PA and negatively with age as shown by Ardawi et al.[43] This also confirmed with a recent study of Ha et al[44] who reported that serum vitamin D levels were positively associated with PA, including low,

Table 4 Beta coefficients and cumulative R^2* values derived from stepwise multiple regression models

Parameters	Muscle fatigue (VAS score, 0–100 mm)					
	Mild (5–44)		Moderate (45–74)		Severe (75–100)	
	R^2* (β)	95% CI	R^2* (β)	95% CI	R^2* (β)	95% CI
Body weight (kg)	22.5 (0.42)	85 (60–87)	18.2 (0.22)	95 (86–100)	16.9 (0.32)	86 (81–100)
Age (years)	12.1 (0.65)	75 (60–95)	17.4 (0.35)	94 (87–100)	10.2 (0.55)	94 (85–98)
BMI (kg/m^2)	7.5 (0.25)	94 (86–98)	7.5 (0.15)	90 (73–97)	6.9 (0.25)	89 (87–100)
Vitamin D (nmol/L)	24.1 (0.25)	82 (75–97)	14.1 (0.45)	76 (72–100)	11.8 (0.85)	94 (73–98)
Calcium (mg/dL)	9.3 (0.44)	82 (75–97)	5.8 (0.24)	89 (87–100)	6.1 (0.34)	95 (88–100)
Physical activity level (MET min/week)	6.9 (0.25)	85 (56–96)	2.5 (0.75)	95 (88–100)	3.8 (0.49)	90 (46–95)
TAC (nmol/μL)	3.4 (0.25)	94 (72–100)	8.6 (0.65)	89 (87–100)	5.7 (0.48)	69 (52–96)
ΣR^2 (%)	85.8	96 (87–100)	74.1	95 (88–100)	61.4	90 (46–95)

Note: *P<0.05.
Abbreviations: BMI, body mass index; CI, confidence interval; MET, metabolic equivalent; TAC, total antioxidant capacity; VAS, visual analog scale.

moderate, and vigorous levels. The positive association between PA and 25(OH)D level found in this study may be related to the presence of an extravascular pool in muscle through which 25(OH)D vitamin circulates.[45]

In the present study, anthropometric indices for obesity have been widely used to study the association between obesity and vitamin D levels in healthy subjects. The results indicated that serum 25(OH)D vitamin levels decreased in physically inactive subjects with overweight/obese status and were negatively correlated with BMI, hips, and waist circumference; these findings are consistent with the previous studies which demonstrated inverse correlations of serum 25(OH)D levels with body weight, BMI, and waist circumference,[45,46] the mechanism by which lower levels of serum 25(OH)D were reported in obese subjects may be related to sequestration in fat or lower rates of lipolysis in the obese subjects.[47,48] However, in moderate to physically active subjects, the increased level of serum 25(OH)D vitamins is significantly associated with an improvement in the anthropometric indices for obesity. This matched with other studies, which reported vigorous PA to be a strong and modifiable contributor to vitamin D status.[49,50] Matching with our results, Brock et al reported that higher obesity, lower PA, and poorer calcium administrations are linked with lower vitamin D status. They proved that these parameters might be considered valuable predictors of vitamin D deficiency.[51]

In physically active subjects, there was an increase in local bone mass, reduction in calcium excretion, and increase in absorption efficiency, consequently leading to an increase in serum calcium, which ultimately enhances sparing of serum vitamin D.[52] Also, in human bodies with reduced body weight, vitamin D serum levels were shown to increase as a result of higher rates of lipolysis, which enhances its mobilization from adipose tissue.[53–55] However, another study reported that low levels of vitamin D may be related to changes in vitamin metabolism or lifestyle of obese subjects, such as sunlight exposure. Whereas, the bioavailability of 25(OH)D or its precursors stored in adipose tissue and muscles was shown to be poor.[56]

Previous reports suggested that low levels of serum 25(OH)D vitamins correlated significantly with metabolic disorders,[57,58] but little is known of the link between 25(OH)D level and different components of the lipid disorders related, such as elevated triglyceride.

In this study, we found an inverse association between 25(OH)D level and lipid profile, including triglycerides, total cholesterol, HDL-cholesterol, and LDL-cholesterol. Serum vitamin D levels were inversely related to total cholesterol; triglycerides in all subjects with different physical activity. The data obtained from stepwise linear regression model showed that both low serum vitamin D levels and decreased vigorous physical activity were dependent predictors for individual variation in the clustering of metabolic risk factors such as lipid profile in this study sample.

The correlative negative association between 25(OH)D and triglycerides reported in our study were in line with other results noticed in a previous study as well.[59] However, in clinical trials, it was difficult to link any association between the improvement in triglyceride level and vitamin D administration.[60] Association of 25(OH)D with glucose regulation and type 2 diabetes has been shown in several studies.[61,62] The levels of 25(OH)D vitamin and fasting glucose were shown to inversely correlate with PA profile.[44] In this study, all subjects showed that serum vitamin D levels were inversely related to fasting glucose, whereas physically active subjects showed significant improvement in glucose levels compared to physically inactive cases. The data obtained matched with Jang et al[63] who reported that serum 25(OH)D vitamin had a negative relationship with fasting glucose and insulin resistance index, and that a higher level of 25(OH)D vitamin in physically active subjects has been associated with insulin sensitivity indices[64] and low 25(OH)D has been shown to predict later development of type 2 diabetes.[65]

Oxidative stress is part of metabolic process in all mammalian cells generating free radicals.[66] Several investigators have studied the mechanisms by which physical exercise modulates the expression of antioxidant systems and oxidant–antioxidant relationship in many biological models.[67] However, no conclusive data exist regarding this association.

Regarding PA, we found that regular PA significantly increased the whole blood resistance to free radicals.

In the present study, there was a significant increase from low to moderate and vigorous PA groups in TAC levels. On the contrary, physically inactive subjects with occasional physical exercise as well as sedentary habits were associated with a decreased antioxidant potential. Interestingly, subjects in the higher levels of TAC and the vigorous PA group had significantly higher levels of 25(OH)D vitamins compared to physically inactive subjects with lower levels of TAC and 25(OH)D vitamins, respectively. The increase in 25(OH)D vitamin levels may be to hamper the release of active free radicals.[68] The data were in accordance with Oikonomou et al[69] who concluded that habitual PA is associated with increased TAC. Similarly, other studies have reported a positive association between regular PA and antioxidant enzyme activity, and clearance of both oxidant stress products.[70,71] This adaptive response has been recognized

both in the young as well as the middle aged and elderly.[72] However, it has also been suggested that excessive PA may promote a generation of more oxidative free radicals than antioxidant capacity,[73] which was prohibited in our physically active subjects via improvement of TAC activity supported with significant increase in the levels of 25(OH)D vitamin, which has a promising antioxidant capacity.[74]

For these previous observations, the main strength of our study was the inclusion of PA and vitamin D status in examining the relation to muscle fatigue. Exercise training interventions, health during daily life activities as well as many diseases may be attributed to muscle fatigue as a secondary outcome of abnormal health performance.[75]

Regarding the importance of PA and its affect on muscle fatigue, serum CK, LDH, troponin I, Ca, hydroxyproline, and fatigue score (VAS) were measured in healthy subjects with varying physical status. There was a significant decrease in muscle fatigue markers, CK, LDH, troponin I, hydroxyproline, and VAS score along with increase in serum Ca level among moderate to physically active subjects. Conversely, physically inactive or sedentary subjects showed higher muscle fatigability marked with lower serum Ca level and higher rates of CK, LDH, troponin I, hydroxyproline, and VAS score, respectively.

Previous reports concluded that excitation of muscle membrane, contraction, and energy metabolism depend mainly upon ionic regulation,[76] and that the irregularity in the concentration of muscle lactate, hydrogen (H^+), potassium (K^+), and calcium (Ca^{2+}) ions is linked with fatigue.[77] It was reported that the greatest part of fatigue is due to reduced sarcoplasmic reticulum calcium (Ca^{2+}) release and decreased Ca^{2+} sensitivity of the contractile proteins.[78] Also, it is commonly believed that the appearance of intramuscular enzymes, such as CK and LDH,[79] besides hydoxyproline amino acids and troponin I, is the result of collagen fiber degradation either by overuse or strained muscle damage;[80–82] these markers are potentially used as indicators of skeletal muscle damage.[83] In this study, the increase in the fatigability of physically inactive subjects may be related to changes in muscle metabolism as a result of restriction of PA, deconditioning, and decrease in muscle mass and strength as previously reported.[84] Whereas, in physically active participants of the same study, the broad spectrum activity via exercise training programs increases muscle strength, function, and improves resistance ability of muscles against fatigue in patients and healthy subjects of all ages.[85,86]

In older subjects, vitamin D status was shown to play a potential significant effect on muscle weakness, pain, balance, and fractures as previously reported.[87,88] In the present study, the data obtained showed significant association between serum levels of 25(OH)D vitamin and muscle fatigue biomarkers. In subjects with low, moderate, and vigorous levels of PA, serum 25(OH)D levels correlated positively with serum calcium level and negatively with CK, LDH, troponin I, hydroxyproline, and VAS score, respectively. In physically inactive subjects, the increase in muscle fatigue parameters is associated with a significant deficiency in serum Ca and 25(OH)D vitamin levels, respectively.

Most studies reported the positive correlation between 25(OH)D levels and muscle performance, such as muscle power strength, and function in the elderly,[89,90] and that considerable amounts of vitamin D administration in subjects with low vitamin levels might improve muscle performance, such as muscle strength via an increase in the size and amount of type II (fast twitch) muscle fibers,[91] which are predominant in power and anaerobic activities.

The potential influence of vitamin D status on muscular strength was explained with two postulated mechanisms. First, via a direct role of vitamin [1,25(OH)2D] on vitamin D receptors within the muscle cells,[92–94] and the second mechanism was the ability of vitamin D to increase the efficiency or number of calcium-binding sites involved in muscle contraction by modifications in the transportation of calcium to the sarcoplasmic reticulum muscles.[90]

The ultimate role of vitamin D, via its active 1,25(OH)2D metabolite, is to facilitate the absorption of calcium from the intestine and help maintain normal concentrations of this vital agent. Equally important, 1,25(OH)2D sustains a wide range of metabolic and physiologic functions throughout the body.[95] In cellular level, a link between vitamin D and the mitochondria was proposed in human skeletal muscle, whereas an improvement in muscle fatigue was reported in vitamin D-deficient adults following cholecalciferol therapy.[96]

It was shown that the presence of vitamin D in higher levels may have positive preventive effects against abnormal muscle injury and performance,[89,92,95] whereas induction of skeletal muscle physiological response requires higher amounts of 25(OH)D levels to achieve significant muscle performance;[93,96] this was confirmed in our study where physically active subjects showed higher 25(OH)D levels (48.6–62.3 ng/mL) compared to physically inactive individuals (23.8 ng/mL). In addition, in physically inactive subjects, resolution of vitamin D insufficiency has the potential to impact performance among physically inactive subjects.[96]

Finally, this study considered the importance of calcium and vitamin D levels along with PA status to prevent muscle

fatigue and enhance muscle performance among healthy older volunteers.

Conclusion

The data showed that considerable levels of 25(OH)D concentrations, calcium intake, and lower obesity positively correlated with improvement in muscle relief and performance of physically active participants. These results demonstrate that 25(OH)D concentrations and calcium might prevent muscle fatigue by regulation of the biosynthesis of CK, LDH, troponin I, and hydroxyproline via a proposed antifree radical mechanism reported by higher TAC activity. It was suggested that vitamin D status could be reported as a marker of the improvement of muscle performance, especially in healthy older adults with a lower PA.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Stewart JW, Alekel DL, Ritland LM, Van Loan M, Gertz E, Genschel U. Serum 25-hydroxyvitamin D is related to indicators of overall physical fitness in healthy postmenopausal women. *Menopause*. 2009; 16(6):1093–1101.
2. Bringhurst FR, Demay MB, Kronenberg HM. Hormones and disorders of mineral metabolism. In: Larsen PR, Kronenberg HM, Melmed S, Polonsky KS, editors. *Williams Textbook of Endocrinology*. 10th edition. Philadelphia: Saunders; 2003:1317–1320.
3. Hutchinson MS, Grimnes G, Joakimsen RM, Figenschau Y, Jorde R. Low serum 25-hydroxyvitamin D levels are associated with increased all-cause mortality risk in a general population: the Tromsø study. *Eur J Endocrinol*. 2010;162(5):935–942.
4. Holick MF. Vitamin D: the other steroid hormone for muscle function and strength. *Menopause*. 2009;16(6):1077–1078.
5. Hamilton B. Vitamin D and human skeletal muscle. *Scand J Med Sci Sports*. 2010;20(2):182–190.
6. Ceglia L. Vitamin D and its role in skeletal muscle. *Curr Opin Clin Nutr Metab Care*. 2009;12(6):628–633.
7. Venning G. Recent developments in vitamin D deficiency and muscle weakness among elderly people. *BMJ*. 2005;330:524–526.
8. Cannell JJ, Hollis BW, Sorenson MB, Taft TN, Anderson JJ. Athletic performance and vitamin D. *Med Sci Sports Exerc*. 2009;41(5): 1102–1110.
9. Haskell WL, Lee IM, Pate RR, et al. Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. *Med Sci Sports Exerc*. 2007;39(8):1423–1434.
10. Kostka T, Drygas W, Jegier A, Zaniewicz D. Aerobic and anaerobic power in relation to age and physical activity in 354 men aged 20–88 years. *Int J Sports Med*. 2009;30:225–230.
11. Fatouros IG, Jamurtas AZ, Villiotou V, et al. Oxidative stress responses in older men during endurance training and detraining. *Med Sci Sports Exerc*. 2004;36(12):2065–2072.
12. Vassalle C, Masini S, Carpeggiani C, L'Abbate A, Boni C, Zucchelli GC. In vivo total antioxidant capacity: comparison of two different analytical methods. *Clin Chem Lab Med*. 2004;42:84–89.
13. Opara EC, Abdel-Rahman E, Soliman S, et al. Depletion of total antioxidant capacity in type 2 diabetes. *Metabolism*. 1999;48:1414–1417.
14. Verstuyf A, Carmeliet G, Bouillon R, Mathieu C. Vitamin D a pleiotropic hormone. *Kidney Int*. 2010;78(2):140–145. DOI:10.1038/Ki.2010.17.
15. Bischoff HA, Stähelin HB, Dick W, et al. Effects of vitamin D and calcium supplementation on falls: a randomized controlled trial. *J Bone Miner Res*. 2003;18(2):343–351.
16. Kennel KA, Drake MT, Hurley DL. Vitamin D deficiency in adults: when to test and how to treat. *Mayo Clin Proc*. 2010;85(8):752–757.
17. Pérez-López FR. Vitamin D and its implications for musculoskeletal health in women: an update. *Maturitas*. 2007;58(2):117–137.
18. Ward KA, Das G, Berry JL, et al. Vitamin D status and muscle function in postmenarchal adolescent girls. *J Clin Endocrinol Metab*. 2009;94(2):559–563.
19. Dhesi JK, Jackson SH, Bearne LM, et al. Vitamin D supplementation improves neuromuscular function in older people who fall. *Age Ageing*. 2004;33(6):589–595.
20. Sato Y, Iwamoto J, Kanoko T, Satoh K. Low-dose vitamin D prevents muscular atrophy and reduces falls and hip fractures in women after stroke: a randomized controlled trial. *Cerebrovasc Dis*. 2005;20(3):187–192.
21. Hurley BF. Age, gender, and muscular strength. *J Gerontol A Biol Sci Med Sci*. 1995;50A:41–44.
22. Samson MM, Meeuwsen IB, Crowe A, Duursma SA, Verhaar HJ. Relationships between physical performance measures, age, height and body weight in healthy adults. *Age Ageing*. 2000;29:235–242.
23. van der Wielen RPJ, Löwik MRH, van der Berg H, et al. Serum vitamin D concentrations among elderly people in Europe. *Lancet*. 1995; 346:201–210.
24. Oh JH, Kim SH, Kim JH, Shin YH, Yoon JP, Oh CH. The level of vitamin D in the serum correlates with fatty degeneration of the muscles of the rotator cuff. *J Bone Joint Surg Br*. 2009;91(12):1587–1593.
25. Rodenburg JB, Bär PR, De Boer RW. Relations between muscle soreness and biochemical and functional outcomes of eccentric exercise. *J Appl Physiol*. 1993;2976–2983.
26. Siegel AJ, Januzzi J, Sluss P, et al. Cardiac biomarkers, electrolytes, and other analytes in collapsed marathon runners: implications for the evaluation of runners following competition. *Am J Clin Pathol*. 2008; 129:948–951.
27. Schuback K, Essén-Gustavsson B, Persson SG. Incremental treadmill exercise until onset of fatigue and its relationship to metabolic response and locomotion pattern. *Equine Vet J*. 1999;30:337–341.
28. Friden J, Seger J, Ekblom B. Sublethal muscle fiber injuries after high-tension anaerobic exercise. *Eur J Appl Physiol Occup Physiol*. 1988; 57:360–368.
29. Shave R, Ross P, Low D, George K, Gaze D. Cardiac troponin I is released following high-intensity short-duration exercise in healthy humans. *Int J Cardiol*. 2010;145:337–339.
30. Bull FC, Maslin TS, Armstrong T. Global physical activity questionnaire (GPAQ): nine country reliability and validity study. *J Phys Act Health*. 2009;6:790–804.
31. Trinh OT, Nguyen ND, van der Ploeg HP, Dibley MJ, Bauman A. Test-retest repeatability and relative validity of the Global Physical Activity Questionnaire in a developing country context. *J Phys Act Health*.2009; 6 Suppl 1:S46–S53.
32. Bijur PE, Silver W, Gallagher EJ. Reliability of the visual analog scale for measurement of acute pain. *Acad Emerg Med*. 2001;8:1153–1157.
33. Todd KH, Funk KG, Funk JP, Bonacci R. Clinical significance of reported changes in pain severity. *Ann Emerg Med*. 1996;27:485–489.

34. Behre CJ, Bergström G, Schmidt CB. Increasing leisure time physical activity is associated with less prevalence of the metabolic syndrome in healthy middle-aged men. *Angiology*. 2011;62:509–512.
35. Gerber Y, Myers V, Goldbourt U, Benyamini Y, Scheinowitz M, Drory Y. Long-term trajectory of leisure time physical activity and survival after first myocardial infarction: a population-based cohort study. *Eur J Epidemiol*. 2011;26:109–116.
36. Scragg R, Camargo CA Jr. Frequency of leisure-time physical activity and serum 25-hydroxyvitamin D levels in the US population: results from the Third National Health and Nutrition Examination Survey. *Am J Epidemiol*. 2008;168:571–586.
37. Lucas JA, Bolland MJ, Grey AB, et al. Determinants of vitamin D status in older women living in a subtropical climate. *Osteoporos Int*. 2005; 16:1641–1648.
38. Hintzpeter B, Mensink GB, Thierfelder W, Müller MJ, Scheidt-Nave C. Vitamin D status among German adults. *Eur J Nutr*. 2007;62(9): 1079.
39. Hirani V, Mosdol A, Mishra G. Predictors of 25-(OH)D status among adults in two British national surveys. *Br J Nutr*. 2008;17:1–5.
40. Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007;357(3):266–281.
41. Neyestani T, Gharavi A, Kalayi A. Iranian diabetics may not be vitamin D deficient more than healthy subjects. *Acta Med Iran*. 2008;46: 337–341.
42. Hashemipour S, Larijani B, Adibi H, et al. The status of biochemical parameters in varying degrees of vitamin D deficiency. *J Bone Miner Metab*. 2006;24:213–218.
43. Ardawi MS, Qari MH, Rouzi AA, Maimani AA, Raddadi RM. Vitamin D status in relation to obesity, bone mineral density, bone turnover markers and vitamin D receptor genotypes in healthy Saudi pre- and postmenopausal women. *Osteoporos Int*. 2011;22:463–475.
44. Ha CD, Cho JK, Lee SH, Kang HS. Serum vitamin D, physical activity, and metabolic risk factors in Korean children. *Med Sci Sports Exerc*. 2013;45(1):102–108.
45. Abboud M, Puglisi DA, Davies BN, et al. Evidence for a specific uptake and retention mechanism for 25-hydroxyvitamin D (25OHD) in skeletal muscle cells. *Endocrinology*. 2013;154(9):3022–3030.
46. McGill AT, Stewart JM, Lithander FE, Strik CM, Poppitt SD. Relationships of low serum vitamin D3 with anthropometry and markers of the metabolic syndrome and diabetes in overweight and obesity. *Nutr J*. 2008;7:4.
47. Petersen L, Schnohr P, Sorensen T. Longitudinal study of the long-term relation between physical activity and obesity in adults. *Int J Obes Relat Metab Disord*. 2004;28:105–112.
48. Frayn K. Obesity and metabolic disease: is adipose tissue the culprit? *Proc Nutr Soc*. 2005;64:7–13.
49. Freedman DM, Chang SC, Falk RT, et al. Serum levels of vitamin D metabolites and breast cancer risk in the prostate, lung, colorectal, and ovarian cancer screening trial. *Cancer Epidemiol Biomark Prev*. 2008;17(4):889–894.
50. Brock K, Graubard B, Fraser DR, et al. Predictors of vitamin D biochemical status in a large sample of middle-aged male smokers from Finland. *Eur J Clin Nutr*. 2010;64(3):280–288.
51. Lenders CM, Feldman HA, Von Scheven E, et al. Relation of body fat indexes to vitamin D status and deficiency among obese adolescents. *Am J Clin Nutr*. 2009;90:459–467.
52. Specker BL. Evidence for an interaction between calcium intake and physical activity on changes in bone mineral density. *J Bone Miner Res*. 1996;11:1539–1544.
53. Tzotzas T, Papadopoulou FG, Tziomalos K, et al. Rising serum 25-hydroxy-vitamin D levels after weight loss in obese women correlate with improvement in insulin resistance. *J Clin Endocrinol Metab*. 2010; 95:4251–4257.
54. Zittermann A, Frisch S, Berthold HK, et al. Vitamin D supplementation enhances the beneficial effects of weight loss on cardiovascular disease risk markers. *Am J Clin Nutr*. 2009;89:1321–1327.
55. Mastaglia SR, Seijo M, Muzio D, Somoza J, Nuñez M, Oliveri B. Effect of vitamin D nutritional status on muscle function and strength in healthy women aged over sixty-five years. *J Nutr Health Aging*. 2011;15(5): 349–354.
56. Heaney RP, Horst RL, Cullen DM, Armas LA. Vitamin D3 distribution and status in the body. *J Am Coll Nutr*. 2009;(3):252–256.
57. Hypponen E, Boucher BJ, Berry DJ, Power C. 25-hydroxyvitamin D, IGF-1, and metabolic syndrome at 45 years of age: a cross-sectional study in the 1958 British Birth Cohort. *Diabetes*. 2008;57(2):298–305.
58. Kayaniyil S, Harris SB, Retnakaran R, et al. Prospective association of 25(OH)D with metabolic syndrome. *Clin Endocrinol (Oxf)*. 2013;80(4): 502–507.
59. Jorde R, Grimnes G. Vitamin D and metabolic health with special reference to the effect of vitamin D on serum lipids. *Prog Lipid Res*. 2011;50(4): 303–312.
60. Jorde R, Sneve M, Torjesen P, Figenschau Y. No improvement in cardiovascular risk factors in overweight and obese subjects after supplementation with vitamin D for 1 year. *J Intern Med*. 2010;267:462–472.
61. Forouhi NG, Ye Z, Rickard AP, et al. Circulating 25-hydroxyvitamin D concentration and the risk of type 2 diabetes: results from the European Prospective Investigation into Cancer (EPIC)-Norfolk cohort and updated meta-analysis of prospective studies. *Diabetologia*. 2012;55(8):2173–2182.
62. Hurskainen AR, Virtanen JK, Tuomainen TP, Nurmi T, Voutilainen S. Association of serum 25-hydroxyvitamin D with type 2 diabetes and markers of insulin resistance in a general older population in Finland. *Diabetes Metab Res Rev*. 2012;28(5):418–423.
63. Jang HB, Lee HJ, Park JY, Kang JH, Song J. Association between serum vitamin d and metabolic risk factors in Korean schoolgirls. *Osong Public Health Res Perspect*. 2013;4(4):179–186.
64. Chiu KC, Chu A, Go VL, Saad MF. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. *Am J Clin Nutr*. 2004; 79(5):820–825.
65. Pittas AG, Sun Q, Manson JE, Dawson-Hughes B, Hu FB. Plasma 25-hydroxyvitamin D concentration and risk of incident type 2 diabetes in women. *Diabetes Care*. 2010;33(9):2021–2023.
66. Urso ML, Clarkson PM. Oxidative stress, exercise, and antioxidant supplementation. *Toxicology*. 2003;189:41–54.
67. Aguiló A, Tauler P, Fuentespina E, Tur JA, Córdova A, Pons A. Antioxidant response to oxidative induced by exhaustive exercise. *Physiol Behav*. 2005;84:1–847.
68. Mukhopadhyaynee Sardar S, Singh M, Chatterjee M. Vitamin D3 as a modulator of cellular antioxidant defence in murine lymphoma. *Nutr Res*. 2000;20:91–102.
69. Oikonomou E, Siasos G, Chrysohoou C, et al. The impact of physical activity on total antioxidant capacity and endothelial function: Ikaria Study. *Eur Heart J*. 2013;34:1089.
70. McArdle A, Jackson MJ. Exercise oxidative stress and aging. *J Anat*. 2000;197:539–541.
71. Jenkins RR, Krause K, Schofield LS. Influence of exercise on clearance of oxidant stress products and loosely bound iron. *Med Sci Sports Exerc*. 1993;25:213–217.
72. Tenabe K, Masuda K, Sugawara J, et al. Effects of different types of training on blood antioxidant capacity and redox balance in middle-aged and elderly women. *J Exerc Sport Physiol*. 2003;10:65–76 (in Japanese).
73. Marzatico F, Pansarasa O, Bertorelli L, Somenzini L, Della Valle G. Blood free radical antioxidant enzymes and lipid peroxides following long-distance and lactacidemic performances in highly trained aerobic and sprint athletes. *J Sports Med Phys Fitness*. 1997;37:235–239.
74. Karmakar R, Banik S, Chatterjee M. Inhibitory effect of vitamin D3 on 3′methyl-4-dimethyl-amino-azobenzene-induced rat hepatocarcinogenesis: A study on antioxidant defense enzymes. *J Exp Ther Oncol*. 2002;2:193–199.
75. Rimmer JH, Schiller W, Chen MD. Effects of disability-associated low energy expenditure deconditioning syndrome. *Exerc Sport Sci Rev*. 2012;40:22–29.

76. Allen D, Lamb GD, Westerblad H. Skeletal muscle fatigue: cellular mechanisms. *Physiol Rev*. 2008;88:287–332.
77. McKenna MJ, Bangsbo J, Renaud JM. Muscle K+, Na+, and Cl disturbances and Na+- K+ pump inactivation: implications for fatigue. *J Appl Physiol*. 2008;104:288–295.
78. Ghyasi R, Sepehri G, Mohammadi M, Badalzadeh R, Ghyasi A. Effect of mebudipine on oxidative stress and lipid peroxidation in myocardial ischemic-reperfusion injury in male rat. *J Res Med Sci*. 2012;17(12): 1150–1155.
79. Nie J, Tong TK, Shi Q, Lin H, Zhao J, Tian Y. Serum cardiac troponin response in adolescents playing basketball. *Int J Sports Med*. 2008; 29:449–452.
80. Shave R, Baggish A, George K, et al. Exercise induced cardiac troponin elevation. *J Am Coll Cardiol*. 2010;56(3):169–176.
81. Mastaloudis A, Leonard SW, Traber MG. Oxidative stress in athletes during extreme endurance exercise. *Free Radic Biol Med*. 2001;31(7): 911–922.
82. Cazorla G, Petibois C, Bosquet L. Lactate et exercice: mythes et realites. *Rev Sci Tech Activ Phys Sport (Grenoble)*. 2001;22(54):63–76.
83. Bishop D, Girard O, Mendez-Villanueva A. Repeated-sprint ability–part II: recommendations for training. *Sports Med*. 2011;41:741–756.
84. Hurley BF, Hanson ED, Sheaf AK. Strength training as a counter measure to aging muscle and chronic disease. *Sports Med*. 2011;41:289–306.
85. Campbell PM, Allain TJ. Muscle strength and vitamin D in older people. *Gerontology*. 2006;52:335–338.
86. Ceglia L. Vitamin D and skeletal muscle tissue and function. *Mole Aspects Med*. 2008;29:407–414.
87. Ceglia L, Harris SS. Vitamin D and its role in skeletal muscle. *Calcif Tissue Int*. 2013;92:151–162.
88. Bischoff-Ferrari HA, Dietrich T, Orav EJ, et al. Dawson-Hughes, B. Higher 25-hydroxyvitamin D concentrations are associated with better lower-extremity function in both active and inactive persons aged > or =60 y. *Am J Clin Nutr*. 2004;80:752–758.
89. Foo LH, Zhang Q, Zhu K, et al. Low vitamin D status has an adverse influence on bone mass, turnover, and muscle strength in adolescent female girls. *J Nutr*. 2009;139:1002–1007.
90. Wacker M, Holick MF. Vitamin D Effects on skeletal and extra skeletal health and the need for supplementation. *Nutrients*. 2013;5:111–148.
91. Wang Y, DeLuca HF. Is the vitamin D receptor found in muscle? *Endocrinology*. 2011;152:354–363.
92. Holick MF. Vitamin D: the underappreciated D-lightful hormone that is important for skeletal and cellular health. *Curr Opin Endocrinol Diabetes*. 2002;9:87–98.
93. Sinha A, Hollingsworth K, Ball S, Cheetham T. Improving the vitamin D status of vitamin D deficient adults is associated with improved mitochondrial oxidative function in skeletal muscle. *J Clin Endocrinol Metab*. 2013;31:98(3):E509–E513.
94. Close GL, Leckey J, Patterson M, et al. The effects of vitamin D(3) supplementation on serum total 25[OH]D concentration and physical performance: a randomised dose-response study. *Br J Sports Med*. 2013; 47(11):692–696.
95. Close GL, Russel J, Cobley JN, et al. Assessment of vitamin D concentration in non-supplemented professional athletes and healthy adults during the winter months in the UK: implications for skeletal muscle function. *J Sports Sci*. 2013;31:344–353.
96. Girgis CM, Clifton-Bligh RJ, Hamrick MW, Holick MF, Gunton JE. The roles of vitamin D in skeletal muscle: form, function, and metabolism. *Endocr Rev*. 2013;34:33–83.