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
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.\(^24\)\(^-\)\(^29\) 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; 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 as well as well-defined thresholds for meaningful change in pain intensity.

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 (Granitest 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., Milpitas, CA, USA). 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.
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 1 General characteristics of subjects based on level of PA

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Physically inactive (≤500 METs min/week)</th>
<th>Moderate PA (500–2,500 METs min/week)</th>
<th>Physically active (≥2,500 METs min/week)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>25</td>
<td>25</td>
<td>35</td>
</tr>
<tr>
<td>Male/female</td>
<td>10/15</td>
<td>16/9</td>
<td>22/13</td>
</tr>
<tr>
<td>Age (years)</td>
<td>62.7±4.3</td>
<td>63.2±5.6</td>
<td>62.9±3.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.1±7.6</td>
<td>23.4±6.3*</td>
<td>18.9±4.3*</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>76.1±27.9</td>
<td>69.7±18.5</td>
<td>64.6±16.5</td>
</tr>
<tr>
<td>Hips (cm)</td>
<td>85.9±24.3</td>
<td>85.7±22.3</td>
<td>85.4±21.7</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>111.2±9.5</td>
<td>108.9±10.3</td>
<td>109.5±10.5</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>72.5±13.7</td>
<td>72.5±11.3</td>
<td>72.8±8.5</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.7±0.85</td>
<td>5.4±0.65**</td>
<td>4.8±0.75**</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>0.85±0.45</td>
<td>0.81±0.65*</td>
<td>0.8±0.41*</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.3±0.56</td>
<td>4.2±0.68*</td>
<td>3.9±0.98*</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.5±0.35</td>
<td>1.2±0.30*</td>
<td>1.0±0.28*</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>3.6±0.72</td>
<td>3.4±0.67*</td>
<td>3.0±0.61*</td>
</tr>
</tbody>
</table>

Notes: Values are expressed as mean ± SD; ***P<0.001, **P<0.01, *P<0.05. Significance at P<0.05.

Abbreviations: BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ME, metabolic equivalent; PA, physical activity; TG, triglyceride; SD, standard deviation.

### 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)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Physically inactive, N=25 (≤500 METs min/week)</th>
<th>Moderate PA, N=25 (500–2,500 METs min/week)</th>
<th>Physically active, N=35 (≥2,500 METs min/week)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH) vitamin D (nmol/L)</td>
<td>23.8±2.9</td>
<td>48.6±4.8**</td>
<td>62.3±7.61***</td>
</tr>
<tr>
<td>sTCA¹ (mmol/L)</td>
<td>1.9±0.56</td>
<td>2.50±4.4**</td>
<td>5.70±4.1***</td>
</tr>
<tr>
<td>sf-Ca² (mmol/L)</td>
<td>1.1±0.7</td>
<td>1.6±0.2**</td>
<td>2.7±0.2**</td>
</tr>
<tr>
<td>CK (IU/L)</td>
<td>309.8±7.9</td>
<td>276.3±3.7**</td>
<td>245.1±5.3***</td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td>65.3±11.6</td>
<td>48.5±7.1**</td>
<td>32.8±4.9**</td>
</tr>
<tr>
<td>Troponin I (ng/mL)</td>
<td>1.9±0.18</td>
<td>1.16±0.12**</td>
<td>0.82±0.05**</td>
</tr>
<tr>
<td>Hydroxyproline (ng/mL)</td>
<td>3.9±0.15</td>
<td>2.65±0.21**</td>
<td>1.75±0.31***</td>
</tr>
<tr>
<td>TAC (mmol/L)</td>
<td>16.5±6.3</td>
<td>22.9±11.7**</td>
<td>38.3±13.8**</td>
</tr>
<tr>
<td>VAS score</td>
<td>55.3±20.5</td>
<td>36.7±12.3**</td>
<td>22.4±9.3**</td>
</tr>
</tbody>
</table>

Notes: Values are expressed as mean ± SD; ***P<0.001, **P<0.01, *P<0.05. Significance at P<0.05.

Abbreviations: CK, creatine kinase; LDH, lactate dehydrogenase; ME, metabolic equivalent; PA, physical activity; SD, standard deviation; sf-Ca², serum-free calcium; sTCA¹, serum total calcium; TAC, total antioxidant capacity; VAS, visual analog scale.
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 prevalence of vitamin D deficiency was 17.4% among physically active subjects. Whereas, the prevalence of vitamin D deficiency was 29.4% among physically inactive subjects, the prevalence of vitamin D deficiency was 17.4% among physically active subjects.41 This also confirmed with a recent study of Ha et al42 who reported that serum vitamin D levels were positively associated with PA, including low,

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Physically inactive, N=25 (≤500 METs min/week)</th>
<th>Moderate PA, N=25 (500–2,500 METs min/week)</th>
<th>Physically active, N=25 (≥2,500 METs min/week)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>–0.28*</td>
<td>–0.2*</td>
<td>–0.18*</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>–0.011*</td>
<td>–0.14*</td>
<td>–0.21***</td>
</tr>
<tr>
<td>Hips (cm)</td>
<td>–0.07*</td>
<td>–0.25*</td>
<td>–0.59***</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>–0.014*</td>
<td>–0.27*</td>
<td>–0.31***</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>–0.041*</td>
<td>–0.59*</td>
<td>–0.72**</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>–0.09*</td>
<td>–0.085*</td>
<td>–0.067**</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>–0.011*</td>
<td>–0.017*</td>
<td>–0.021**</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>–0.006*</td>
<td>–0.015*</td>
<td>–0.031**</td>
</tr>
<tr>
<td>sTCa²⁺ (mmol/L)</td>
<td>0.042*</td>
<td>0.051*</td>
<td>0.038**</td>
</tr>
<tr>
<td>sf-Ca²⁺ (mmol/L)</td>
<td>0.016*</td>
<td>0.035*</td>
<td>0.046**</td>
</tr>
<tr>
<td>CK (UI/L)</td>
<td>–0.21*</td>
<td>–0.25*</td>
<td>–0.29**</td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td>–0.34*</td>
<td>–0.51*</td>
<td>–0.37**</td>
</tr>
<tr>
<td>Troponin I (ng/mL)</td>
<td>–0.61*</td>
<td>–0.47*</td>
<td>–0.54**</td>
</tr>
<tr>
<td>Hydroxyproline (ng/mL)</td>
<td>–0.27*</td>
<td>–0.35*</td>
<td>–0.47**</td>
</tr>
<tr>
<td>TAC (nmol/L)</td>
<td>0.32*</td>
<td>0.41*</td>
<td>0.59**</td>
</tr>
<tr>
<td>VAS score</td>
<td>–0.12*</td>
<td>–0.15*</td>
<td>–0.17**</td>
</tr>
<tr>
<td>PA score</td>
<td>0.23*</td>
<td>0.52*</td>
<td>0.71**</td>
</tr>
</tbody>
</table>

*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-Ca²⁺, serum-free calcium; sTCa²⁺, serum total calcium; TAC, total antioxidant capacity; TG, triglyceride; VAS, visual analog scale; WC, waist circumference.

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Muscle fatigue (VAS score, 0–100 mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mild (5–44)</td>
</tr>
<tr>
<td></td>
<td>$R^2$ (β)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>22.5 (0.42)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>12.1 (0.65)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>7.5 (0.25)</td>
</tr>
<tr>
<td>Vitamin D (nmol/L)</td>
<td>24.1 (0.25)</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>9.3 (0.44)</td>
</tr>
<tr>
<td>Physical activity level (MET min/week)</td>
<td>6.9 (0.25)</td>
</tr>
<tr>
<td>TAC (nmol/L)</td>
<td>3.4 (0.25)</td>
</tr>
<tr>
<td>Σ² (%)</td>
<td>85.8</td>
</tr>
</tbody>
</table>

*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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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;\textsuperscript{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.\textsuperscript{56}

Previous reports suggested that low levels of serum 25(OH)D vitamins correlated significantly with metabolic disorders,\textsuperscript{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.\textsuperscript{59} However, in clinical trials, it was difficult to link any association between the improvement in triglyceride level and vitamin D administration.\textsuperscript{60} Association of 25(OH)D with glucose regulation and type 2 diabetes has been shown in several studies.\textsuperscript{61,62} The levels of 25(OH)D vitamin and fasting glucose were shown to inversely correlate with PA profile.\textsuperscript{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\textsuperscript{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\textsuperscript{64} and low 25(OH)D has been shown to predict later development of type 2 diabetes.\textsuperscript{55}

Oxidative stress is part of metabolic process in all mammalian cells generating free radicals.\textsuperscript{65} Several investigators have studied the mechanisms by which physical exercise modulates the expression of antioxidant systems and oxidant–antioxidant relationship in many biological models.\textsuperscript{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.\textsuperscript{68} The data were in accordance with Oikonomou et al\textsuperscript{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.\textsuperscript{70,71} This adaptive response has been recognized.
both in the young as well as the middle aged and elderly. However, it has also been suggested that excessive PA may promote a generation of more oxidative free radicals than antioxidant capacity, 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.

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.

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, and that the irregularity in the concentration of muscle lactate, hydrogen (H\(^+\)), potassium (K\(^+\)), and calcium (Ca\(^{2+}\)) ions is linked with fatigue. 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. Also, it is commonly believed that the appearance of intramuscular enzymes, such as CK and LDH, besides hydroxyproline amino acids and troponin I, is the result of collagen fiber degradation either by overuse or strained muscle damage; these markers are potentially used as indicators of skeletal muscle damage. 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. 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.

In older subjects, vitamin D status was shown to play a potential significant effect on muscle weakness, pain, balance, and fractures as previously reported. 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 fatigability 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, 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, 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, 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.

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. 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.

It was shown that the presence of vitamin D in higher levels may have positive preventive effects against abnormal muscle injury and performance, whereas induction of skeletal muscle physiological response requires higher amounts of 25(OH)D levels to achieve significant muscle performance; 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.

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 con-
centrations, calcium intake, and lower obesity positively corre-
lated 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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