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ORIGINAL RESEARCH

Effects of vitamin D and quercetin, alone and in combination, on cardiorespiratory fitness and muscle function in physically active male adults

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Introduction: Vitamin D and the antioxidant quercetin, are promising agents for improving physical performance because of their possible beneficial effects on muscular strength and cardiorespiratory fitness.

Purpose: The purpose of this study was to determine the effects of increased intakes of vitamin D, quercetin, and their combination on antioxidant status, the steroid hormone regulators of muscle function, and measures of physical performance in apparently healthy male adults engaged in moderate-to-vigorous-intensity exercise training.

Methods: A total of 40 adult male participants were randomized to either 4,000 IU vitamin D/d, 1,000 mg/d quercetin, vitamin D plus quercetin, or placebo for 8 weeks. Measures of cardiorespiratory fitness and muscle function, blood markers for antioxidant and vitamin D status, and hormones 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) and testosterone were measured pre- and postsupplementation.

Results: At enrollment, 88.6% of participants were vitamin D sufficient (serum 25-hydroxyvitamin D >50 nmol/L) and had normal serum testosterone levels. Supplementation with vitamin D significantly increased serum 25(OH)D concentration (by 87.3% in the vitamin D group, $P<0.001$) and was associated with an increasing trend of testosterone concentration. There were no changes in concentration of $1,25(OH)_2D_3$ and markers of antioxidant status associated with vitamin D or quercetin supplementation. No improvements in physical performance measures associated with vitamin D and quercetin supplementation were found.

Conclusion: The findings obtained demonstrate that long-term vitamin D and quercetin supplementation, alone or in combination, does not improve physical performance in male adults with adequate vitamin D, testosterone, and antioxidant status.

Keywords: 25-hydroxyvitamin D, oxidative stress, physical performance, antioxidant status, testosterone

Introduction

Dietary supplementation in athletes is popular for the purposes of maintaining health and improving physical performance.[1,2] Combining multiple supplements such as flavonoids and vitamins will affect multiple biological targets, offering advantages over treatments with a single product. This strategy is often used in an attempt to improve physical performance.[3–7]

Supplementation with antioxidants has been suggested to improve physical performance through reducing reactive oxygen species.[8–11] Quercetin, a flavanol abundant in many commonly consumed fruits and vegetables, is one of the most potent antioxidants effective in reducing reactive oxygen species in vitro and in vivo.[12–14] However, it remains unclear whether quercetin supplementation increases antioxidant status

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or reduces oxidative stress, leading to enhanced physical performance.[4,15–18]

Interest in vitamin D supplementation has increased recently due to reports indicating that low cardiorespiratory fitness[19–22] and impaired muscular strength[23–26] in athletes are associated with low vitamin D status. A significant percentage (57%–91%) of athletes are reported to have insufficient vitamin D status.[27–29] Most commonly, vitamin D insufficiency is due to inadequate sun exposure and affects athletes who train indoors, train early morning or late evening hours, live in far northern or far southern latitudes, or have dark skin.[30]

Vitamin D_3 is a precursor to a steroid hormone, 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$).[26,31–33] Vitamin D receptors are located in numerous types of cells within the body, including those of skeletal and cardiac muscle, endothelium, and the nervous system.[26,31,33,34] In addition, vitamin D receptors are expressed in the male reproductive system and appear to influence the production of testosterone,[35,36] which can improve muscular strength.[37] The hormone $1,25(OH)_2D_3$ also functions (as other steroid hormones) via nongenomic mechanisms, including the mechanisms converging on cellular Ca^{2+} signaling.[26,38–41]

Low vitamin D status may affect skeletal and cardiac muscle because it is associated with decreased amino acid uptake, prolonged time to peak muscle contraction and relaxation, dysregulation of intracellular Ca^{2+}, muscle weakness, myalgia, impaired neuromuscular function, and hypotonia.[42,43] Supplementation with vitamin D has the potential to improve physical performance through its role in regulation of Ca^{2+} signaling in the muscle as well as biosynthesis of the muscle contractile proteins.[33,39,44,45] Several authors studied the association of vitamin D status with physical performance, but results were inconclusive.[46–49] It is interesting to note that, similar to quercetin, vitamin D has been shown to reduce oxidative stress.[50] Moreover, vitamin D has anti-inflammatory properties,[51] and vitamin D status was observed to be inversely associated with inflammation in runners.[52]

It is possible that supplementation with vitamin D and quercetin could improve physical performance through the direct and indirect effects in reducing oxidative stress and increasing production of hormones – testosterone and $1,25(OH)_2D_3$ – involved in regulation of muscle function; the combined supplementation could be more effective because it will target multiple pathways for improving muscle functioning.

This study was undertaken to determine whether dietary supplementation with vitamin D_3 and quercetin, alone or in combination, improves physical performance in male adults. We hypothesized that vitamin D_3 and quercetin supplementation will increase the steroid hormone regulators of muscle function (testosterone, $1,25(OH)_2D_3$), improve antioxidant status, and reduce oxidative stress, resulting in improved physical performance. The results obtained do not support these hypotheses: supplementation with vitamin D and quercetin did not influence steroid hormone regulators of muscle function and antioxidant status, resulting in no improvements in physical performance.

Materials and methods

Participants

A total of 40 physically active males volunteered to participate after preliminary screening and explanation of all procedures. Written informed consent was obtained from all participants in accordance to the experimental procedures approved by the University of South Dakota (Institutional Review Board [IRB] Protocol number: 2012.098), South Dakota State University (IRB Protocol number: IRB-1205004-EXP), and University of Sioux Falls (IRB Protocol number: E-02) Institutional Review Boards. Following consent, all volunteers completed a medical history, an exercise history, and a Physical Activity Readiness Questionnaires (PAR-Q). Volunteers were included if they met American College of Sports Medicine's (ACSM's) standards for fitness (moderate to vigorous physical exercise 3–5 days per week for 30–90 min/d), exercised primarily indoors, and were males between the age of 25 and 45. Volunteers were excluded if they used indoor tanning and had two or more of the following ACSM risk factors: family history of heart disease, cigarette smoking, hypertension, dyslipidemia, impaired fasting glucose, obesity, or sedentary lifestyle. Other exclusions included supplementation with vitamin D, quercetin, testosterone, and prescription medications, such as anticoagulants or antibiotics.

The participants were instructed to maintain their current training program and not alter the frequency, intensity, duration, or mode of exercise during the study. Participants were also instructed to maintain their dietary habits, record 24-hour diet analysis pretesting to consume the same foods posttesting, and refrain from any supplementation. Study personnel contacted participants weekly to record physical activity and to monitor adherence.

Participants were randomly assigned in a double-blind manner to the vitamin D_3 (4,000 IU/d), quercetin (1,000 mg/d), vitamin D_3 plus quercetin (4,000 IU/d plus 1,000 mg/d, respectively), or placebo groups. The

"plotblockrand" function in R 3.0.2 was used to create the randomization cards used when assigning participants to the groups. Treatment started after the completion of the fourth laboratory visit and continued for 8 weeks (Figure 1).

Supplementation

The quercetin supplement contained 1,000 mg of quercetin (PureBulk, Roseburg, OR, USA). This amount was based on the previous research regarding safety, bioavailability, and human plasma quercetin concentrations achieved with supplementation.[15] The vitamin D supplement (PureBulk) contained 4,000 IU of vitamin D_3 (cholecalciferol), which is the recommended tolerable upper intake level (UL) for this vitamin.[53] The supplier provided certificates of analysis for quercetin and vitamin D_3. The supplements were also tested and certified by an independent laboratory (Novatia, Monmouth Junction, NJ, USA) (quercetin, 96.7%; vitamin D_3, 94.3% by liquid chromatography/mass spectroscopy). Microcrystalline cellulose (Professional Compunding Centers of America, Houston, TX, USA) was used as a placebo. All supplements were in powder form, dyed for identical color, and packaged in visually identical capsules and containers (Pharmacy Specialties, Sioux Falls, SD, USA).

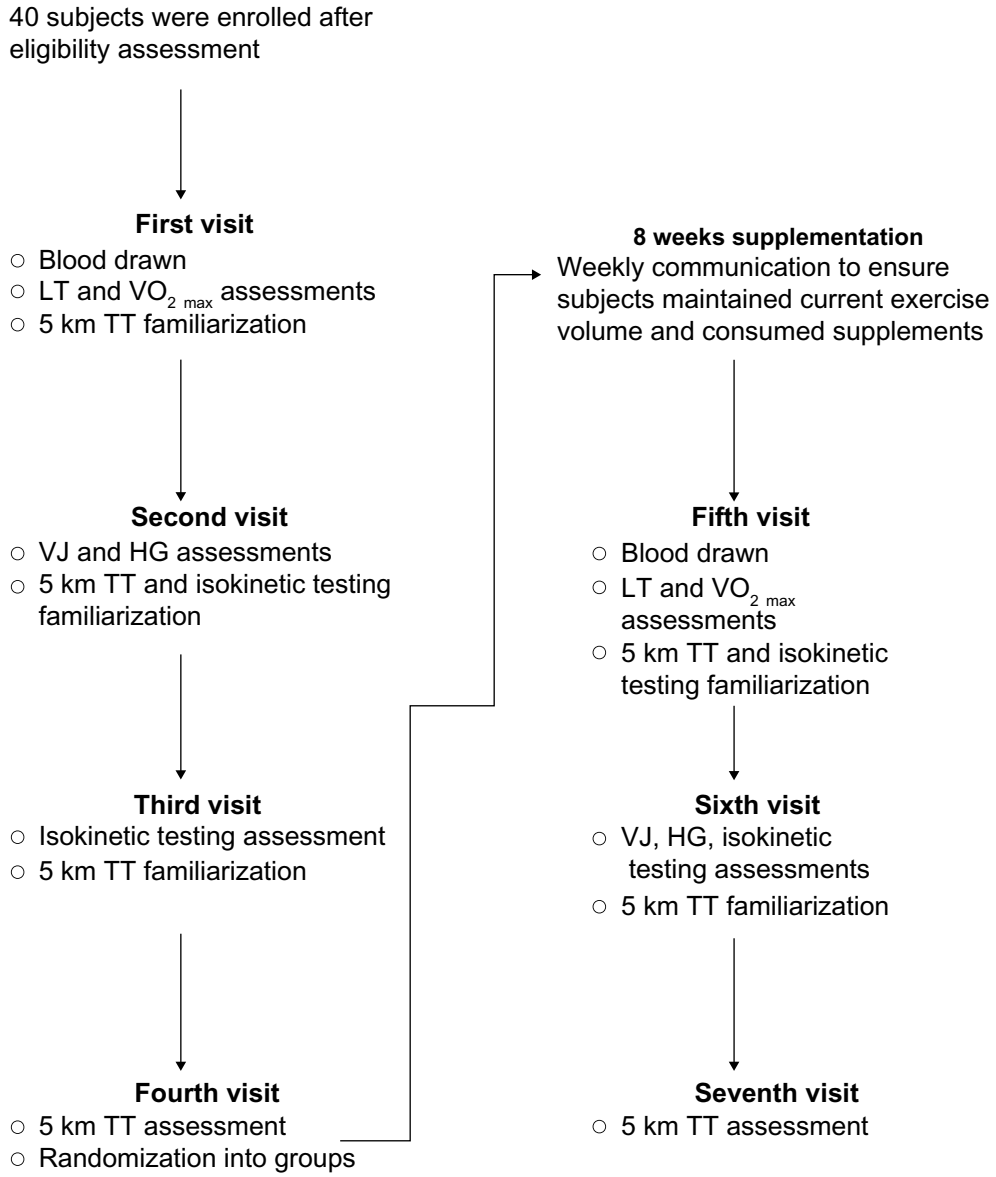

Figure 1 Methods schematic.
Notes: The time between visits was at least 24 hours to allow for recovery. Participants completed testing in seven total visits, with 8 weeks of supplementation separating the pre- and posttesting period.
Abbreviations: LT, lactate threshold; $VO_{2\ max}$, maximal oxygen consumption; 5 km TT, 5 km time trial; VJ, vertical jump; HG, hand grip strength.

Cardiorespiratory fitness

$VO_{2\ max}$

$VO_{2\ max}$ (maximal oxygen consumption) (aerobic capacity) was determined for all the participants using a previously published graded protocol[54] on a motorized treadmill (TrackMaster Model TMX325C), with expired gases analyzed using a MedGraphics CPX/D automated metabolic cart (St Paul, MN, USA).

Lactate threshold

After a 5-minute warm-up, the initial treadmill speed was set according to the participant's fitness and increased by 1 km/h, between each of the 3-minute successive stages. Each stage was separated by a 30-second period of rest, during which a fingertip capillary blood sample was collected and analyzed using a Lactate Pro blood lactate test meter (Arkray Inc., Kyoto, Japan). Lactate threshold (LT) was defined using the running speed at which an abrupt increase in blood lactate occurred.[55]

5 km time trial

Participants were habituated with the 5 km time trial (5 km TT) by including three familiarization sessions prior to the test performance. The 5 km TT was performed in an airconditioned laboratory (temperature: 18.0°C±1.2°C) using a TrackMaster treadmill, with a fan positioned 1 m in front of the subject to provide cooling. Participants started with a 5-minute warm-up followed by stretching. Before the 5 km TT, participants were instructed to complete the 5 km TT in the least amount of time as possible (ie, to race the 5 km TT). During the 5 km TT, participants were allowed to adjust the treadmill speed as desired. Participants were given feedback with respect to distance and time elapsed, but were not allowed to know their running speed. Participants were informed of their final 5 km TT time at completion. Water consumption was encouraged and allowed ad libitum. Exercise heart rates were measured using a wireless transmitter system (Polar Electro Inc., Woodbury, NY, USA).

Muscle function

Hand grip strength

The JAMAR hand dynamometer (Lafayette Instrument, Lafayette, IN, USA) was used to measure grip strength. The test was performed with the subject in a seated position using the dominant hand, with the shoulder adducted, elbow flexed at 90°, and a neutral wrist position. Participants were instructed to squeeze as hard as possible. This was repeated three times with a 60-second rest between each repetition. The mean value of the repeated measures was reported.

Vertical jump

Vertical jump (VJ) height was assessed using the VERTEC (Questtek Corp, Northridge, CA, USA). The height of the VERTEC was adjusted in accordance with the manufacturer's guidelines. Participants were given three attempts to reach their maximum height using counter movement jump. The highest of the three trials was recorded.

Isokinetic leg strength

Isokinetic knee extension (IKE) and isokinetic knee flexion (IKF) of the dominant limb were determined using a computerized dynamometer in an isokinetic mode (Biodex Multi-joint System 3, Shirley, NY, USA), using a previously published protocol.[56] Following warm-up, participants performed five repetitions of knee flexion and extension at angular velocities of 60°/s, 120°/s, and 300°/s, with a 3-minute rest between each set. Corrections were made for gravity.[56] Participants were given one familiarization session 24–48 hours before testing. The maximum torque produced in a single repetition was used as the measure of strength.

Markers of antioxidant, vitamin D, and testosterone status

Blood samples were drawn into tubes with no additives, serum was aliquoted, immediately frozen, and stored at –80°C until analysis. The following kits were used: 25-hydroxyvitamin D ELISA (enzyme-linked immunosorbent assay) (Immunodiagnostics Systems, Scottsdale, AZ, USA), $1,25(OH)_2$ D ELISA (Immunodiagnostics Systems), testosterone ELISA (ALPCO Immunoassays, Salem, NH, USA), Total Antioxidant Capacity Assay, OxiSelect Superoxide Dismutase Activity Assay, OxiSelect MDA Adduct ELISA, OxiSelect Protein Carbonyl ELISA (Cell Biolabs, Inc., San Diego, CA, USA). The kits for measuring vitamin D metabolites employ highly specific 25(OH)D and $1,25(OH)_2D_3$ antibodies. Solid-phase monoclonal anti-$1,25(OH)_2D_3$ was used for immunoextraction of $1,25(OH)_2D_3$ prior to detection of the hormone by ELISA.

Statistical analysis

The participants were randomized into four groups: vitamin D_3 (D, n=14), quercetin (Q, n=6), vitamin D_3 plus quercetin (D + Q, n=6), and placebo (P, n=14). A multiple ANOVA with interactions was used to compare differences between supplementation groups. A Tukey post hoc test was run for

significant tests. Significance for all comparisons was set at $P<0.05$. This data analysis allowed group comparisons for individual assays and performance measures. All data were analyzed using R version 3.0.2.

Results

Subject description and assessment

Around 40 participants were recruited for the study during winter of 2013 (January–February), with follow-up occurring 8 weeks after enrollment. One of these participants was excluded because of not meeting training requirements during the 8-week time period. Following analysis of presupplementation data, four participants (three from the D group, one from the P group) were removed from statistical analysis as outliers because of high serum testosterone concentrations (defined as significantly outside the normal range of 4.5–28.0 nmol/L; the values were confirmed as outliers using interquartile range [IQR] test, R 3.0.2). Statistical analysis was performed with and without the participants with high testosterone level. The exclusion of these participants did not change the significance or nonsignificance of results for any parameter. Final statistical analysis was completed using 35 participants (P, n=12; D, n=11; Q, n=6; Q + D, n=6). Upon enrollment, 31/35 (88.6%) had 25(OH)D concentration >50 nmol/L and 9/35 (25.7%) had 25(OH)D concentration >75 nmol/L. The serum 25(OH)D concentration increased by 58.7±30.5 nmol/L in the D group and by 31.0±14.0 nmol/L in the D + Q group at the end of supplementation period (4,000 IU/d of vitamin D_3 for 8 weeks). Descriptive statistics of the participants are presented in Table 1. The age, height, and weight of the participants ranged from 25 to 42 years, 172.7 to 193.0 cm, and 65.9 to 108.2 kg, respectively. At the completion of the study, ethnic groups were 97% white and 3% black, which is similar to the local population demographics. Data collection was performed in Sioux Falls, SD, USA (latitude: 43.5°N).

Table 1 Subject characteristics

Group	Age (yr)	Height (m)	BW (kg)	BMI (kg/m^2)	Physical activity (h/d)
Placebo	29.9±1.5	1.85±0.05	90.0±4.7	26.2±4.5	4.1±0.4
Vitamin D	32.8±1.7	1.83±0.12	78.8±2.3	23.4±1.8	4.5±1.8
Quercetin	30.3±1.4	1.87±0.08	90.9±8.4	25.9±1.9	3.3±0.6
Vitamin D + quercetin	32.2±3.7	1.82±0.08	76.1±5.7	23.1±3.2	4.0±0.5

Notes: There were no significant differences ($P<0.05$) between groups for any variables. Values are mean ± SEM.

Abbreviations: BW, body weight; BMI, body mass index; SEM, standard error of mean; yr, year; h/d, hour/day.

Cardiorespiratory fitness and muscle function

Physical performance outcomes are presented in Tables 2 and 3. The treatment groups were well-matched at the baseline for all outcome measures except $VO_{2\ max}$ (the Q group had somewhat lower average $VO_{2\ max}$ values, but there were no significant changes of $VO_{2\ max}$ in this group from pre- to postsupplementation). There were no significant changes in physical performance measures (VJ, HG [hand grip strength], IKF, IKE, $VO_{2\ max}$, 5 km TT, and LT) between any groups from pre- to postsupplementation. Isokinetic muscular performance outcomes are presented in Table 2. At baseline, the vitamin D group tended to have smaller torque values than other groups, but this difference was not statistically significant for any rotational joint speed. As expected, torque values decreased with increasing knee flexion and extension velocities.

Markers of antioxidant, vitamin D, and testosterone status

Figures 2 and 3 show serum markers of the vitamin D, testosterone, and antioxidant status before and after supplementation with vitamin D_3 and quercetin. At baseline, no statistically significant differences in the vitamin D status (serum 25(OH)D concentration) and the steroid hormone regulators of muscle function (serum testosterone and $1,25(OH)_2D_3$) were detected between the groups. As expected, supplementation with vitamin D_3 resulted in a significant increase in serum 25(OH)D concentration in the D (by 87.3%, $P<0.001$) and D + Q groups (by 35.5%, $P=0.045$) (Figure 2A). It is important to note that an increase in 25(OH)D in the D and D + Q groups was not accompanied by significant increases in physical performance (as measured by VJ, HG, IKF, IKE, $VO_{2\ max}$, 5 km TT, and LT). The concentration of the hormonal form of vitamin D, $1,25(OH)_2D$, remained within the narrow normal physiological range in all groups (Figure 2B).

There were no statistically significant changes in the testosterone concentration from pre- to postsupplementation (Figure 2C). Specifically, the testosterone concentration was higher by 3.7% in the P group ($P=0.834$), 41.2% in the D group ($P=0.216$), 0% in the Q group ($P=0.998$), and 3.4% in the D + Q group ($P=0.849$). These findings imply a strong increasing trend of testosterone concentration associated with vitamin D_3 supplementation in participants with the normal initial testosterone levels. When participants with abnormally high baseline testosterone levels were included in statistical analysis, there was only 1.7% increase in the D group

Table 2 The muscle performance outcomes

	Presupplementation	Postsupplementation	*P*-value
Knee flexion 60°/s (Nm)			
Placebo	119.98±8.93	120.30±8.01	0.948
Vitamin D	103.08±6.85	107.26±5.15	0.632
Quercetin	117.96±12.65	114.74±13.06	0.863
Vitamin D + quercetin	109.73±11.35	117.73±9.53	0.601
Knee extension 60°/s (Nm)			
Placebo	213.53±18.02	209.58±22.15	0.901
Vitamin D	204.78±14.66	209.41±13.61	0.786
Quercetin	241.51±28.98	239.13±30.22	0.956
Vitamin D + quercetin	213.34±25.22	231.84±22.19	0.594
Knee flexion 120°/s (Nm)			
Placebo	100.13±7.27	103.65±7.67	0.732
Vitamin D	96.49±4.85	98.36±4.26	0.789
Quercetin	105.59±11.27	104.51±10.78	0.946
Vitamin D + quercetin	106.77±11.14	108.02±10.17	0.936
Knee extension 120°/s (Nm)			
Placebo	193.04±14.10	195.96±14.79	0.829
Vitamin D	173.61±11.23	180.97±10.36	0.623
Quercetin	200.59±20.68	211.23±25.75	0.754
Vitamin D + quercetin	199.60±21.16	208.57±18.81	0.758
Knee flexion 300°/s (Nm)			
Placebo	75.38±5.78	79.74±5.54	0.584
Vitamin D	70.73±3.89	73.46±3.86	0.704
Quercetin	72.77±6.22	80.29±6.58	0.425
Vitamin D + quercetin	81.62±8.12	81.34±8.34	0.982
Knee extension 300°/s (Nm)			
Placebo	129.11±7.90	131.96±8.23	0.748
Vitamin D	119.51±6.95	123.59±6.49	0.598
Quercetin	138.67±14.33	142.88±15.90	0.848
Vitamin D + quercetin	136.69±12.72	138.97±9.98	0.890
Vertical jump (cm)			
Placebo	54.29±2.96	56.09±2.81	0.668
Vitamin D	52.76±2.05	54.38±1.90	0.631
Quercetin	56.52±3.92	56.52±4.05	0.999
Vitamin D + quercetin	67.31±5.58	72.14±6.37	0.581
Hand grip strength (N)			
Placebo	506.3±29.1	534.1±28.7	0.477
Vitamin D	516.6±18.9	516.6±15.3	0.841
Quercetin	589.2±63.8	590.0±53.3	0.992
Vitamin D + quercetin	547.5±54.9	580.6±43.5	0.658

Notes: The duration of the supplementation period was 8 weeks. Concentric isokinetic knee flexion and extension were tested at 60°/s, 180°/s, and 300°/s. There were no significant changes ($P<0.05$) between any variable from pre- to postsupplementation. Values are mean ± SEM.
Abbreviation: SEM, standard error of mean.

Table 3 The cardiorespiratory performance outcomes

	Presupplementation	Postsupplementation	*P*-value
$VO_{2\ max}$ (mL/(kg·min))			
Placebo	50.21±2.56	49.92±2.27	0.943
Vitamin D	56.46±2.93	55.77±2.66	0.849
Quercetin	43.56±1.65	42.70±1.79	0.733
Vitamin D + quercetin	50.45±1.71	51.97±1.96	0.572
5 km time trial (minute)			
Placebo	27.25±1.32	26.46±1.29	0.711
Vitamin D	23.71±1.50	23.32±1.46	0.809
Quercetin	28.82±2.52	28.74±2.40	0.983
Vitamin D + quercetin	26.28±1.98	25.87±2.32	0.895
Lactate threshold (km/h)			
Placebo	10.70±0.45	11.15±0.34	0.512
Vitamin D	12.02±0.72	12.28±0.73	0.550
Quercetin	10.06±0.77	10.14±0.82	0.945
Vitamin D + quercetin	10.68±0.46	11.15±0.52	0.513

Notes: There were no significant changes ($P<0.05$) between any variable from pre- to postsupplementation. There was a significant difference at presupplementation for $VO_{2\ max}$ between the Q and P groups ($P=0.034$); however, there was no significant change in $VO_{2\ max}$ from pre- to postsupplementation in the Q and P groups. Values are mean ± SEM.
Abbreviations: $VO_{2\ max}$, maximal oxygen consumption; SEM, standard error of mean; Q, quercetin; P, placebo.

(*P*=0.963), 2.5% in the P group (*P*=0.949), 0% in the Q group (*P*=0.998), and 3.4% in the D + Q group (*P*=0.849).

The evaluation of antioxidant staus revealed significant variations in total antioxidant capacity (TAC) in serum, including a decrease in TAC values for all the treatment groups and the placebo group from pre- to postsupplementation (Figure 3A). This may indicate that a relatively intensive long-term training program depletes antioxidant capacity and that the quercetin and vitamin D supplementations are not effective in preventing this decrease in TAC. The superoxide dismutase (SOD) activity in serum, which can serve as a marker of oxidative stress by indicating a release of the enzyme from the damaged tissues,[57] was increased in the P group at the end of the evaluation period (Figure 3B). The combined vitamin D_3 plus quercetin supplementation (the D + Q group) resulted in a dramatic decrease (71.7%, $P<0.001$) of serum SOD, possibly implying a protective antioxidant effect of the supplementation. No significant differences in lipid peroxidation (MDA [malondialdehyde]) and protein oxidation (protein carbonyl) were found between groups (Figure 3C and D), although a trend to the normalization (decrease) of postsupplementation MDA values and a significant decrease of the protein carbonyl postsupplementation values (by 19.3%, $P=0.023$) were observed in the D + Q group. Taken together, these findings imply that supplementation with vitamin D_3 plus quercetin can be moderately effective in reducing chronic oxidative stress associated with a high level of long-term physical activity. However, no significant changes in physical performance between groups that can be associated with vitamin D_3 and quercetin supplementation were observed.

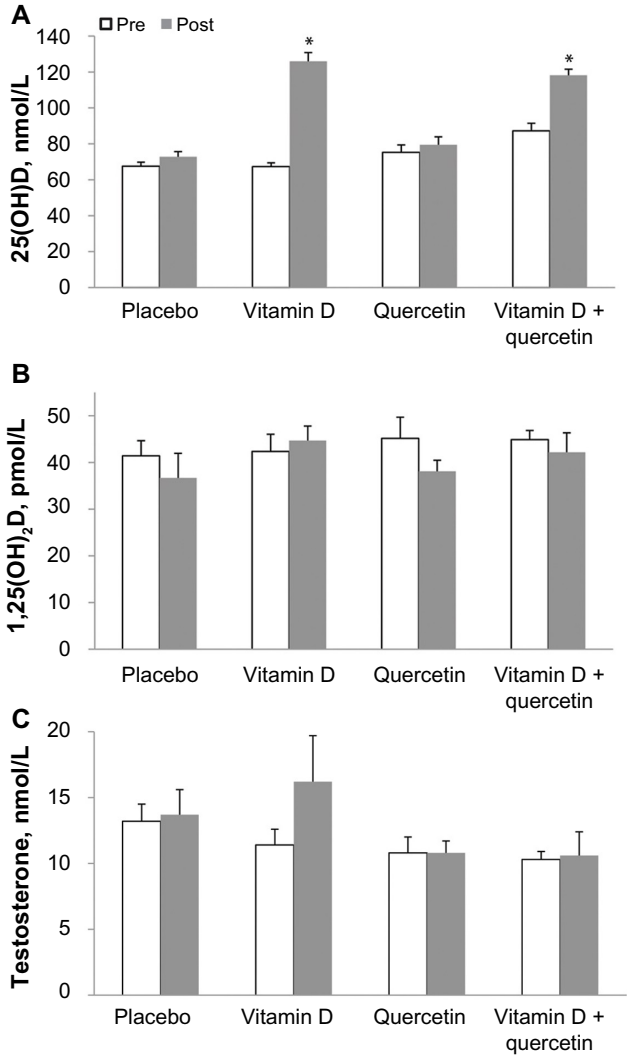

Figure 2 Concentrations of 25(OH)D, $1,25(OH)_2D$, and testosterone in serum.
Notes: (**A**) Vitamin D_3 supplementation significantly increased serum 25(OH)D concentration in the vitamin D_3 ($P<0.001$) and D + Q ($P=0.045$) groups. (**B**) No pre- to postsupplementation changes in $1,25(OH)_2D$ concentration were observed. (**C**) Testosterone concentration increased by 41.2% ($P=0.216$) with vitamin D_3 supplementation. Values are mean ± SEM. The asterisk indicates significant difference from pre- to postsupplementation ($P<0.05$).
Abbreviations: $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; D + Q, vitamin D + quercetin; SEM, standard error of mean.

Discussion

The aims of this study were to evaluate the effects of vitamin D and quercetin supplementation on the cardiorespiratory and muscular fitness outcomes and determine the possible mechanisms of these effects, converging on the steroid hormone regulators of muscle function and linked to maintaining antioxidant status. The main findings obtained demonstrate that long-term supplementation of physically active male adults with vitamin D_3 alone, quercetin alone, or their combination does not increase physical performance and does not produce significant changes in markers associated with antioxidant status and hormones (testosterone, $1,25(OH)_2D_3$) involved in regulation of the muscle function.

The Institute of Medicine recommends serum 25(OH)D concentration >50 nmol/L as adequate for bone and overall health in healthy adult individuals.[58] Our findings indicate that 31 of 35 (88.6%) physically active male adults were vitamin D sufficient (25(OH)D >50 nmol/L) during winter months at a northern latitude (43.5°N). The Endocrine Society defines vitamin D sufficiency as serum 25(OH)D concentration >75 nmol/L.[59] At this level, 9 of 35 (25.7%) study participants had sufficient vitamin D status. Several earlier studies indicate a wide range (9%–43%) of vitamin D sufficient status (defined as 25(OH)D concentration >50 nmol/L) in athletes.[27–29,60,61]

Based on previous studies, we expected that vitamin D_3 supplementation with 4,000 IU/d would increase serum 25(OH)D concentration by approximately 50 nmol/L and peak after 4–5 weeks of supplementation.[62–64] This statistically significant increase was achieved in the vitamin D_3 group but not in the vitamin D_3 plus quercetin group, possibly implying an antagonistic effect of quercetin supplementation on vitamin D status. Sufficient vitamin D status and normal $1,25(OH)_2D$ concentration at presupplementation may explain the failure of vitamin D_3 supplementation to increase physical performance in this study. The circulating $1,25(OH)_2D$ concentration is homeostatically regulated and is not decreased until 25(OH)D drops below 25 nmol/L or even significantly less ("undetectable").[31,45] $1,25(OH)_2D$ concentration in all participants of this study was within the normal and narrow range of physiological values (approximately 40 pmol/L).

Previous studies have shown a positive association between serum 25(OH)D and testosterone.[36,65,66] We found a nonsignificant increase in testosterone level with an increase in serum 25(OH)D. Despite an increasing trend in testosterone levels with vitamin D supplementation, physical performance outcomes remained unchanged. A much larger increase in testosterone concentration may be necessary for improved physical performance.[67] Additionally, the healthy young males of this study responded to vitamin D_3 supplementation with only limited increase in testosterone concentration, probably because they have already achieved a high testosterone status associated with an increased physical activity and a decreased body mass index.[68]

The observed increase in serum 25(OH)D concentration was not accompanied by significant changes in muscular performance. These results are consistent with meta-analyses performed using older adults.[46,47] Although an increase in proximal lower limb muscle strength with vitamin D supplementation in vitamin D deficient (<25 nmol/L) elderly participants was demonstrated in one study,[69] we did not find

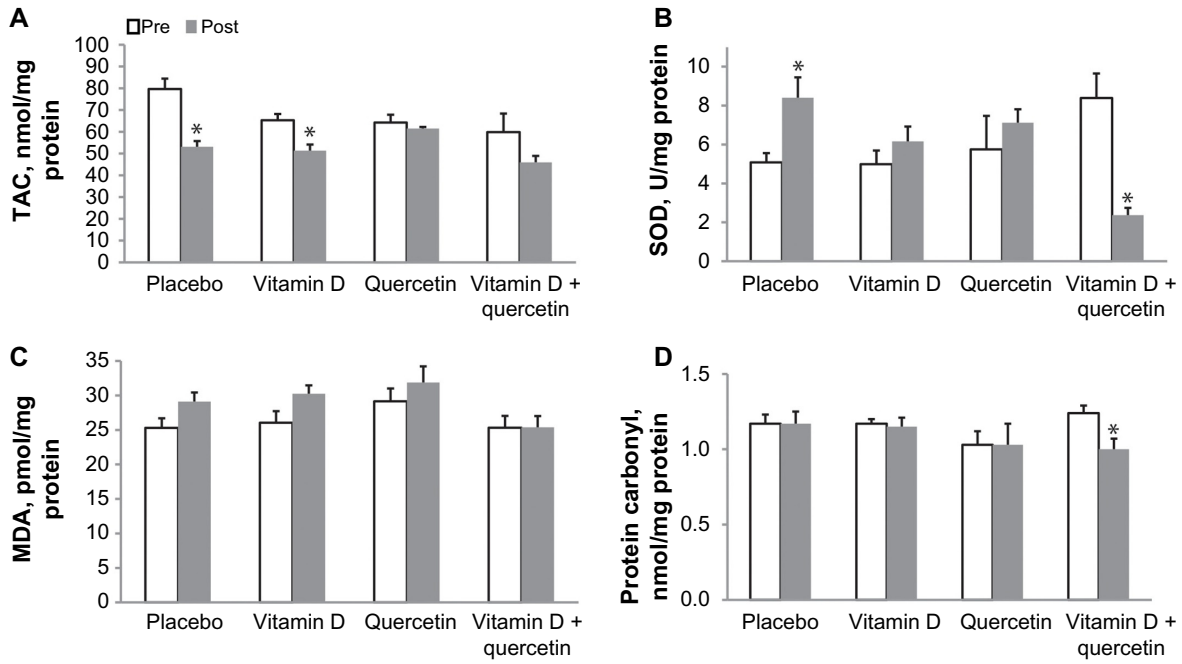

Figure 3 Markers of antioxidant status in serum.

Notes: (**A**) TAC values were decreased from pre- to postsupplementation in the placebo ($P<0.001$), vitamin D_3 ($P=0.002$), and D + Q ($P=0.155$) groups. (**B**) SOD activity was significantly increased in the placebo group ($P=0.009$) and significantly decreased in the D + Q group ($P<0.001$) group, possibly implying a protective antioxidant effect of the combined supplementation. (**C**) No significant changes in MDA levels (lipid peroxidation) were found. (**D**) A significant decrease in the protein carbonyl level (protein oxidation) was found for the D + Q ($P=0.023$) group, possibly implying a protective antioxidant effect of the combined supplementation. Values are mean ± SEM. The asterisk indicates significant difference from pre- to postsupplementation ($P<0.05$).

Abbreviations: TAC, total antioxidant capacity; SOD, superoxide dismutase; D + Q, vitamin D + quercetin; MDA, malondialdehyde; SEM, standard error of mean.

any improvement in muscular strength of vitamin D sufficient (>50 nmol/L) healthy adult males. These differences may be due to a young age and a sufficient baseline serum 25(OH)D levels (>50 nmol/L) of participants in our study.

Quercetin supplementation has been associated with improved cardiorespiratory fitness, possibly through its role as an antioxidant. Davis et al[70] reported significant improvements in time to fatigue and $VO_{2\,max}$ in nonathletic individuals supplemented with 500 mg/d of quercetin for 7 days, indicating the potential importance of quercetin for enhancing aerobic performance. We previously reported that supplementation of trained participants with 1,000 mg/d of quercetin for 6 weeks tends to increase $VO_{2\,max}$, suggesting a slight improvement in cardiorespiratory fitness through reducing oxidative stress.[18]

In this study, quercetin and vitamin D supplementation did produce significant changes in antioxidant status. However, these biomarker changes were not in parallel with one another limiting the strength of these findings.[71] Furthermore, these changes were not significant enough to enhance physical performance ($VO_{2\,max}$, 5 km TT, or LT), possibly due to the high initial antioxidant status of the participants. These null findings in trained participants are similar to two other studies that reported no effects on several measures of oxidative stress and antioxidant capacity with quercetin supplementation for 6–12 weeks.[72,73]

An association between vitamin D staus and cardiorespiratory fitness ($VO_{2\,max}$) in healthy adults has been reported.[19,21] The current investigation assessed the effect of vitamin D_3 supplementation on outcomes of cardiorespiratory fitness, but did not find a significant increase in $VO_{2\,max}$, 5 km TT, or LT with a significant increase in vitamin D status. The participants in this study were young, physically fit, vitamin D sufficient males, with a body mass index of 25.3 and $VO_{2\,max}$ of 51.8 mL/(kg·min). These factors could limit the potential beneficial effects of vitamin D supplementation. It is possible that a higher (>4,000 IU/d) intake of vitamin D_3 further increases 25(OH)D concentration and could result in an improved physical performance, as found in two studies using athletic populations.[60,74,75] However, it was not possible for us to supplement with >4,000 IU/d, as we were restricted by the Institutional Review Board from exceeding the Upper Limit set by the Institute of Medicine due to concerns of toxicity. Finally, previous evidence suggests quercetin supplementation has a small effect size for improving performance. Our study may have been underpowered to find such an effect. However, the addition of vitamin D should probably have strengthened this effect.

It would have been interesting to have utilized an athletic team which would have the advantages of a similar training plan, performance status, age, and meal plan for all subjects. Additionally, future approaches could incorporate greater bouts of intensity to elicit a greater amount of oxidative stress, recruitment of vitamin D deficient (<30 nmol/L) subjects incorporating an adequate repletion regiment, and increased supplementation amounts of quercetin or vitamin D with or without other flavonoids. Finally, a study designed to improve measures of fitness would investigate the added training effects of supplementation with quercetin or vitamin D.

Conclusion

The results obtained demonstrated that vitamin D_3 supplementation at a tolerable UL (4,000 IU/d) for 8 weeks significantly increases vitamin D status (serum 25(OH) D concentration). However, vitamin D_3 alone, quercetin alone, and their combination did not produce significant changes in the markers associated with antioxidant status or the steroid hormone regulators of muscle function and did not influence physical performance outcomes in physically active males. These findings indicate that long-term vitamin D and quercetin supplementation do not improve physical performance in male adults with adequate vitamin D, testosterone, and antioxidant status and do not support the supplementation with vitamin D and quercetin, singularly or in combination, for enhancing physical performance in physically active male adults.

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Author contributions

The sequence of authors reflects their relative contributions. Conceived and designed the study: SDS and INS; acquired and interpreted the data: all authors; carried out biomarkers assays: QS; performed statistical analysis: CBB; wrote manuscript: SDS and INS; critical revision and approval of the final version of the manuscript: all authors.

Disclosure

The authors report no conflicts of interest in this work.

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