The Effect of Vitamin D Supplementation on Serum Total 25(OH)D Levels and Biochemical Markers of Skeletal Muscles in Runners Vitamin D Supplementation in Marathon Runners

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Abstract:	The study aimed to evaluate the effects of a 3-week vitamin D supplementation on serum 25(OH)D levels and skeletal muscle biomarkers (i.e. troponin, myoglobin, creatine kinase and lactic dehydrogenase) of endurance runners. Twenty-four runners were examined at baseline and in response to eccentric exercise before and after two dietary protocols (dose of 2000 IU for three weeks or placebo). Significant differences between pre- and post-intervention in 25(OH)D levels were observed (36.1±6.0 versus 40.0±5.2 ng/ml, p <0.05). A higher post intervention 25(OH)D level was observed after vitamin D diet compared to placebo (40.0±5.2 versus 31.8±4.2 ng/mL, respectively; p <0.01). The vitamin D supplementation decreased 1 h and 24 h post-exercise troponin (p <0.05, p <0.01, respectively), myoglobin concentration (p <0.05, p <0.01, respectively) and 24 h post exercise creatine kinase (CK) activity (p <0.01). A negative correlation was observed between post exercise 25(OH)D levels and myoglobin levels (r=-0.57; p <0.05), 25(OH)D levels and CK (r=-0.60; p <0.05), and 25(OH)D levels and TNFα (r=-0.58; p <0.05). These findings suggested that an increase in 25(OH)D release in response to vitamin D supplementation attenuated the muscle biomarker levels following eccentric exercise and might play a key role in prevention of skeletal muscle injury.
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Is this study a clinical trial?<hr>A clinical trial is defined by the World Health Organisation as 'any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes'.	No

The Effect of Vitamin D Supplementation on Serum Total 25(OH)D Levels and Biochemical Markers of Skeletal Muscles in Runners

Vitamin D Supplementation in Marathon Runners

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Abstract

The study aimed to evaluate the effects of a 3-week vitamin D supplementation on serum 25(OH)D levels and skeletal muscle biomarkers (*i.e.* troponin, myoglobin, creatine kinase and lactic dehydrogenase) of endurance runners. Twenty-four runners were examined at baseline and in response to eccentric exercise before and after two dietary protocols (dose of 2000 IU for three weeks or placebo). Significant differences between pre- and post-intervention in 25(OH)D levels were observed (36.1±6.0 *versus* 40.0±5.2 ng/ml, $p<0.05$). A higher post intervention 25(OH)D level was observed after vitamin D diet compared to placebo (40.0±5.2 *versus* 31.8±4.2 ng/mL, respectively; $p<0.01$). The vitamin D supplementation decreased 1 h and 24 h post-exercise troponin ($p<0.05$, $p<0.01$, respectively), myoglobin concentration ($p<0.05$, $p<0.01$, respectively) and 24 h post exercise creatine kinase (CK) activity ($p<0.01$). A negative correlation was observed between post exercise 25(OH)D levels and myoglobin levels (r=-0.57; $p<0.05$), 25(OH)D levels and CK (r=-0.60; $p<0.05$), and 25(OH)D levels and TNFα (r=-0.58; $p<0.05$). These findings suggested that an increase in 25(OH)D release in response to vitamin D supplementation attenuated the muscle biomarker levels following eccentric exercise and might play a key role in prevention of skeletal muscle injury.

Key words: vitamin D; muscle biomarkers; eccentric exercise; fatigue; marathon.

Introduction

Strenuous exercise has been associated with adaptive changes in skeletal muscle, such as an ability to use oxygen to generate energy for muscle work, a decrease in oxygen demand for the same level of external work performed, as well as an improvement of mechanisms towards decreased exercise-induced muscle damage [1]. In a recent study, a prevalence of vitamin D deficiency in extreme endurance athletes, and an association between delayed physical performance and the deficiency in vitamin D were observed during regular training [2-4]. These physiological responses in muscles were influenced by exercise-induced mechanisms and were probably affected by nutritional athletic status and limitation of sun exposure [2, 5-7].

Long distance running has been shown to induce progressive increase of neuromuscular function and adaptive changes in cardiovascular, as well as immune and endocrine systems [8-11]. The potential mechanisms - through which function of the muscular system might be beneficially modified in response to extreme repeated exercise stress - included improvement of vitamin D status [4]. Several studies supported the theory that functional responses in skeletal muscle were influenced by mechanisms that could be affected by biological effects of an active form of vitamin D and its ability to bind with the membrane and nuclear vitamin D receptors (VDRs) [11, 12]. Besides the importance of vitamin D, especially 25(OH)D (serum 25-hydroxy vitamin D), in the regulation of bones and calcium homeostasis, it was also involved in skeletal muscle performance and in exercise-induced inflammatory processes, neurological functions and cardiovascular health [7, 13-15]. It should be noted that muscle power and force in marathon runners were linked with vitamin D levels [16]. The deficiency in vitamin D increased the risk of muscle myopathy, and impaired cross-bridge formation leading to muscle weakness and fatigue [17-19]. Due to the higher

levels of biomarkers of muscle injury and reduction of total antioxidant capacity and muscle function in response to extreme exercise training, strategies should be developed to maintain an optimal vitamin D level in response to its exercise-induced deficiency. It has been hypothesized that higher exposure to vitamin D - producing ultraviolet light and serum 25(OH)D levels above the normal reference range (up to 50 ng/mL) - could be associated with beneficial adaptations in skeletal muscle consisting of enhanced aerobic performance, both force and power production and decreased recovery time from training [20].

The physiological consequence of intense physical training in response to vitamin D supplementation induced by activation of the serum 25(OH)D status depended on the dosages exceeding the recommendations for vitamin D [21-24]. In elite rowers, maximal oxygen uptake increased significantly in response to supplementation with 6000 IU/day of vitamin D during 8-weeks training, whereas, the dosage of 4000 IU/day for 35 days of vitamin D improved the recovery by the attenuation of the inflammation processes in moderately active adults [25]. Positive effects of supplementation (8 weeks of 5000 IU/day of vitamin D) and increases in force and power production in professional soccer players were also observed [24]. However, optimal vitamin D dosage and serum levels needed for athletic performance and recovery have been controversial [25]. A dosage of 600-800 IU/day and 1000 IU/day of vitamin D might not be sufficient for optimal levels of vitamin D, nor prevent a decline in serum 25(OH)D in response to intense exercise training [21]. There was evidence suggesting that dietary supplementation with 2000 to 5000 IU/day of vitamin D had a positive impact on bone health and skeletal muscle function [23]. However, it was not specified what dose of vitamin D was sufficient to prevent muscle damage and could be effective for accelerating muscle regeneration after intense effort with an eccentric work component [26, 27].

Participation in marathon and ultra-marathon races is becoming an increasingly popular activity, which is encouraged by an increasing number of running events being organized each year. Hence, a number of investigations have been conducted to determine the risk factors of skeletal muscle injury in long-term runners [9, 28]. Considering that fact, there are still, at present, no official recommendations for the treatment of muscle fatigue. Nonspecific treatments with higher vitamin D usage have been used clinically or experimentally, and have shown some positive effects.

Therefore, it seemed important to investigate the association between recommended low vitamin D dosage and an early identification of increased muscle fatigue risk. In previous studies on the assessment of muscle dysfunction, the conventional biomarkers (*e.g.*, Tn, CK, myoglobin, LDH) have been analyzed [29, 30]. These markers had different release times and different times of reaching maximal concentrations [8, 10, 31]. It has been hypothesized that exercise-induced lower muscle biomarker secretion may depend on increased serum 25(OH)D levels and these vitamin levels might be used for early detection of greater muscle resistance to fatigue. There are limited data regarding the effect of lower dosages of vitamin D supplementation on muscle function and optimization of recovery mechanisms of elite ultramarathon runners. It was also hypothesized that higher serum 25(OH)D levels in response to low dosage of vitamin D supplementation might improve this function via the stimulation of 25(OH)D production and release. To verify this, the relationships between eccentric exercise-induced muscle biomarker levels, as measured by troponin, myoglobin concentrations and creatine kinase and lactic dehydrogenase activity and 25(OH)D levels in response to vitamin D supplementation in marathon runners were examined.

Material and Methods

Ethical approval

The experiment was approved by the Ethics Committee of the Academy of Physical Education in Katowice (Ethics Committee decision KBN 3.2016) and conformed to the standards set by the Declaration of Helsinki.

Subjects

Twenty-four male ultramarathon runners who were endurance-trained for about seven years participated in the study. They were randomly assigned to either dietary protocol (*i.e.* placebo or the vitamin D supplementation, placebo-controlled study). All subjects participated in the study during the pre-season period. Study members were recruited from all the competitors of the ultra-marathons held during the Polish Running Championships. The inclusion criteria were participation in at least five marathons and written informed consent to take part in the study. The training status of the subjects included in the supplemented and placebo group expressed as maximal oxygen consumption (VO_2max) was 54.5±9.4 and 50.1±7.4 $ml/kg/min$, respectively. Age, height, body mass, body mass index (BMI) and body composition of the participants (Mean±SD) are presented in Table 1. Mean energy supply with diet, mean daily fat, carbohydrate, protein and vitamin D intake were comparable in the supplemented group and placebo group (Table 2). Biochemical measurements of pre intervention 25(OH)D levels in runners indicate that serum levels of 25(OH)D did not differ between the groups (Table 3).

All subjects reported that they were not taking any medication that could affect the 25(OH)D status. They were instructed to abstain from strenuous exercise for 24 hours

before the ultrasound measurements. No caffeine, supplements, or alcohol were permitted during the 48 hours before the experiment. Three weeks prior to the study all participants were put on a mixed diet (Table 2). The composition of the diet was calculated with dedicated software for each subject (Dietus, B.U.I. InFit. Warsaw, Poland). The diet was continued with vitamin D or placebo administration. To ensure that participants adhered to the dietary regimen, they had to keep daily food intake logs which were inspected during the weekly, obligatory visits in the laboratory. We supplemented our subjects for 3 weeks and before each diet protocol, the biochemical variables and physiological variables were analyzed.

Supplementation procedure and training protocol

All clinical data, including biochemical parameters and exercise examination, were obtained after an overnight fast. Following these measurements, blood samples were taken through a peripheral catheter inserted into the antecubital vein; each participant completed an incremental ergometer exercise test. After initial testing, the vitamin D supplemented group received 50 μg (2 x 1000 IU/day) of vitamin D. The control group received a placebo in the form of gelatin capsules (1.3 g lactose monohydrate). Participants were instructed to take the capsules with meals twice daily for a total of 3 weeks.

Exercise protocols

All subjects participated in the following experiment consisting of three protocols: the incremental exercise test (to determine the intensity of continuous eccentric exercise, downhill running) continuous eccentric exercise before supplementation and continuous eccentric exercise post supplementation (preExE and postExE, respectively). The two laboratory protocols were separated by at least seven days to prevent any possible

interference on the subjects' exercise abilities or fatigue. At the baseline, before treatment protocol (supplementation or placebo), all subjects performed a standard incremental treadmill exercise test (LE 200 treadmill, Jaeger, Frankfurt, Germany) to measure their individual aerobic performance (maximal oxygen uptake, VO_2max). The test started with a 3-min warm-up at 6 km/h and 0° inclination; the intensity was then increased by 2 km/h every 3 min up to 12 km/h and then the intensity was increased and inclination by 2.5° up to maximal exercise intensity or volitional fatigue. Heart rate (HR) (PE-3000 Sport-Tester, Polar Inc., Kempele, Finland) and systolic and diastolic blood pressure (SBP/DBP) were measured (HEM-907 XL, Omron Corporation, Kyoto, Japan) before and immediately after the test. Pulmonary ventilation (VE), oxygen uptake (VO_2), and carbon dioxide output (CO_2) were measured continuously from the 6 minutes prior to exercise test and throughout each stage of the exercise test using the Oxycon Apparatus (CareFusion Germany 234 GMBH, Hoechberg Jaeger, Germany). Physiological characteristics of the participants are presented in Table 1.

In the second phase of the study, the subjects participated in a 30-minuterunning test with an eccentric type of work (ExE) and intensity of their individual 70% VO_2max and treadmill 16° inclination based on a modified test protocol (AR Young Company, Indianapolis)[32]. According to Sorichter *et al.* [32], it has been shown that running down, i.e. eccentric effort, is an effective way to cause such a load on skeletal muscle that it can induce delayed onset muscle soreness (DOMS) symptoms. All subjects participated in the third laboratory protocol after 3 weeks of vitamin D supplementation or placebo according to the same ExE protocol.

Measurements and blood collection

At the beginning of the study (pre intervention) and at the end of each treatment period (post intervention supplementation or placebo protocol) all subjects reported to the laboratory and had venous blood drawn for the determination of levels of 25(OH)D and muscle biomarker concentrations. The blood samples were collected to determine the aforementioned markers immediately before (rest), immediately after the eccentric exercise (max) and during post-workout recovery (60 min and 24 hours after the end of the test).All investigated subjects underwent bioelectric impedance analysis (InBody Data Management System) under resting conditions to determine their body mass. The exercise tolerance was assessed by heart rate (HR) and blood lactate concentrations (LA) in response to eccentric exercise.

Biochemical analyses

For biochemical analysis, antecubital venous blood samples were always drawn at the same time of day, with the subject in a seated position. Venous blood samples were collected at four time points. Blood was allowed to clot at room temperature and then centrifuged. The resulting serum was aliquoted and frozen at -80^{o}Cfor later analyses. The measurements of serum 25(OH)D levels were performed using25OH- Vitamin D ImmunoAssay(DIA source 25OH Vitamin D total RIA CT Kit, Belgium). Intra- and interassay coefficients of variation for 25(OH)D were 5.9 - 3.3 % and 7.4 - 4.9 %, respectively. The measurements of troponin (TN) were performed using Human TNNI1 (Troponin I Type 1, Slow Skeletal ELISA Kit EH-0625, Fine Biological Technology, Co Ltd. Wuhan, China). Intra- and interassay coefficients of variation for TN were <8.0 % and < 10.0 %, respectively. The serum myoglobin (MB) levels were measured using Human Myoglobin Enzyme Immunoassay (Mioglobina ELISA, KIT DRG® Myoglobin, EIA-3955). Intra- and interassay coefficients of variation for MB were 3.9 - 6.6% and 7.8 - 7.2%, respectively.

The lowest detectable level of myoglobin by this assay is estimated to be 5 ng/ml. The proinflammatory cytokines interleukin-6 (IL-6) levels were measured by using Human IL-6 High Sensitive ELISA kit, Diacone, France. Intra- and inter-assay coefficients of variation for of IL-6 were < 4.4% and < 6.4 %, respectively and tumor necrosis factor-alpha (TNF-α) were performed using (TNF-α-EASIA KAP1751 firm DIAsource, Belgium). Intra- and interassay coefficients of variation for TNF-α were < 5.1 % and < 8.6 %, %, respectively. Creatine Kinase (CK) and Lactate Dehydrogenase (LDH) activity were measured using a commercial kit (CK NAC and LDH P-L, RANDOX, UK). Intra- and interassay coefficients of variation for CK were 2.3 - 1.5 % and 3.9 - 3.3%, respectively and for LDH were 3.9 - 1.8 % and 4.0 - 2.8%, respectively. Blood lactate concentrations (LA) were determined using BiosenC_line method (EKF Diagnostic GmbH, Germany). The degree of hemoconcentration (%) was calculated according to formula of subtracting the peak hematocrit with the minimum hematocrit recorded and multiplying by 100; all biochemical variables levels were corrected according to plasma volume.

Statistical Analysis

Shapiro-Wilk, Levene's and Mauchly's tests were used in order to verify the normality, homogeneity and sphericity of the sample's data variances, respectively. The magnitudes of differences between results of pre-test and post-test were expressed as a standardized mean difference (Cohen effect sizes). The criteria to interpret the magnitude of the effect sizes were: <0.2 trivial, 0.2—0.6 small, 0.6—1.2 moderate, 1.2—2.0 large and >2.0 very large. Descriptive statistics were calculated and the results were presented as means and standard deviations (mean±SD). We analyzed differences between pre- and post-intervention (placebo/vitamin D) baseline and post exercise variables. The data were analyzed by two-way ANOVA followed by the Student-Newman-Keuls test when appropriate. The statistical analysis includes a two-way ANOVA (placebo vs. vitamin D) and pre intervention vs. post intervention. Pearson correlation coefficients were analyzed to

determine the inter-variable relationships. All analyses were performed using the Statistica v. 12 statistical software package (StatSoft, Tulsa, OK, USA). Statistical significance was set at $p<0.05$.

Results

The effects of dietary supplementation with vitamin D and placebo administration on serum 25(OH)D, muscle biomarkers and proinflammatory cytokines concentrations in runners were compared after three weeks of each treatment protocol. Analysis of variance revealed a significant effect of vitamin D supplementation on serum 25(OH)D concentration ($F=17.1$; $p<0.001$). Significant differences between pre-intervention and post-intervention baseline serum 25(OH)D levels ($p<0.05$) and post ExE levels were observed after the vitamin D dietary protocol ($p<0.001$). A significantly higher post intervention baseline 25(OH)D level was observed after vitamin D diet compared to placebo (40.0±5.2 *versus* 31.8±4.2 ng/ml, $p<0.05$, respectively). The vitamin D increased baseline 25(OH)D (Δ) by 5.7±2.8 ng/ml and decreased placebo by - 2.2±3.6 ng/ml. ANOVA revealed a significant effect of vitamin D diet on TN levels ($F=11.6$; $p<0.01$). A significantly lower 24 h post exercise TN level was observed in vitamin D diet compared to pre-supplementation values ($p<0.05$). The baseline and max TN levels were significantly lower in vitamin D diet compared to placebo ($p<0.05$ and $p<0.001$, respectively). A significant effect of vitamin D supplementation was observed in response to MB levels ($F=9.0$; $p<0.01$) and TNFα ($F=4.7$; $p<0.05$). A repeated measure of two-way ANOVA revealed the significance of diet and exercise interaction effects on MB ($F=4.5$; $p<0.01$), CK ($F=4.5$; $p<0.01$) and 25(OH)D concentration ($F=3.2$; $p<0.05$).

A significantly lower 24h post ExE CK activity was observed after vitamin D diet compared to the pre intervention and placebo group ($p<0.05$ and $p<0.05$, respectively). No significant effect of vitamin D diet was observed regarding LDH activity at baseline and at post-exercise levels. Significant lower max and 1h post ExE TNFα levels were observed

after vitamin D diet compared to pre-intervention (p<0.01and p<0.01, respectively) and a non-significant trend to lower IL-6 levels (Table 3).

A significant and negative correlation was observed between 25(OH)D concentration and TN level (24 h post ExE) in response to supplementation (r=-0.49; p<0.05) and 25(OH)D (Figure 1) and MB concentration (r=- 0.57; p=0.05) (Figure 2). Importantly, the negative correlation was observed between 25(OH)D concentration and CK activity during the 24h recovery period (r=-0.60; p<0.05) and TNFα levels (r=-0.42; p<0.05) (Figure 3) only in response to vitamin D supplementation. ANOVA did not reveal any significant effect of diet on HRmax (157.0±5.0 *versus* 154.0±3.0 b/min) and serum LA (1.9±0.3 *versus* 1.8±0.3) concentrations in response to ExE (p>0.05).

Discussion

The present study was undertaken to investigate whether vitamin D supplementation might exert a beneficial effect on serum 25(OH)D concentrations, skeletal muscle biomarkers, and an exercise tolerance in marathon runners. Our results have demonstrated that a three-week low dosage of vitamin D supplementation caused elevation of baseline serum 25(OH)D compared to pre-supplementation levels. An increase in baseline and post-exercise serum 25(OH)D were also observed in contrast to the placebo administration. Moreover, the increased 25(OH)D production seem to have significant effect on resting and post eccentric exercise – induced skeletal biomarker levels and proinflammatory cytokines. The major findings of our study are that greater 25(OH)D expression in response to vitamin D diet correlated with biomarkers of muscle damage and that this effect is more pronounced during 24h recovery. Three weeks of supplementation had a beneficial effect on skeletal muscle function. Lower serum levels of biomarkers of skeletal muscle damage and vitamin D status improvement might, in turn, have significantly decreased individual recovery time from eccentric exercise.

Data concerning positive impacts of vitamin D consumption on optimizing athletic performance and recovery in intensely trained athletes are still sparse [5, 20, 33]. Most studies support the benefits of dietary supplementation with vitamin D in healthy untrained adults and people diagnosed with 25-hydroxyvitamin D insufficiency (<30 ng/ml) [24, 34, 35]. These results revealed a positive effect of vitamin D supplementation on global muscle strength, power and mass [14, 17, 36]. Supplementation also seems more effective on people aged 65 years compared to younger subjects. The effectiveness of the vitamin D supplementation was confirmed in athletes, however, the optimal intake and serum 25(OH)D levels have yet to be identified in the athletic population [2]. In the study of Zhang *et al.*, vitamin D

supplementation positively affected lower limb muscle strength, but not muscle power in athletes [37]. It has been suggested that different muscle groups may respond differently to vitamin D supplementation. Significant improvements in muscle function following vitamin D repletion were reported in a study on females[38]. Contrarily, a recent meta-analysis involving 532 athletes found no improvement in measures of physical performance despite the inclusion of vitamin D deficient athletes at baseline and improvements in vitamin D levels over mean 12 weeks of follow-up [5].

It has recently been reported that vitamin D supplementation might influence aerobic performance in athletes [36, 39]. Significant positive correlation was observed between 25(OH)D levels and aerobic performance (VO_2max) and training status. Supplementation with supraphysiological dose of vitamin D (6000 IU/day) during 8-week of training in rowers with sufficient 25(OH)D levels significantly increased VO_2max compared to placebo group [25]. However, no significant effect of vitamin D on athletic performance or association between 25(OH)D levels and an individual's VO_2max were also noted [40, 41].

Several mechanisms have been reported that may be responsible for the protective and ergogenic effect of 25-hydroxycholecalciferol in skeletal muscle[13]. The proposed mechanisms include a role of vitamin D receptors (VDR) that are expressed in skeletal muscle and when bound to $1,25(OH)_2D_3$, exert genomic effects at target sites [24]. Another mechanism includes a role of supplementation with vitamin D in stimulating oxygen uptake in skeletal muscle. It has been hypothesized that positive effects of 25(OH)D on oxygen uptake could be due to the fact that the cytochrome enzymes that activate vitamin D into 1,25-dihydroxycholecalciferol have heme-containing proteins that could potentially affect the binding affinity of oxygen to hemoglobin [42]. A significant effect of both exercise training and vitamin D supplementation on increased force and power output of skeletal

377 muscle perhaps in response to an enhanced cross-bridge cycling and muscular contraction
378 has also been suggested [22, 43, 44].
379
380 In our study we concluded that 25(OH)D production after vitamin D diet has a significant
381 effect on selected biomarkers of skeletal muscle damage and post exercise proinflammatory
382 cytokine levels. Significant negative correlation was observed between 25(OH)D
383 concentration and TN level and 25(OH)D and MB concentration in response to a vitamin D
384 diet. Importantly, the negative correlation was observed between 25(OH)D concentration
385 and CK activity during the 24h recovery period and TNFα levels. These support the
386 findings that lower serum levels of biomarkers of skeletal muscle damage and vitamin D
387 status improvement, might, in turn, have significantly decreased individual recovery time in
388 marathon runners. Lower levels of serum vitamin D have been associated with increased
389 muscle weakness, fatigue and injury incidents [45]. Therefore, the ability to reduce fatigue
390 and decrease the recovery time is important for athletes who train at high and moderate
391 intensity with both concentric and eccentric muscle contraction more frequently. It was also
392 observed that during recovery 1,25-hydroxyvitamin D increases the myogenic
393 differentiation and proliferation, down-regulates myostatin and improved the skeletal
394 muscle regeneration in animal studies [17]. The findings that vitamin D supplementation
395 enhances the recovery process following intense exercise [18] and ultramarathon runs[46] were
396 also supported by human studies. Serum 25(OH)D concentrations correlated positively with
397 physical activity scores, and negatively with body mass index, lipid profile, fatigue scores
398 (visual analog scale), and muscle fatigue biomarkers in healthy older adults [47, 48]. Higher
399 25(OH)D levels were accompanied by lower creatine kinase, troponin I, and lactic acid
400 dehydrogenase activity, the generally used biomarkers for earlier detection of muscle
401 injury, especially muscle soreness following training interventions [34]. In the study of
402 Nowak *et al.*, self-reported fatigue has been linked to low levels of circulating 25-

hydroxyvitamin D (25OHD), a biomarker of vitamin D status, however, vitamin D treatment significantly improved fatigue in healthy persons with vitamin D deficiency [47].

Fatigue is a complex and nonspecific phenomenon with significant response to physical and mental exertion or a feature of illnesses. There is no generally accepted set of criteria for fatigue, and the prevalence of fatigue varies widely depending on the assessment method [49-52]. A previous study demonstrated that vitamin D supplementation attenuated the inflammatory biomarkers immediately following intensive exercise with both eccentric and concentric muscle contractions [19]. Our results revealed lower post exercise TNF-α levels and a tendency towards lower IL-6 concentrations in a specifically trained supplementation group compared to the baseline levels. Regardless of the fact that long-term exercise training might diminish 25(OH)D concentrations, we conclude that a dietary vitamin D supplementation also has a beneficial effect on the function of the immune system by suppressing exercise-induced proinflammatory cytokines in elite athletes. Still, a question arises whether the recommended dosage of 1500-2000 IU/day vitamin D could maintain adequate serum vitamin D concentrations in endurance trained athletes. The optimal levels needed for athletic performance are controversial; lower than 1000 UI/day may not be sufficient, especially for an older athletic population. It has been shown that dosages higher than 2000 UI/day or 3000 UI/day have been sufficient to increase skeletal muscle function and reduce the risk of stress fractures [23, 53, 54]. The possible mechanisms responsible with a detailed characteristic of skeletal muscle functions in response to different dosages of vitamin D diet were not a major issue of the paper. These preliminary findings highlight the requirement for further studies on the effects of different dosages of vitamin D supplementation on skeletal muscle function and optimal performance in athletes.

In summary, our results show that a 3-week vitamin D supplementation had a beneficial effect on skeletal muscle adaptation to running exercise with eccentric muscle contraction. The improvement of muscle function and recovery observed in our study population might have been induced by a decrease in biomarkers of muscle damage and injury associated with higher serum 25(OH)D concentrations a vitamin D-rich diet.

Declarations

Ethics approval and consent to participate

The experiment was approved by the Ethics Committee of the Academy of Physical Education in Katowice (Ethics Committee decision KBN 3.2016) and conformed to the standards set by the Declaration of Helsinki.

Consent for publication

All authors gave their consent for publication

Availability of data and material

Upon request from the first author

Competing interests

The authors declare no conflict of interest.

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Authors' contributions

Conceptualization, A.Ż. and Z.W.; Methodology, A.Z., E.S-K.; Validation, A.S..; Formal Analysis, A.Z.; Investigation, O.Ł. A.Z, E. S-K; Data Curation, T.R., P.N. and B.K.;

Writing – Original Draft Preparation, A.Z.; Writing – Review & Editing, E.B., T.R., P.N. and B.K.; Visualization, T.R., P.N. and B.K.; Supervision, T.R., P.N. and B.K.; Project Administration, T.R., P.N. and B.K.

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Table 1 Subject characteristics (mean, SD)

Variables	EXP *n*=12	CON *n*=12
Age (years)	33.7 ± 7.5	35.9 ± 5.3
Body mass (kg)	74.7 ± 10.6	75.3 ± 8.6
Body Height (cm)	176.8 ± 6.0	178.2 ± 6.8
BMI (kg/m^2)	23.8 ± 2.2	23.7 ± 2.1
FAT (%)	13.7 ± 3.3	13.5 ± 4.4
SMM (kg)	36.5 ± 5.1	36.9 ± 4.5
TBW (L)	47.2 ± 6.4	47.5 ± 5.4
VO_2max (mL/kg/min)	54.5 ± 9.4	54.5 ± 9.4
Peak power (Watt)	321.5 ± 77.9	351.4 ± 68.3
HR max (b/min)	181.0 ± 11.0	186.0 ± 9.0

BMI- body mass index, FAT- percent of body fat, SMM – skeletal muscle mass, TBW – total body water, VO_2max – maximal oxygen uptake, HR max – heart rate maximum.

661 **Table 2** Mean energy supply with diet, mean daily fat, carbohydrate, protein and vitamin D
662 intake in the supplemented group and placebo group (mean, SD).
663

Variables	EXP (*n*=12)	CON (*n*=12)
Energy [kcal/kg/day]	29.6 ± 3.0	28.0 ± 2.0
Fat intake [%]	31.7 ± 9.6	30.8 ± 8.3
Carbohydrate intake [%]	46.1 ± 6.6	46.7 ± 8.5
Protein intake [%]	22.8 ± 5.4	22.4 ± 3.3
Vitamin D [µg/day]	7.8 ± 7.1	8.4 ± 7.3

664

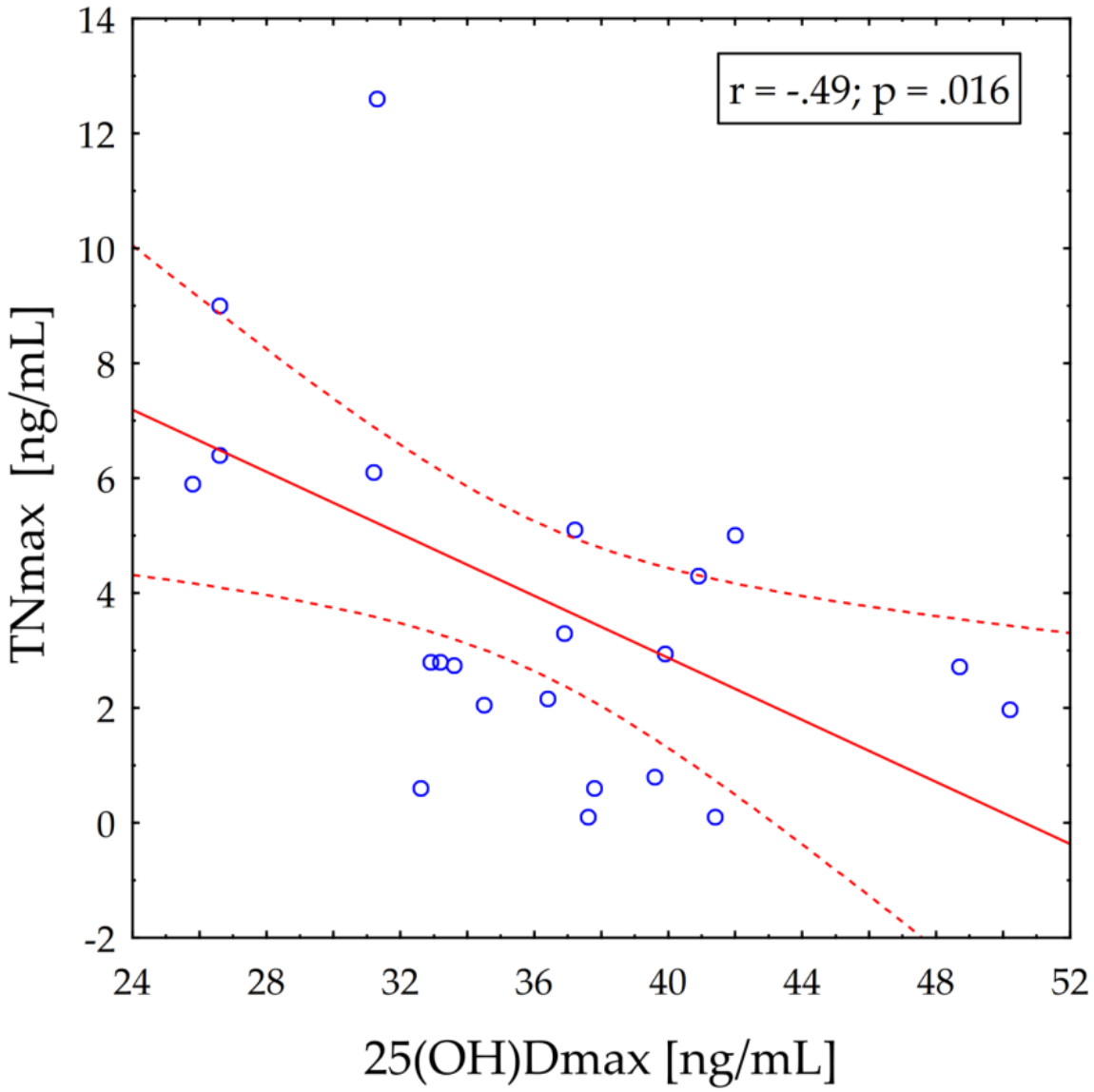
665 **Table 3** Serum 25(OH)D levels and biochemical markers of muscle damaged of the subjects

666

Variables	EXP		CON		*P*	Effect size
	Pre-Suppl	Post-Suppl	Pre-Placebo	Post-placebo	Post Suppl vs post Placebo	Cohen d
25(OH)rest [ng/ml]	34.9 ± 4.7	40.3 ± 4.9 *	33.9 ± 4.8	31.8 ± 4.2	0.05	1.86 / Large
25(OH)max [ng/ml]	36.5 ± 3.3	44.9 ± 4.9 ***	34.7 ± 8.1	39.2 ± 7.6	ns	0.89 / Moderate
25(OH)D1 h [ng/ml]	40.0 ± 8.8	45.5 ± 4.7	33.3 ± 3.4	38.5 ± 9.7	0.05	0.92 / Moderate
25(OH)D24h [ng/ml]	36.2 ± 6.2	41.2 ± 5.0 *	30.0 ± 6.4	35.7 ± 6.9	0.05	0.91 / Moderate
TN rest [ng/ml]	2.9 ± 1.9	2.0 ± 1.6	7.2 ± 1.9	5.6 ± 4.2	0.05	1.13 / Moderate
TN max [ng/ml]	5.1 ± 1.7	2.7 ± 1.6	8.9 ± 6.2	5.3 ± 4.1	0.001	0.84 / Moderate
TN 1 h [ng/ml]	4.9 ± 2.0	2.9 ± 2.0 *	4.4 ± 3.2	4.7 ± 2.4	ns	0.81 / Moderate
TN 24 h [ng/ml]	6.3 ± 3.7	3.7 ± 1.2 *	3.1 ± 1.2	3.1 ± 1.2	ns	0.5 / Small
MB rest [ng/ml]	44.7 ± 23.1	40.6 ± 17.6	44.4 ± 11.8	37.1 ± 21.8	ns	0.18 / Trivial
MB max [ng/ml]	73.9 ± 32.0	58.7 ± 27.6	93.4 ± 33.1	73.5 ± 43.7	ns	0.4 / Small
MB 1h [ng/ml]	173.6 ± 104.5	92.6 ± 48.9	102.6 ± 59.5	83.9 ± 50.0	ns	0.18 / Trivial
MB 24h [ng/ml]	93.2 ± 56.2	59.5 ± 37.8 ***	98.3 ± 26.7	93.0 ± 50.7	ns	0.75 / Moderate
CK rest [U/l]	151.0 ± 59.5	166.4 ± 95.5	234.2 ± 88.9	248.4 ± 179.0	ns	0.57 / Small
CKmax [U/l]	226.1 ± 141.0	212.7 ± 112.0	276.2 ± 118.2	286.6 ± 191.5	ns	0.47 / Small
CK 1h [U/l]	248.0 ± 161.8	214.3 ± 109.0	276.8 ± 122.3	213.2 ± 113.4	ns	0.01 / Trivial
CK 24 h [U/l]	361.3 ± 228.9	243.3 ± 91.5 *	434.3 ± 143.9	332.0 ± 255.6	0.05	0.46 / Small
LDH rest [U/l]	337.1 ± 73.5	333.1 ± 80.5	339.4 ± 47.8	333.1 ± 60.1	ns	0 / Trivial
LDH max [U/l]	400.5 ± 108.0	395.9 ± 68.6	401.4 ± 63.8	413.5 ± 79.6	ns	0.24 / Small
LDH 1h [U/l]	361.4 ± 87.8	354.2 ± 69.4	355.0 ± 44.9	368.6 ± 72.2	ns	0.2 / Small

LDH 24h [U/l]	344.9 ± 75.5	313.5 ± 66.6	339.1 ± 56.8	321.1 ± 31.1	ns	0.15 / Trivial
TNFα rest [pg/ml]	9.7 ± 5.7	5.6 ± 2.6	13.7 ± 7.4	12.5 ± 4.4	ns	1.91 / Large
TNFα max [pg/ml]	23.9 ± 15.2	10.5 ± 4.6 **	22.9 ± 13.7	22.7 ± 17.4	ns	0.96 / Moderate
TNFα 1h [pg/ml]	21.9 ± 16.8	8.4 ± 3.7 **	18.7 ± 11.4	21.3 ± 12.2	ns	1.43 / Large
TNFα 24h [pg/ml]	19.8 ± 14.2	11.6 ± 5.7	13.9 ± 6.7	13.7 ± 7.3	ns	0.32 / Small
IL-6 rest [pg/ml]	1.4 ± 1.3	1.9± 1.8	1.5 ± 1.3	2.2 ± 2.0	ns	0.16 / Trivial
IL-6 max [pg/ml]	2.0 ± 1.9	1.7 ± 1.0	2.7 ± 1.5	2.5 ± 2.3	ns	0.45 / Small
IL-6 1h [pg/ml]	2.7 ± 2.3	2.3 ± 1.3	3.1 ± 2.0	3.0 ± 1.9	ns	0.43 / Small
IL-6 24h [pg/ml]	1.8 ± 1.2	1.0 ± 0.9	2.0 ± 1.2	2.4 ± 1.6	ns	1.08 / Moderate

667



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669 **Figure 1** Correlation between 25(OH)D concentration and TNmax level in response to
670 vitamin D supplementation.

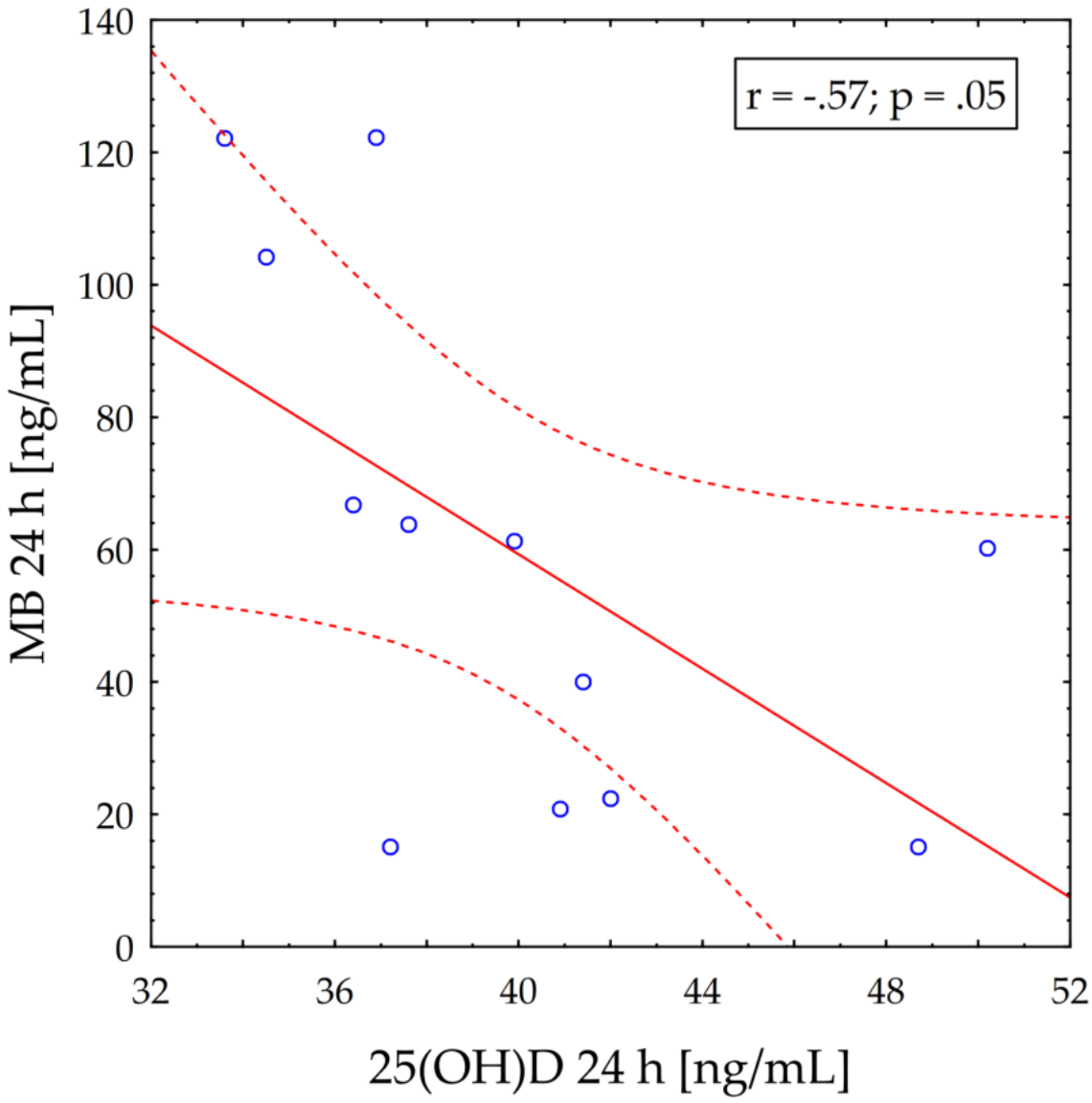

Figure 2 Correlation between 25(OH)D concentration and myoglobin (MB) level (24 h post ExE) in response to vitamin D supplementation.

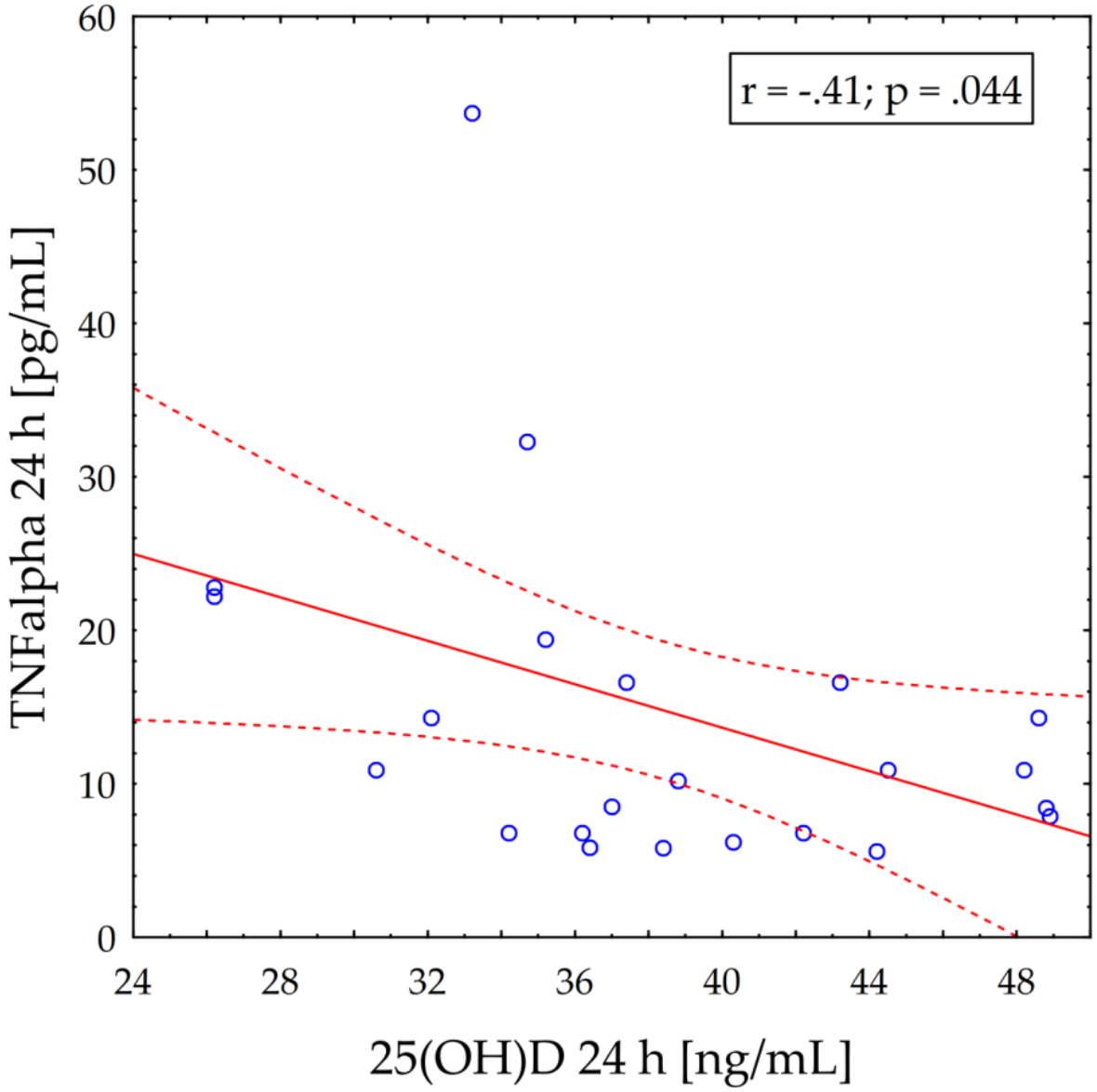

Figure 3 Correlation between 25(OH)D concentration and TN alpha level (24 h post ExE) in response to vitamin D supplementation.