Journal of Human Kinetics volume 50/2016, 71-77 DOI: 10.1515/hukin-2015-0144
Section II- Exercise Physiology & Sports Medicine

DE GRUYTER OPEN

25(OH)D_3 Levels Relative to Muscle Strength and Maximum Oxygen Uptake in Athletes

by
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Vitamin D is mainly known for its effects on the bone and calcium metabolism. The discovery of Vitamin D receptors in many extraskeletal cells suggests that it may also play a significant role in other organs and systems. The aim of our study was to assess the relationship between 25(OH)D_3 levels, lower limb isokinetic strength and maximum oxygen uptake in well-trained professional football players. We enrolled 43 Polish premier league soccer players. The mean age was 22.7±5.3 years. Our study showed decreased serum 25(OH)D_3 levels in 74.4% of the professional players. The results also demonstrated a lack of statistically significant correlation between 25(OH)D_3 levels and lower limb muscle strength with the exception of peak torque of the left knee extensors at an angular velocity of 150˚/s (r=0.41). No significant correlations were found between hand grip strength and maximum oxygen uptake. Based on our study we concluded that in well-trained professional soccer players, there was no correlation between serum levels of 25(OH)D_3 and muscle strength or maximum oxygen uptake.

***Key words**: vitamin D, muscle strength, VO_{2max}, athletes.*

Introduction

Vitamin D is a lipophilic prohormone that may originate from the diet, although its principal source is endogenous synthesis of the vitamin in response to sunlight (Larson – Meyer et al., 2010).

The fundamental roles of Vitamin D in the human body are the regulation of the calcium and phosphate metabolism and bone mineralisation (Haussler et al., 2013). Low Vitamin D levels stimulate PTH production and PTH could have direct effects on skeletal muscle. PTH may induce muscle catabolism, reductions in calcium transport (calcium–ATPase activity), impairment of energy availability (reduction in intracellular phosphate and mitochondrial oxygen consumption) and the metabolism (reduction in creatinine phosphokinases and oxidation of long-chain fatty acids) in skeletal muscle (Ceglia, 2008).

The presence of Vitamin D receptors (VDRs) in most human extraskeletal cells may suggest that it plays an important role in many other organs and systems, including skeletal muscles. These processes are mediated through genomic and non-genomic mechanisms. VDRs are involved in transcriptional activation of genes which leads to calcium transport into the cells and a phospholipid metabolism in muscles resulting in muscle contraction. VDRs with non-genomic mechanisms involve activation of a multitude of cell signalling pathways, including cAMP/PKA, PKC, calmodulin/CaM-kinase, PKB/Akt, and multiple MAPKs. This results in the activation of protein synthesis and an increased number of

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Authors submitted their contribution to the article to the editorial board.
Accepted for printing in the Journal of Human Kinetics vol. 50/2016 in March 2016.

type II muscle fibres, which in turn translates into an increased muscle contraction rate and strength (Ardestani et al., 2011, Dirks – Naylor and Lennon – Edwards, 2011).

Vitamin D deficiency may contribute to myopathies, decreased muscle tone, an increased rate of falls, and cause deficits in strength, balance and atrophy and fatty degradation of type II muscle fibres (Bischoff – Ferrari et al., 2004; Dirks – Naylor and Lennon – Edwards, 2011; Wicherts et al., 2007). While the available literature includes a large number of reports on the effects of Vitamin D on the muscular system in elderly people (Bischoff – Ferrari et al., 2004; Neal et al., 2015; Snijder et al., 2006; Wicherts et al., 2007), little is known about the relationship between $25(OH)D_3$ levels and muscle strength in professional athletes, particularly high-rank professional athletes.

It should also be pointed out that low serum 25-hydroxycholecalciferol ($25(OH)D_3$) levels can contribute to myocardial hypertrophy, increased blood pressure, endothelial dysfunction and decreased maximum oxygen uptake (VO_{2max}). While the available literature abounds in data on the relationship between Vitamin D levels and cardiorespiratory fitness in subjects with a low level of physical activity (Turkbey et al., 2010), reports on similar studies investigating individuals with a high level of physical activity are scarce.

The aim of our study was to assess the relationship between $25(OH)D_3$ levels, muscle strength and VO_{2max} in professional soccer players.

Material and Methods

Subjects

We enrolled 43 Polish premier league soccer players. The mean age was 22.7±5.3 years, body height 182.0±6.7 cm, body mass 76.3±7.4 kg, and the body mass index (BMI) 23.1±1.5 kg/m^2. The mean career duration was 14.7±4.5 years, while average VO_{2max} was 55.8±4.0 ml/kg/min. The study was conducted during a winter season in Wroclaw, Poland, which is situated at the latitude of 51°10′ N. The uniforms covered 80% of the competitors' bodies. All the players were in the competitive period and had similar training loads. None of the subjects used any food supplements containing Vitamin D and calcium.

Blood testing

Blood sampling was carried out at 8 am after a 12 h fast and a 24 h period without training. Serum was separated and stored at –70°C. Serum levels of 25-hydroxycholecalciferol were measured by electrochemiluminescence (ECLIA) using the Elecsys system (Roche, Switzerland). The intra- and interassay coefficients of variation (CV) for $25(OH)D_3$ were 5.6% and 8.0%, respectively, and the limit of detection was 4 ng/ml (10 nmol/l).

Lower limb muscle torque testing under isokinetic conditions

Lower limb strength was tested with a Biodex's Multi-Joint 4 Isokinetic Dynamometer (Biodex Medical System, New York, USA).

Peak torque (PTQ) and average power (AVGP) of the right (R) and left (L) knee flexors (F) and extensors (E) were determined on a station for isokinetic studies. The measurements were taken between 12 pm and 2 pm, and involved flexors and extensors of the knee.

Prior to each test, a chair, the dynamometer and a proper unit were adjusted in such a way that the dynamometer's tip was placed in the axis of rotation of the examined joint. The extents of flexion and extension were the same for all the participants at 90° (S 0-0-90), and the measurements were corrected for gravitation. The thigh and pelvis of the examined athlete were stabilised with belts fixed to the chair in order to eliminate movements in the adjacent joints. The baseline position was set as the maximum flexion of the knee joint.

The test started with a warm-up: each participant performed up to 3 submaximal flexions and extensions of each knee joint, followed by one maximal movement, in order to familiarise with the load. The warm-up was followed by the proper test, i.e. measurement of muscle torque at three different angular velocities, namely 30°/s, 60°/s and 150°/s.

Each subject performed three repetitions at 60°/s, 90°/s, 120°/s, 150°/s. Functional parameters of the muscles, i.e. PTQ (N-m) and AVGP (W), were registered at three angular velocities. The tests at different angular velocities were separated by a 1 min recovery period. The athletes were asked to achieve their maximum muscle power in the shortest time possible during each movement.

Hand grip strength testing

Hand grip strength measurements were taken with a manual dynamometer (TAKEI, Japan) at resolution of 0.1 kg and accuracy of 0.5 kg. Prior to testing each subject was instructed on the correct performance of the task. Each subject was asked to comfortably hold the dynamometer with his fingers and palm tight on the device. Then he lowered his upper limb along the trunk, while keeping a certain distance so that neither the elbow nor the hand touched the body, and gripped the dynamometer using maximum muscle power. Throughout the test subjects were asked to stand with their feet apart and the other upper limb freely along the body. The measurements were taken in kilograms (kg).

Aerobic capacity

VO_{2max} values during exercise testing with increasing loads were determined with a portable system K4 b^2 (Cosmed, Italy). Oxygen uptake measurements in this device are based on the standard Douglas method (Bruce et al., 1963). The system is fitted with rapid analysers and low-resistance turbines which enable it to evaluate changes in concentration and to measure oxygen uptake and carbon dioxide output using the breath-by-breath approach. Before each use, the K4 b^2 system was switched on an hour prior to calibration in order to achieve the required internal sensor temperature of 31–32ºC. This was followed by calibration directly before taking the measurements, as recommended by the manufacturer, that included calibration with atmospheric air, with the reference gas mixture (16.00% of O_2, 5.00% of CO_2), delay calibration, and calibration of the flowmeter turbine.

Ethical approval

The study was approved by the Bioethics Committee of the University School of Physical Education, Wroclaw, Poland.

Statistical analysis

Statistical analysis was conducted with PQStat ver. 1.6. The differences in muscle strength between the subgroups of various levels of $25(OH)D_3$ were assessed with univariate analysis of variance (ANOVA). The relationship between serum levels of $25(OH)D_3$, the absolute isokinetic peak torque hand grip and the cardiorespiratory fitness test (VO_{2max}) was assessed using the Pearson correlation coefficient, and the statistical analysis was performed using *Statistica* 10.

Results

The results of our study are presented in Tables 1–3.

The mean serum $25(OH)D_3$ level was 16.9±8.4 ng/ml. Assuming the levels of serum $25(OH)D_3$ of 30–50 ng/ml as the physiological norm (Holick, 2011), we found that 74.4% of the subjects had levels consistent with Vitamin D deficiency.

Table 1

Absolute isokinetic peak torque (mean±SD) relative to $25(OH)D_3$ levels

	Serum $25(OH)D_3$ levels		
	<20 ng/ml n=33	**≥20 ng/ml n=10**	***p***
Left lower extremity			
Knee ext concentric 30˚/s	286.3±48.3	316.4±65.8	0.2
Knee flex concentric 30˚/s	143.2±21.2	157.1±18.1	0.12
Knee ext concentric 60˚/s	234.2±37.5	267.1±57.4	0.09
Knee flex concentric 60˚/s	129.4±20.0	133.4±18.4	0.63
Knee ext concentric 150˚/s	172.6±22.2	195.3±36.0	0.05*
Knee flex concentric 150˚/s	103.7±15.2	108.2±16.6	0.5
Right lower extremity			
Knee ext concentric 30˚/s	274.32±47.0	306.8±66.6	0.2
Knee flex concentric 30˚/s	143.2±21.2	157.1±18.1	0.1
Knee ext concentric 60˚/s	233.6±33.8	252.4±52.2	0.27
Knee flex concentric 60˚/s	117.4±23.0	134.3±23.9	0.1
Knee ext concentric 150˚/s	174.8±20.0	186.6±29.4	0.23
Knee flex concentric 150˚/s	96.8±18.8	105.3±17.4	0.28

*Ext, extension; flex, flexion. Statistical significance at *p≤0.05*

Table 2

Average power (mean±SD) relative to 25(OH)D3 levels

	Serum 25(OH)D3 levels		
	<20 ng/ml n=33	**≥20 ng/ml n=10**	***p***
Left lower extremity			
Knee ext concentric 30°/s	100.3±16.1	110.7±29.1	0.24
Knee flex concentric 30°/s	50.7±9.1	51.2±12.3	0.73
Knee ext concentric 60°/s	167.0±24.4	180.1±45.80	0.32
Knee flex concentric 60°/s	80.1±17.0	81.6±22.3	0.9
Knee ext concentric 150°/s	281.3±35.0	295.8±69.0	0.47
Knee flex concentric 150°/s	149.3±37.0	156.2±36.0	0.7
Right lower extremity			
Knee ext concentric 30°/s	97.5±17.4	110.0±26.0	0.16
Knee flex concentric 30°/s	47.1±10.7	51.6±14.7	0.39
Knee ext concentric 60°/s	166.1±26.3	178.1±34.0	0.33
Knee flex concentric 60°/s	785.9±18.6	87.8.4±14.	0.78
Knee ext concentric 150°/s	286.7±36.3	302.7±47.3	0.35
Knee flex concentric 150°/s	145.5±40.7	145.7±31.0	1.0

Ext, extension; flex, flexion

Table 3

Correlations between 25(OH)D3 levels and the following variables: absolute isokinetic peak torque, the hand grip (left and right) and cardiorespiratory fitness

	25(OH)D3 (ng/ml) **(n=43)**
Knee ext concentric 150°/s	0.41*
Hand grip (left)	0.01
Hand grip (right)	0.07
VO_{2max}	0.02

*Ext, extension. Statistical significance at *p≤0.05*

The values of peak torque (PTQ) of the right (R) and left (L) knee flexors (F) and extensors (E) are presented in Table 1. Subjects with 25(OH)D3 levels of ≤20 ng/ml had significantly lower PTQ values only for the left knee extensors (150°/s, p=0.05) compared to subjects with higher levels of 25(OH)D3. However, when these results were corrected for body mass, relative values of muscle PTQ did not differ between the study groups. The other results did not differ significantly between the study groups in terms of PTQ (Table 1).

The values of AVGP achieved by the subjects are presented in Table 2. The AVGP results did not differ significantly between the study groups.

Table 3 shows the Pearson correlation coefficient values for correlations between serum 25(OH)D3 and the following variables: PTQ, hand grips (left and right) and VO_{2max}. The results show a statistically significant positive correlation between 25(OH)D3 levels and PTQ only for the

left knee extensors (150˚/s, p=0.04). No significant correlations were found for the remaining data.

Discussion

Vitamin D plays an essential role in the regulation of calcium and phosphate homoeostasis and affects muscle tissue morphology, which may have an impact on athletic performance in well-trained athletes (Ceglia, 2008; Ceglia, 2009; Hamilton et al., 2014). Many studies in athletes, particularly during the winter season, showed serum 25(OH)D_3 levels below the recommended range (Holick, 2011; Halliday et al., 2011; Kopec et al., 2013; Solarz et al., 2014). Our study showed decreased serum 25(OH)D_3 levels in 74.4% of the professional soccer players.

We also observed a lack of significant differences between 25(OH)D_3 levels and PTQ values, similarly to Hamilton et al. (2014), who showed no relationship between 25(OH)D_3 levels and PTQ in members of the first teams from clubs playing in the Qatar's premier "Star" League soccer division. This study merely showed 25(OH)D_3 to be significantly correlated with non-dominant leg hamstring concentric 300˚/s and eccentric 60˚/s. Subjects with higher levels of Vitamin D achieved higher PTQ values compared to those with 25(OH)D_3 levels below 10 ng/ml. However, when these results were corrected for body mass, relative PTQ values were no longer statistically significant. Our study showed similar results for the non-dominant (left) leg during isokinetic measurement at an angular velocity of 150˚/s, which may suggest that the non-dominant leg generates higher power when the player stops on the pitch compared to the dominant leg. Our subjects were all right-handed. Hand grip strength testing showed no significant correlation of 25(OH)D_3 with the hand grip (left and right), which is consistent with the findings of Nieman et al. (2013), who showed a lack of any relationship between Vitamin D levels and hand grip strength in the dominant hand in pit crew athletes.

In contrast to our study, Valtuena et al. (2013) observed a significant positive correlation between 25(OH)D_3 levels and hand grip strength, but only for healthy adolescent females, while no such correlation was found in adult males.

Our study showed no correlation between 25(OH)D_3 levels and muscle power measured with the isokinetic method. Barker et al. (2012) found no increase in AVGP or PTQ following Vitamin D supplementation at 200 IU and 4000 IU in females and males involved in leisure sport activities.

Opposite findings were obtained by Wyon et al. (2014) in a study of elite ballet dancers, which demonstrated an increase in muscle power in those subjects who had received Vitamin D supplementation at 2000 IU for 4 months. This discrepancy may have been caused by a different method of muscle power measurement Wyon et al. (2014) used (a vertical jump). Therefore, Vitamin D deficiency may affect muscle groups that were not evaluated in isokinetic testing.

A low level of Vitamin D and physical inactivity may cause muscle atrophy in the elderly, which is not the case of healthy, young and fit populations. The benefits of training may outweigh the negative impacts of 25(OH)D_3 deficiency. Therefore, subjects with the lowest level of physical activity may receive a greater benefit from increasing 25(OH)D_3 levels by changes in muscle mass and fibre type than those who are already involved in physical activity (Ardestani et al., 2011; Hamilton et al., 2014).

Our study did not show a significant correlation between 25(OH)D_3 levels and VO_{2max} in soccer players. Ardestani et al. (2011) documented a positive association between 25(OH)D_3 and cardiorespiratory fitness in healthy adults independent of their age, gender or the BMI. It should be added that the correlation between VO_{2max} and Vitamin D levels was greatest in those with the lowest levels of physical activity. Valtuena et al. (2013) conducted a study in European adolescents and also showed a significant positive correlation between VO_{2max} and 25(OH)D_3 levels in adolescent males. This may have been due to the differences in aerobic capacity between the studied subjects. VO_{2max} is an important determinant of individual work capacity. VO_{2max} is limited by cardiac output, arterial oxygen content, shunting of blood to active muscles and extraction of oxygen by these muscles. Low 25(OH)D_3 levels may decrease cardiac output and increase peripheral vascular resistance, resulting in decreased VO_{2max}. Ardestani et al. (2011) suggested that 25(OH)D_3 could have a greater beneficial effect on VO_{2max} in

subjects with a low level of physical activity than in those who were already involved in physical activity at a high level.

The main limitation of this study was a relatively small sample size; furthermore, we did not evaluate serum levels of parathormone (PTH) and calcium in athletes and did not monitor their dietary intake, what limits generalisability of the research findings.

Conclusions

Our study revealed reduced levels of 25(OH)D3 in 74.4% of the professional soccer players we investigated. We also showed a lack of correlation between serum 25(OH)D3 levels and the following variables: peak torque, average power measured under isokinetic conditions, and maximal oxygen uptake.

Based on our results it may be concluded that in well-trained professional athletes, there is no correlation between serum levels of 25(OH)D3 and such variables as peak torque assessed under isokinetic conditions or maximal oxygen uptake.

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