

## **An evaluation of the levels of 25-hydroxyvitamin D<sub>3</sub> and bone turnover markers in professional football players and in physically inactive men**

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**Short title:** The level of vitamin D and bone markers in football players.

### **Abstract**

Vitamin D is synthesised in the skin during exposure to sunlight and its fundamental roles are the regulation of calcium and phosphate metabolism and bone mineralisation.

The aim of our study was to evaluate serum levels of 25-hydroxyvitamin D<sub>3</sub>, PTH and bone turnover markers (P1NP, OC,  $\beta$ -CTx, OC/ $\beta$ -CTx) and the intake of calcium and vitamin D in Polish Professional Football League (Ekstraklasa) players and in young men with a low level of physical activity. Fifty healthy men aged 19 to 34 years were included in the study. We showed that 25(OH)D<sub>3</sub> and P1NP levels and OC/ $\beta$ -CTx were higher in the group of professional football players than in the group of physically inactive men. The daily vitamin D and calcium intake in the group of professional football players was also higher. We showed a significant relationship between 25(OH)D<sub>3</sub> levels and body mass, body cell mass, total body water, fat-free mass, muscle mass,

vitamin D and calcium intake. Optimum 25(OH)D<sub>3</sub> levels were observed in a mere 16.7% of the football players and vitamin D deficiency was observed in the physically inactive men.

The level of physical activity, body composition, calcium and vitamin D intake and the duration of exposure to sunlight may significantly affect serum levels of 25(OH)D<sub>3</sub>.

**Key words:** bone turnover markers, football players, physical activity. 25(OH)D<sub>3</sub>

## **Introduction**

Vitamin D is a lipophilic secosteroid synthesised in the skin in response to sunlight. Ultraviolet B (UVB) radiation causes the conversion of 7-dehydrocholesterol to previtamin D<sub>3</sub>. If the exposure to sunlight is adequate, previtamin D<sub>3</sub> undergoes rapid conversion to vitamin D<sub>3</sub> (cholecalciferol). Vitamin D<sub>3</sub> is converted to 25(OH)D<sub>3</sub> in the liver and is subsequently converted to the active form, 1,25(OH)<sub>2</sub>D<sub>3</sub>, in the kidneys (Holick 2007). Diet may be a source of much lower amounts of vitamin D.

Receptors for the active form of vitamin D, vitamin D receptors (VDR), have been identified in the cells of the intestinal epithelium, renal tubules, bone and other tissues and organs, which indicates a broad spectrum of action of calcitriol (Larson-Meyer and Willis 2010). 1,25-dihydroxyvitamin D<sub>3</sub> receptors have also been identified in the muscle tissue, which may suggest an important role of 25(OH)D<sub>3</sub> in the normal functioning of skeletal muscles by regulating calcium metabolism on genomic and non-genomic levels (Hamilton 2010). 25(OH)D<sub>3</sub> may therefore play a role in increasing serum calcium levels, which facilitates metabolic processes in the bone.

It has been suggested in the literature that low levels of vitamin D in athletes may lead to reduced muscle strength and may significantly increase the risk of bone injuries (Willis KS *et al.* 2008; Tukaj 2008). Some authors suggest that decreased levels of vitamin D may reduce exercise capacity of professional athletes (Cannell *et al.* 2009). Several reports have been published in the

recent years that question the role of vitamin D in the functioning of muscles, particularly in highly trained young athletes. According to Hamilton *et al.* (2013), there is little evidence that vitamin D deficiency may significantly affect exercise capacity.

It should be noted that the literature data on the levels of 25(OH)D<sub>3</sub> and bone turnover markers in athletes versus individuals with a low level of physical activity are inconclusive (Maïmoun and Sultan 2009; Maïmoun and Sultan 2011).

Comparing the levels of 25(OH)D<sub>3</sub> and bone turnover markers and the intake of calcium and vitamin D between professional football players and men with a low level of physical activity who work indoors may be important for assessing the impact of a high level of physical activity and selected elements of diet and lifestyle (duration of exposure to sunlight) on bone metabolism.

The aim of our study was to evaluate serum levels of 25(OH)D<sub>3</sub>, PTH and bone turnover markers (P1NP, OC,  $\beta$ -CTx, OC/ $\beta$ -CTx) and the intake of calcium and vitamin D in Polish Professional Football League (Ekstraklasa) players and in young men with a low level of physical activity.

## **Material and methods**

Fifty healthy men aged 19 to 34 years were included in the study, which was conducted in April.

Twenty-four of the study participants were professional football players of an average career length in this discipline of 14.75 $\pm$ 4.53 years. The mean age of the players was 26.46 $\pm$ 3.41 years. All the players were in the competitive phase in April and had a similar exercise load. In the winter season, the players had trained outdoors for about 2 hours daily. They did not use any food supplements containing vitamin D or calcium.

The control group consisted of 26 healthy, non-smoking men whose mean age was 25.92 $\pm$ 3.22 years and whose level of physical activity was low (1357.36 $\pm$ 1720.48 MET x h/week).

All the men in the control group worked indoors and did not use any food supplements containing vitamin D or calcium.

Table 1 summarises the anthropometric characteristics of both study groups.

Height was measured with a stadiometer and body mass with an electronic scale. Body composition (fat mass, fat-free body mass, water content and muscle mass) was evaluated with the bioelectric impedance analyser BIA manufactured by Akern Bioresearch (Italy). The level of physical activity in the control group was determined using the Polish version International Physical Activity Questionnaire (IPAQ, short form).

Food intake (foods and beverages consumed over 24 hours for 5 days) was evaluated using a dietary recall. The computer software Dieta 4.0 was used to calculate the quantities of vitamin D and calcium ingested by each of the football players and each of the controls.

#### *Biochemical analysis*

Blood in the group of footballers was sampled at 8.00am after 12 hours of fasting and 24 hours without training. Blood in the control group was sampled at 8.00am after 12 hours of fasting. The serum samples were separated and stored at  $-70^{\circ}\text{C}$ . Serum levels of 25(OH)D<sub>3</sub>, osteocalcin (OC), parathormone (PTH), procollagen type 1 N-terminal propeptide (P1NP), and beta-CrossLaps (beta-CTx) were determined by electrochemiluminescence (ECLIA) using the Elecsys system (Roche, Switzerland). For 25(OH)D<sub>3</sub>, the intra- and interassay coefficients of variation (CVs) were 5.6% and 8.0%, respectively, and the limit of detection was 10 nmol/l. The respective values for PTH were: 4.5%, 4.8% and 1.20 pg/ml, those for P1NP were 2.3%, 2.8% and <5 ng/ml, those for OC were 2.9%, 4.0% and <5 ng/ml, and those for beta-CTx were 2.5%, 3.5% and 0.01 ng/ml. Serum calcium was determined by colorimetry using the Konelab 60 system from bioMérieux (France).

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

## Statistical analyses

Statistical analyses were performed using PQStat for Windows (version 1.4.4.126). The comparison of serum levels of bone metabolic markers between the two groups, the football players and the controls, were analysed with parametric tests (the t-Student test) for normally distributed variables and with non-parametric tests (the Wilcoxon signed-rank test) for variables that did not meet the criterion for normal distribution. The relationship between 25(OH)D<sub>3</sub> levels and anthropometric parameters, body composition, the levels of bone turnover markers and the intake of calcium and vitamin D was analysed by estimating the Spearman rank correlation coefficient. Data are presented as means  $\pm$  SD with  $p < 0.05$  being indicative of statistical significance.

## Results

The results of our study are summarised in Tables 1, 2 and 3.

There were no significant differences in the values of anthropometric parameters or body mass composition between the group of football players and the control group.

**Table 1.** Characteristics of the study participants.

	<b>Football players (n=24)</b>	<b>Controls (n=26)</b>
Age (years)	26.46 $\pm$ 3.41	25.92 $\pm$ 3.22
Body mass (kg)	79.61 $\pm$ 6.71	73.64 $\pm$ 9.49
Height (m)	1.84 $\pm$ 0.06	1.79 $\pm$ 0.07
Body cell mass (kg)	39.11 $\pm$ 4.34	32.68 $\pm$ 4.21
Total body water (l)	47.28 $\pm$ 4.81	41.97 $\pm$ 4.99
Extracellular water (l)	18.79 $\pm$ 2.10	17.77 $\pm$ 2.05
Intracellular water (l)	28.49 $\pm$ 3.22	24.09 $\pm$ 2.21
Fat mass (kg)	15.55 $\pm$ 3.18	16.65 $\pm$ 5.12
Fat-free mass (kg)	64.50 $\pm$ 6.69	57.00 $\pm$ 6.39
Muscle mass (kg)	47.24 $\pm$ 5.14	39.81 $\pm$ 5.01

Table 2 shows the mean values of serum levels of 25(OH)D<sub>3</sub>, PTH, bone turnover markers (P1NP, OC, β-CTx, OC/β-CTx) and calcium and the mean levels of calcium and vitamin D intake in the football players and the controls.

The mean levels of 25(OH)D<sub>3</sub> and P1NP were significantly higher in the group of football players than in the group of physically inactive men. The players also had higher values of OC/β-CTx and a significantly higher daily dietary intake of vitamin D and calcium.

**Table 2.** Serum levels of bone turnover markers (mean ± SD) and intake of dietary calcium and vitamin D in the football players and the controls.

	<b>Football players (n=24)</b>	<b>Controls (n=26)</b>	<b>Paired t-test, Wilcoxon signed rank test, p value</b>
25(OH)D <sub>3</sub> (nmol/l)	62,64±24,78	34,65±14,48	<0.0001***
PTH (pg/ml)	25.37±7.95	29.23±10.16	NS
P1NP (ng/ml)	79.83±22.57	59.89±23.68	0.002**
OC (ng/ml)	33.00±9.05	28.91±8.57	NS
β-CTx (ng/ml)	0.68±0.22	0.71±0.23	NS
OC/β-CTx	50.60±11.34	43.38±13.66	0.04*
Calcium (mmol/l)	2.46±0.08	2.44±0.15	NS
Vitamin D intake (µg/d)	6.30±4.30	2.81±1.88	0.0009***
Calcium intake (mg/d)	1172.6±271.16	842.32±325.25	0.0004***

\*p<0.05; \*\*p<0.01; \*\*\*p<0.001

Table 3 shows the values of the Spearman rank correlation coefficient for correlations between serum levels of 25(OH)D<sub>3</sub> and the anthropometric parameters and between bone marker turnover serum levels of 25(OH)D<sub>3</sub> and the bone turnover markers. No statistically significant relationship was shown between serum levels of PTH, P1NP, OC, β-CTx, OC/β-CTx and calcium levels and serum levels of 25(OH)D<sub>3</sub>. When we analysed the correlation between the anthropometric parameters and the level of 25(OH)D<sub>3</sub> we found a highly significant relationship with body mass (r=0.31), body cell mass in kg (r=0.48), body cell mass in percent (r=0.37), total body water (r=0.41), extracellular water (r=0.32), intracellular water (r=0.46), fat free mass

( $r=0.42$ ) and muscle mass ( $r=0.48$ ). We also found a statistically significant positive correlation between serum levels of 25(OH)D<sub>3</sub> and the intake of calcium and vitamin D.

**Table 3.** The Spearman rank correlation coefficient ( $r$ ) between serum levels of 25(OH)D<sub>3</sub> and the anthropometric parameters and between serum levels of 25(OH)D<sub>3</sub> and the bone turnover markers.

	<b>Football players (n=24)</b>
Body mass (kg)	0.31*
Height (cm)	0.18
Body cell mass (kg)	0.48**
Body cell mass (%)	0.37**
Total body water (l)	0.41**
Extracellular water (l)	0.32*
Intracellular water (l)	0.46**
Fat mass (kg)	-0.07
Fat free mass (kg)	0.42**
Muscle mass (kg)	0.48**
PTH (pg/ml)	-0.05
TP1NP (ng/ml)	0.27
OC (ng/ml)	0.08
$\beta$ -CTx (ng/ml)	0.07
OC/ $\beta$ -CTx	0.02
Serum calcium (mmol/l)	-0.06
Vitamin D intake ( $\mu$ g /d)	0.38**
Calcium intake (mg/d)	0.30*

\* $p<0.05$ ; \*\* $p<0.01$

## Discussion

There are numerous reports in the literature on serum levels of 25(OH)D<sub>3</sub> in representatives of various sport disciplines versus individuals with a low level of physical activity but they are inconclusive. A study in a group of bodybuilders showed higher levels of 25(OH)D<sub>3</sub> compared to the control group (Bell *et al.* 1988). Similar results were obtained by Zittermann (2003), who

compared representatives of various disciplines (triathletes, play sports and track and field sports) with individuals leading a sedentary lifestyle. On the other hand, a study conducted by Maïmoun et al. (2004) in cyclists and swimmers did not show elevated levels of 25(OH)D<sub>3</sub>.

Our study showed that serum levels of 25(OH)D<sub>3</sub> in professional football players were considerably higher (by 80.09%) than those in the control group. It may stem from the fact that athletes were exposed to more sunlight and ingested higher amounts of vitamin D with their diets (by 124.2%) compared to men in the control group.

However, it should also be noted that the level of 25(OH)D<sub>3</sub> in our football players was 39.1% lower than that in the Italian Serie A representatives, who were also investigated in springtime (Angelini F *et al.* 2011). This difference may be due to the different geographic location and, possibly, a greater exposure to sunlight in Italy than in Poland. When we compared our results to those of those obtained in the British Premier League footballers (Morton *et al.* 2012) investigated in wintertime (24 December), who train at a similar latitude (53° N) we found that the British players had similar levels of vitamin D to those in our players (51±19 nmol/l vs 62,4±24,76 nmol/l).

It should be emphasised that only 16.7% (n=4) of our players had normal levels of 25(OH)D<sub>3</sub>, while 45.8% (n=11) were vitamin D insufficient and 37.5% (n=9) were vitamin D deficient. In the control group, none of the subjects had normal levels of 25(OH)D<sub>3</sub> with 7.7% (n=2) being vitamin insufficient and 92.2% (n=24) vitamin D deficient. Measurement of serum levels of 25(OH)D<sub>3</sub> may pose a problem due to the discrepant physiological ranges for this parameter. In our study, in line with the Endocrine Society guidelines, the normal range for serum levels of 25(OH)D<sub>3</sub> was defined as 75–150 nmol/l, vitamin D insufficiency was defined as serum levels of 50,1–74,9 nmol/l and vitamin D deficiency as serum levels below 50 nmol/l (Holick, 2007).

A study in Qatar's professional football league players showed that only 15.8% of the subjects had 25(OH)D<sub>3</sub> levels above 75 nmol/l, 28.7% were vitamin D insufficient and 55.6%

deficient. Although they train and play in a geographical region heavily exposed to sunlight, the players still have 25(OH)D<sub>3</sub> deficiency. The authors suggest that this might be due to the fact that due to the high temperatures during the day the players most often train after the sunset. According to Hamilton *et al.* (2013), factors that determine serum levels of 25(OH)D<sub>3</sub> may include the skin colour, use of sun protection cosmetics and the geographic region from which the players originate.

Dietary intake of vitamin D contributes to serum levels of 25(OH)D<sub>3</sub> in less than 10% (Tukaj 2008). Our study showed a statistically significant relationship between the dietary intake of vitamin D and its serum levels.

When we analysed the diets followed by the players and by the controls we found that vitamin D supply although twice as high in the group of footballers than that in the group of physically inactive men still did not meet the minimum daily requirement. Dietary intake of vitamin D in the control group was nearly four times lower than the recommended daily dietary allowance, which suggests the need for nutritional education.

In both groups, on the other hand, we found that dietary calcium supply was within normal limits (>800 mg/d) (Sumida *et al.* 2012). We also found a significant positive correlation between the appropriate dietary calcium supply with the diet and serum levels of vitamin D. This correlation was consistent with the relationship observed by Bescós García and Rodríguez Guisado (2011), who found a positive correlation between calcium intake and vitamin D levels.

Bonjour *et al* (2009) suggest that the fortified soft plan cheese consumed by elderly women with vitamin D insufficiency can reduce the level of bone resorption markers by positively influence Ca and protein economy as expressed by decreased PTH and increased IGF-1 level.

It should be emphasised that there are conflicting literature reports on the impact of physical activity on serum levels of PTH. In the case of athletes pursuing performance disciplines, serum levels of PTH were lower than in physically inactive subjects (Brahm *et al.* 1997).

Our study did not, however, show any differences in PTH levels between the study groups.

Our results are consistent with those of Bell *et al* (1988), who conducted a study in representatives of strength disciplines. No differences in PTH levels between athletes and individuals with a low level of physical activity were also reported in cyclists, swimmers and triathlonists (Maïmoun *et al.* 2004).

Maïmoun *et al.* (2009) suggest that the level of physical activity may indirectly affect the levels of PTH and 25(OH)D<sub>3</sub> levels, as the exercise stimulus causes serum levels of phosphate and calcium to decrease, which results in increased secretion of PTH and may finally lead to increased renal synthesis of calcitriol. Ljunghall *et al.* (1988) found that the postexercise increase in PTH levels did not significantly affect blood electrolyte levels and therefore may have very little impact on bone mineral density.

The literature data on the levels of bone turnover markers (P1NP, OC,  $\beta$ -CTx, OC/ $\beta$ -CTx) in the context of high levels of physical activity are also inconsistent.

Some studies indicate that strength/resistance exercise positively affects the skeletal system to a larger degree than does performance exercise or leisure physical activity (Maïmoun and Sultan 2011). In the case of strength disciplines, there are reports showing that serum levels of bone turnover markers may be considerably higher in athletes than in individuals with a low level of physical activity. Serum levels of OC, for instance, are 44%, 59.8%, 97% and 41.5% higher in representatives of strength disciplines, decathlonists, volleyball players and judokas, respectively, than in controls (Bell *et al.* 1988; Maïmoun L *et al.* 2008; Nishiyama *et al.* 1988; Prouteau *et al.* 2006). The results obtained by the latter may suggest an effect of strength/resistance exercise on the activity of osteoblasts.

Our study did not show statistically significant differences in OC levels between the professional footballers and the controls, which is consistent with the findings of Brahm *et al.* (1997), who assessed long-distance runners, and with the findings of Maïmoun *et al.* (2004), who assessed cyclists and triathlonists. A study by Karlsson *et al.* (2003), on the other hand,

demonstrated a relationship between the level of physical activity and OC levels and showed the latter to be 29.6% higher in football players than in the control group. It should be noted, however, that the study by Karlsson et al. (2003) did not show any significant differences between the players and the controls in the levels of other bone turnover markers, such as total alkaline phosphatase (tALP) or C-terminal propeptide of type 1 procollagen (PICP).

In our study, we did not find any statistically significant differences in serum levels of  $\beta$ -CTx between the study groups, which is consistent with the study by Maïmoun *et al.* (2004), who also showed no statistically significant differences in the levels of this marker a marker of bone resorption processes — in triathlons and cyclists.

Our study did, however, show that a high level of physical activity could affect the levels of N-terminal propeptide of type 1 procollagen (P1NP). We found that P1NP levels are significantly higher in football players than in individuals with a low level of physical activity. Scott *et al.* (2010) showed that intensive physical exercise did not affect the levels of those bone turnover markers that are responsible for bone formation (P1NP and bone alkaline phosphatase [bALP]) directly after training and after 96-hour restitution. The authors did, however, suggest that the levels of these markers could increase after a long period of time, which may positively affect bone formation.

Our study showed OC/ $\beta$ -CTx to be considerably higher in the group of footballers than in controls. This may suggest an increased activity of osteoblasts relative to the activity of osteoclasts in football players. In individuals with a higher level of physical activity, bone formation processes may be relatively more dynamic than bone resorption processes.

High levels of bone turnover markers (e.g. type I collagen C-telopeptide, C-terminal cross-linked telopeptide of type I collagen, type I collagen N-telopeptide) are believed to adversely affect the bone. However, in the case of athletes, high levels of both bone resorption markers and bone formation markers may indicate dynamic changes occurring in the skeleton, which may result

from the need to repair microinjuries caused by increased physical activity. The increased bone remodelling rate may in turn lead to reduced risk of fractures (Maïmoun *et al.* 2008).

Our study showed a significant positive relationship between serum levels of vitamin D and the following parameters: fat-free mass (kg), muscle mass (kg), body cell mass (kg, %), total body water (l), extracellular water (l), intracellular water (l) and body mass (kg).

Hamilton *et al.* (2013) showed a positive correlation between 25(OH)D<sub>3</sub> levels and total fat-free body mass. In athletes with a considerable vitamin D deficiency in the serum (<25 nmol/l), low total body mass and low fat-free mass values (Hamilton *et al.* 2013).

Parikh *et al.* (2004), on the other hand, found that obese individuals had lower levels of 25(OH)D<sub>3</sub> compared to individuals with a normal body mass. The reduced 25(OH)D<sub>3</sub> levels in individuals with a higher amount of adipose tissue may be due to vitamin D sequestration in the adipose tissue, although the exact underlying mechanism is still unclear (Halliday *et al.* 2011).

## **Summary and conclusions**

Our study showed that football players had significantly higher levels of 25(OH)D<sub>3</sub>, P1NP and OC/β-CTx compared to individuals with a low level of physical activity, which may indicate a positive impact of intensive physical exercise on bone formation. Normal (according to the definitions suggested by the Endocrine Society) serum levels of 25(OH)D<sub>3</sub> were found in a mere 16.7% of the footballers. The remaining footballers and all the men in the control group had vitamin D insufficiency or deficiency. Daily dietary supply of vitamin D was also shown to be insufficient in both groups, which may indicate the need to modify the diet. Our study did not show any significant differences in the levels of PTH, OC, β-CTx or calcium between individuals with a high level of physical activity and the control group. We showed a significant relationship between serum levels of 25(OH)D<sub>3</sub> and the following parameters: anthropometric parameters (body mass, body cell mass, total body water, fat-free mass, muscle mass) and the dietary intake of calcium and

vitamin D. The level of physical activity, body mass composition, intake of calcium, intake of vitamin D and duration of exposure to sunlight may significantly affect serum levels of 25(OH)D<sub>3</sub>.

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