

Is vitamin D status reflected by testosterone concentration in elite athletes?

AUTHORS: Jarosław Krzywański¹, Andrzej Pokrywka², Marcel Młyńczak³, Tomasz Mikulski⁴

¹ National Centre for Sports Medicine, Warsaw, Poland

² Department of Biochemistry and Pharmacogenomics, Faculty of Pharmacy, Medical University of Warsaw, Warsaw, Poland

³ Institute of Metrology and Biomedical Engineering, Faculty of Mechatronics, Warsaw University of Technology, Warsaw, Poland

⁴ Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

ABSTRACT: Vitamin D is a nutrient whose active form affects tissues as a hormone and possibly enhances performance. One plausible mechanism is by increasing testosterone concentration, which is established as an important factor for athletic performance. Therefore the aim of the study was to examine the relationship between plasma concentration of 25(OH)D and testosterone in Polish elite track and field athletes depending on vitamin D status, season, training period, body composition, sex, type of training, sun exposure and vitamin D supplementation. Plasma concentrations of 25(OH)D and testosterone were measured in all seasons within two years in athletes (70 females, 79 males) who represent strength ($n = 103$) and endurance ($n = 46$) kinds of sports, in the preparatory-competitive season and transition period. There were no differences in 25(OH)D concentration between male and female athletes, insufficiency [$25(\text{OH})\text{D} < 30 \text{ ng/ml}$] was observed in 32.9%, whereas deficiency [$25(\text{OH})\text{D} < 20 \text{ ng/ml}$] in 3.2%. Circannual rhythm was noted for vitamin D but not for testosterone concentration; no correlations between them were found either in strength or endurance athletes or between 25(OH)D and body composition. Testosterone concentration was higher in the transition period than in the preparatory-competition period only in male athletes. Higher 25(OH)D was observed in athletes who trained during winter in Africa (higher sun exposure) or used oral supplementation, whereas the respective testosterone levels were unchanged. In athletes, testosterone concentration did not reflect vitamin D status. The widespread of inadequate vitamin D status among athletes, makes it vital to recommend them the regular monitoring of 25(OH)D concentration and use of reasonable supplementation.

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Corresponding author:

Tomasz Mikulski

Department of Applied
Physiology

Mossakowski Medical Research
Centre, Polish Academy
of Sciences

Pawinskiego 5

02-106 Warsaw, Poland

E-mail: tomik@imdik.pan.pl

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INTRODUCTION

Vitamin D is a nutrient whose active form – calcitriol [1,25(OH)D] – affects tissues as a hormone not only responsible for calcium homeostasis but in many studies its pleiotropic, extraskeletal actions were described, constituting a link between its deficiency and many diseases and mortality [1–3]. Vitamin D status was evaluated on the basis of blood concentrations of 25(OH)D – 25-hydroxycholecalciferol – which is substrate for synthesis of the active form of vitamin D. Biological effects of vitamin D are mediated by the vitamin D receptor (VDR), a transcription factor that belongs to the same nuclear receptor family as testosterone. The presence of VDR and CYP27B1 (α -1 hydroxylase – the enzyme responsible for synthesis of the active form of vitamin D in kidneys) have been found in many cells of the reproductive system [4, 5]. Therefore, a potential influence of vitamin D on fertility is currently under debate [6]. In several studies involving older people, the relationship between vitamin D and testosterone concentration has been confirmed, but the association is strongest in deficient individuals [7–10].

The discovery of the presence of VDR in skeletal muscles raised interest in the possible role of vitamin D in muscle strength and function. Most randomized, controlled studies have confirmed such a relationship, but mainly in older subjects [11, 12]. For this purpose, vitamin D became popular in athletes, since some studies have confirmed its ergogenic effects on strength and endurance [13, 14]. Nevertheless, the majority of studies showed no influence of vitamin D on performance and more studies are needed to clarify that [15–18].

The main effect of vitamin D in muscles might be linked with satellite cell activity. Activation of VDR in satellite cells regulates cell fate decisions, whether it differentiates and divides or remains in the stem cell pool. In consequence the amount of type II muscle fibres is increased, and it transfers into greater speed and strength [19, 20]. The expression of IGF-1 is also increased after VDR activation, producing the hypertrophy and remodelling of myocytes [20]. The other mechanism of influence of VDR on muscles is a rapid non-genomic action by VDRs located in the muscle cell membrane and

is linked with regulation of calcium ions influx into the muscle cell [19, 20].

Another potential influence of vitamin D on performance might be related to testosterone concentration [21, 22]. Testosterone undoubtedly enhances performance by increasing muscle growth, reducing body fat and improving psychological aspects (aggression, motivation, etc.), but its exogenous administration is prohibited in sport and clearly defined as doping [23–26]. Therefore, any practices which might legally promote endogenous testosterone concentration are cordially welcome by athletes. Despite lacking evidence, vitamin D is very popular among athletes as an ergogenic aid, frequently overdosed or prescribed by self-appointed experts [1, 27, 28]. The outcome of such practice is difficult to predict nowadays.

The data concerning the interaction between vitamin D and testosterone in athletes are scanty and equivocal. For example, in 45 soccer players the relationship between 25(OH)D and testosterone concentrations was confirmed [29], whereas in 50 hockey players it was not found [30].

The aim of the present study was to assess the relationship between plasma concentration of 25(OH)D and testosterone in Polish elite track and field athletes according to the vitamin D status, season, training period, body composition, sex, type of event performed, higher sun exposure in winter and vitamin D supplementation.

MATERIALS AND METHODS

Participants

The participants were recruited from the Polish national track and field team. In this retrospective, observational study, 284 samples were taken from 149 male and female elite athletes. The basic characteristics is presented in Table 1. All the athletes were Caucasians with skin type I-III according to Fitzpatrick's scale [31].

The study was conducted according to the Declaration of Helsinki and was approved by the Committee on Bioethics at the Medical University of Warsaw (permission AKBE/111/15). Each subject had

signed the consent form for routine medical monitoring, including the statement of agreement for the use of the results for scientific purposes. Because the study was retrospective neither written nor verbal consent for this particular study was obtained.

Training periods

Two training periods were distinguished: the transition period, when athletes do not train or compete (Sep-Nov), and the preparatory-competitive period, when athletes train hard and compete (Dec-Aug). The usual training hours were 10 a.m. – 1 p.m. and 4 – 7 p.m. with weather-dependent sun exposure. During training abroad the daily sunshine exposure was longer than the one which provides recommended daily skin vitamin D synthesis [32].

Type of sports

The athletes were divided according to different types of athletic events based on character of training into strength (sprinters, jumpers and throwers) and endurance (distance runners and race walkers) groups.

Factors influencing vitamin D status

The following groups were distinguished for the purposes of the study: 0 (n = 110) – athletes who trained in Poland at the latitude of 49–54° N; Sun (n = 42) – athletes exposed to sun during Polish winter (Jan-Mar) in the Republic of South Africa (RSA), at the latitude of 27° S and on Tenerife, at the latitude of 28° N, who were sampled within 1–4 weeks after their return to Poland; Supl (n = 51) – athletes with a deficit or insufficient vitamin D status who trained in Poland and were orally supplemented. The deficient athletes received individually adjusted vitamin D (cholecalciferol) supplementation of 4000–8000 IU daily, depending on the scale of deficiency, whereas the dose for the insufficient ones was established at 2000 IU in accordance with the experts' guidelines for the general population in Poland and Central Europe and in athletes [28, 33]. The effectiveness

TABLE 1. Descriptive information of the athletes (n = 149).

	Females (n = 70)		Males (n = 79)	
	Strength (n = 47)	Endurance (n = 23)	Strength (n = 56)	Endurance (n = 23)
Age [years]	25.0 ± 0.3	25.0 ± 0.5	26.0 ± 0.3	26.0 ± 0.7
Height [m]	1.73 ± 0.01	1.70 ± 0.01	1.87 ± 0.01	1.81 ± 0.01
Body mass [kg]	64 ± 1	56 ± 1	90 ± 2	68 ± 1
Body mass index [kg/m ²]	20.9 ± 0.5	19.1 ± 0.2	25.5 ± 0.5	20.7 ± 0.3
Muscle mass [kg]	27.2 ± 1.2	24.5 ± 0.9	40.2 ± 1.8	33.2 ± 1.5
Fat mass [kg]	15.3 ± 1.4	9.4 ± 1.0	13.8 ± 1.2	7.8 ± 0.5
Fat percentage [%]	20.9 ± 1.1	16.4 ± 1.1	18.4 ± 1.6	11.9 ± 1.1

of supplementation was evaluated after 8–12 weeks of treatment and only fully compliant athletes who confirmed strict follow-up of the prescribed doses were included in the Supl group.

Blood measurements

Fasting blood samples were collected in the morning from the ante-cubital vein into the clot activator tubes. Vitamin D status was evaluated on the basis of blood concentrations of 25(OH)D. The serum concentration of 25(OH)D was determined with the Liaison diagnostic system (DiaSorin, Stillwater, MN, USA) by a chemiluminescent immunoassay (CLIA); range of detection 4–150 ng/ml, precision 5.0% CV, accuracy SD 1.2.

In the present study the following serum 25(OH)D concentration criteria were assumed: < 20 ng/ml was defined as deficiency, 21–29 ng/ml as insufficiency, 30–50 ng/ml as normal, 50–100 ng/ml as high and > 100 ng/ml as toxic [32–34]. Deficiency and insufficiency were classified as inadequate vitamin D status.

Testosterone concentration was assessed with the electrochemiluminescent method on a Cobas analyser using Roche commercial kits.

Statistical analyses

The obtained data were analysed for normality of distribution with Shapiro-Wilk's test and when the data were not normally distributed Wilcoxon's test was used to compare groups. The within-subject (repeated measures, ANOVA) approach was used to examine the effect of the training periods and circannual rhythm on concentrations of 25(OH)D and testosterone. Analyses in pairs were conducted in subjects who were measured before and after the training camp abroad, during summer in Poland and after winter sun exposure abroad and before and after oral supplementation. The relationship between concentrations of 25(OH)D and testosterone were analysed on combined data using the Pearson correlation coefficient. The values are shown as mean \pm SE and a *P* value less than 0.05 was considered statistically significant. Statistical analyses were performed using the Statistica 6 software.

RESULTS

There were no differences in 25(OH)D concentration between male and female athletes. The annual average 25(OH)D concentration was 36.0 ± 12.4 ng/ml, vitamin D insufficiency [25(OH)D < 30 ng/ml] was observed in 32.9% of athletes, whereas deficiency [25(OH)D < 20 ng/ml] in 3.2%.

The seasonal pattern of 25(OH)D concentration was confirmed – significantly higher in summer than in autumn ($p < 0.01$) and winter ($p < 0.05$) and in spring than in autumn ($p < 0.001$) and winter ($p < 0.01$, Figure 1). There were no seasonal differences in plasma testosterone concentrations in both female and male athletes (Figure 2).

Plasma concentration of 25(OH)D was significantly higher in the samples collected during the training and competition months

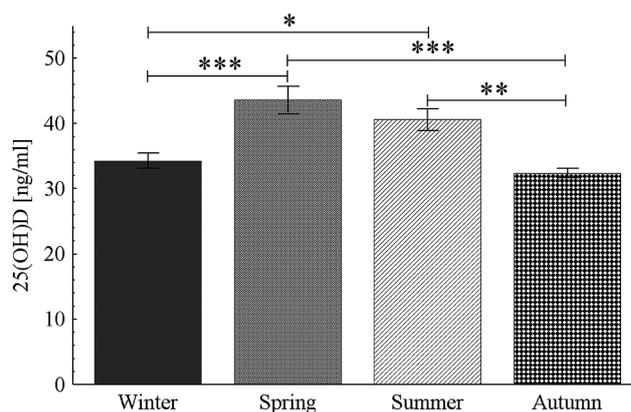


FIG. 1. Seasonal pattern of 25(OH)D concentration in blood in all athletes;

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

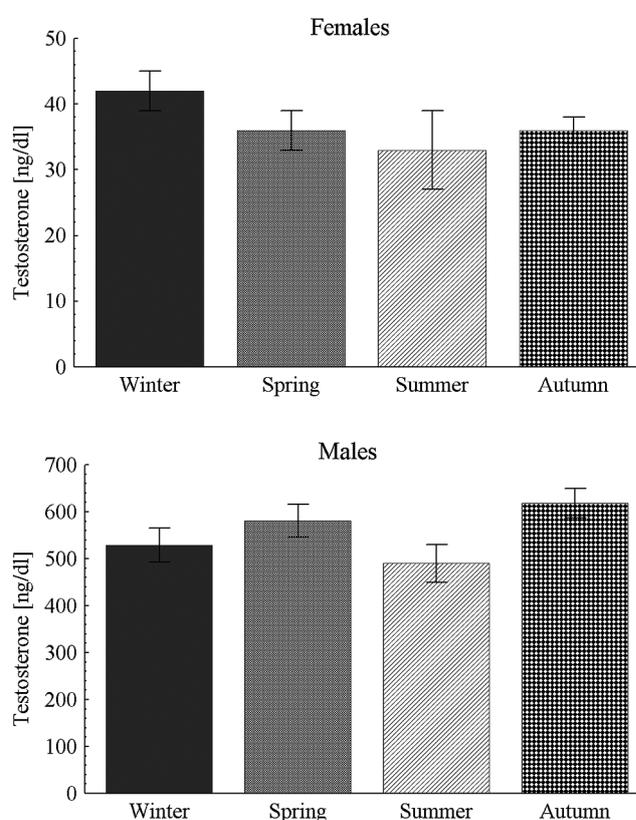


FIG. 2. Seasonal pattern of testosterone concentration in blood in female and male athletes.

Dec-Aug (preparatory-competitive period) than recovery months Sep-Nov (transition period) in both male (40.1 ± 1.6 ng/ml vs. 32.8 ± 0.9 ng/ml, $p < 0.01$) and female (37.0 ± 1.2 ng/ml vs. 31.9 ± 1.0 ng/ml, $p < 0.01$) athletes. The testosterone concentration was significantly lower during the preparatory-competitive period only in male athletes, 539.8 ± 21.7 ng/dl vs. 618.1 ± 31.2 ng/dl

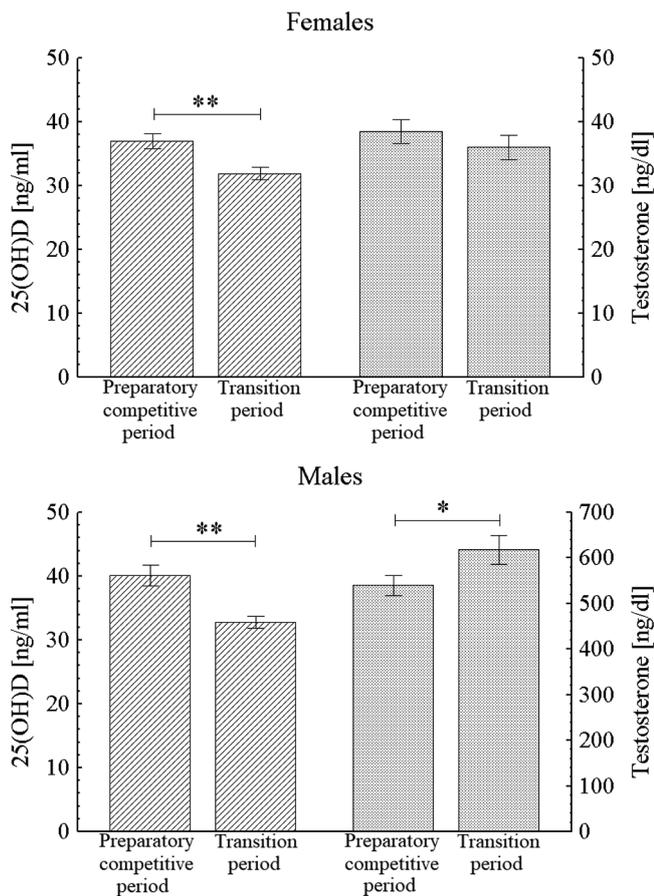


FIG. 3. Blood concentration of 25(OH)D and testosterone during the training and competition months Dec-Aug (preparatory-competitive period) and recovery months Sep-Nov (transition period) in male and female athletes; * $p < 0.05$, ** $p < 0.01$.

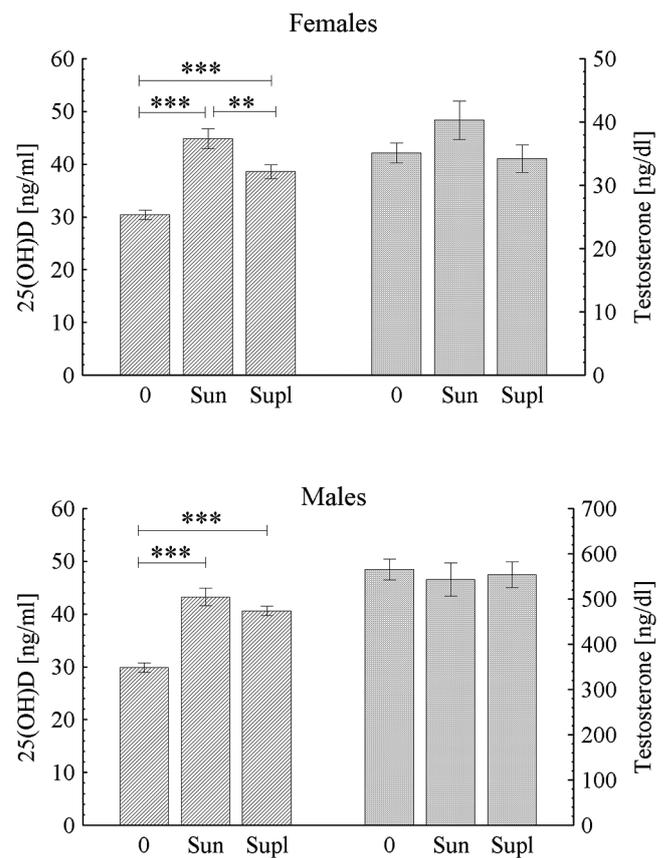


FIG. 4. Blood concentration of 25(OH)D and testosterone in female and male athletes who trained during winter in Poland (0), after the sun exposure in winter (Sun) and oral supplementation (Supl); ** $p < 0.01$, *** $p < 0.001$.

in transition period, $p < 0.05$, whereas in females it was 38.5 ± 1.9 ng/dl vs. 36.0 ± 1.9 ng/dl, respectively, n.s. (Figure 3).

A significantly higher 25(OH)D concentration was observed both after the sun exposure in winter (Sun) and oral supplementation (Supl) than in non-supplemented athletes who trained during winter in Poland (0), whereas testosterone levels were not influenced (Figure 4).

For the whole year in both female and male athletes there were no significant correlations between 25(OH)D and testosterone, as well as for the athletes with inadequate vitamin D status [25(OH)D below 30 ng/ml] separately (Figure 5). There were also no significant correlations between 25(OH)D and testosterone concentrations in either female or male athletes in each season separately, in strength or endurance disciplines separately, according to BMI and muscle mass, in athletes with proper vitamin D status [25(OH)D over 30 ng/ml] only and both in preparatory-competitive and transition periods. No correlation between percentage of body fat or muscle mass and testosterone concentration was found in either female or male athletes.

DISCUSSION

The recent changes of lifestyle, namely more time spent indoors and use of clothes and sunscreens, resulted in common vitamin D deficiency all over the world [35, 36]. The results obtained in the present study revealed an inadequate status of vitamin D in 30% of Polish elite athletes (annual average), which is similar to the values described in the sedentary population and in other athletes [37–40].

In the present study the seasonal rhythm of 25(OH)D concentration was observed with higher values in spring and summer. It is consistent with the data available in the literature for countries situated above the latitude of 40° N (Poland is situated in a moderate climate/latitude between 49° and 54° N) for both athletes and the rest of the population [39, 41–45]. The significant seasonal differences in vitamin D status emphasize the importance of the availability of ultraviolet B (UVB) radiation originating from the sun, which is mostly influenced by the latitude. The higher 25(OH)D concentration observed in the present study in spring results from the training camps at the lower latitude in RSA and Tenerife in which most

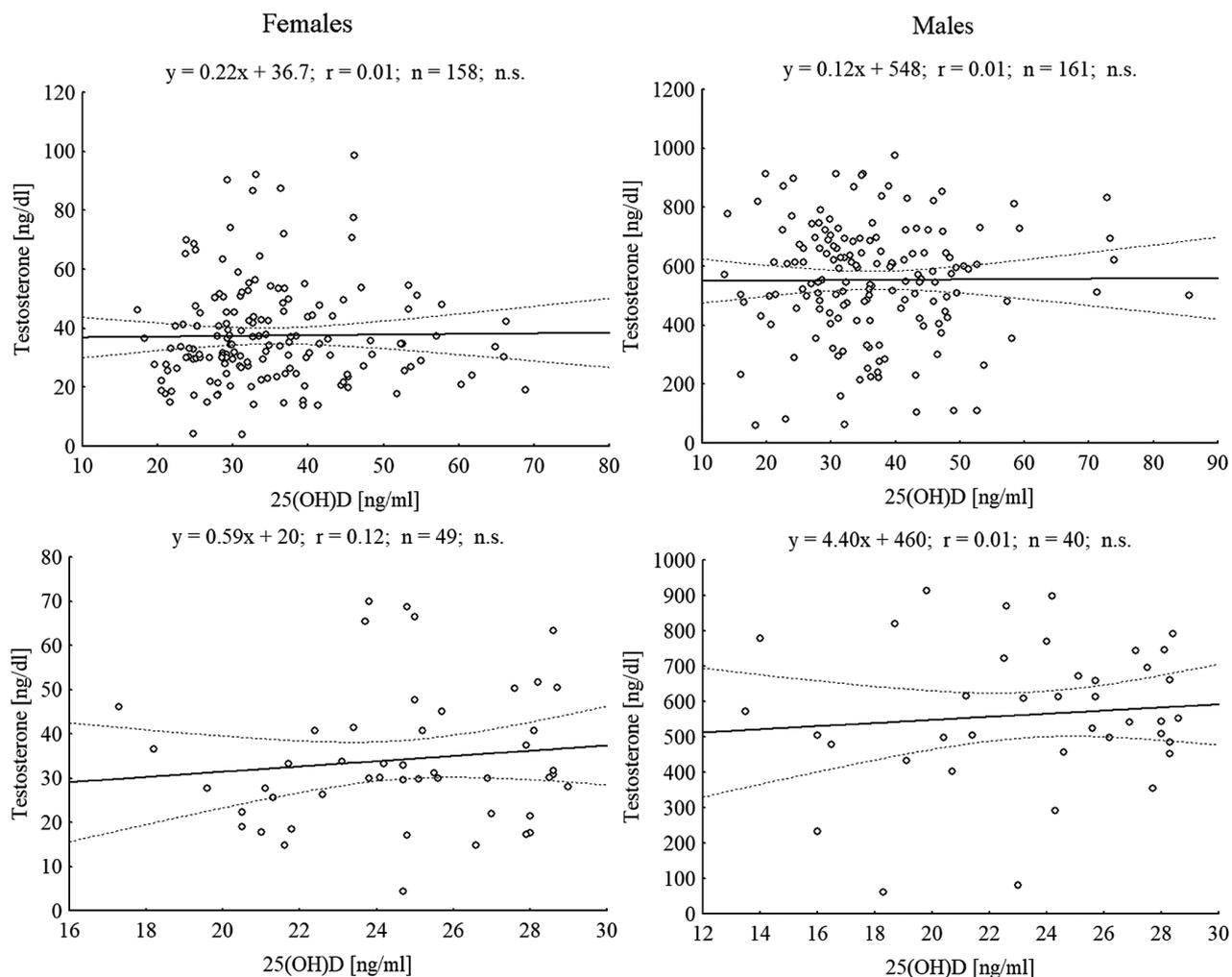


FIG. 5. Correlations between 25(OH)D and testosterone for the whole year in female and male athletes in all subjects (top) and in athletes with inadequate vitamin D status [25(OH)D below 30 ng/ml] separately (bottom).

athletes participate. Those locations are closer to the equator than Poland by approx. 21°, which proved to be a much stronger stimulus for vitamin D synthesis than the sun during summer in Poland. This is consistent with most studies in which the inverse correlation between latitude and 25(OH)D concentration was described (the average 25(OH)D concentration near the equator is 40 ng/ml, whereas in the far north or south it is just 15 ng/ml) [46, 47] and with our previous data [39]. The mechanism of more efficient vitamin D synthesis at lower latitudes results from better availability of UVB radiation due to a more favourable zenith angle.

In the present paper no seasonal pattern in testosterone concentration was observed. Similar findings were described in Finland (24 young adults sampled monthly), Belgium (5028 men aged 50–70 sampled over 3 years), California (915 old men), Boston (121 men, different races, 6 samples from each subject) and Southwest US (11000 patients grouped by months and seasons) [48–52]. Nevertheless, in several articles a seasonal pattern of testosterone concentration was identified,

but there was no consistency in the months of peak and nadir [53–58]. Other authors have concluded that in the general population testosterone concentration depends more on age of subjects, sleep patterns (diurnal manner that is dependent on sleep) and region (the length of day and temperature) than on seasonal rhythm [59, 60]. Sim et al. even linked the higher testosterone observed in Koreans in winter with seasonal dietary variation in consumption of seafood, affecting via zinc the male reproductive function [58]. Summing up the available literature, any seasonal variations in testosterone can only be characterized as inconsistent and occur in a specific population when highly influenced by one factor [61]. It is also hypothesized that higher testosterone observed in summer is linked with greater physical activity undertaken at that time.

Exercise is a potent stimulus for the production of testosterone and the hypothalamo-pituitary-gonadal axis was defined as more sensitive in younger men due to their higher level of physical activity [59, 62].

Studies on the seasonal rhythm of testosterone in athletes are very limited. Martinez *et al.* studied 12 Spanish basketball players and sampled testosterone in pre-season (October), while training (December) and in competition (March-April). A higher concentration of testosterone was observed in the competition period, when athletes have decreased the training volume [63]. Lombardi *et al.* in 167 Italian soccer players have found the peak testosterone concentration in June, when athletes were on holiday, supporting the hypothesis that testosterone depends mostly on exercise load [29].

In the present study testosterone concentration was higher only in male athletes during the transition period with no training or competition (September-November), which also supports the above-mentioned hypothesis that in athletes the testosterone concentration is mostly affected by the training loads and this effect overlaps (if any are present) seasonal fluctuations. It can be concluded that leisure time physical activity promotes testosterone production, whereas strenuous professional training decreases it. Similar findings comparing the influence of exercise were obtained in several other studies [64–70].

Studies on women's seasonal variations in testosterone are rare and show conflicting results; most of the available literature refers to male subjects [71–73]. The lack of significant differences observed in female athletes in the present study might be due to high variations within the menstrual cycle and use of hormonal contraceptives [74–75]. Those factors were not considered at the time of blood sampling and the majority of female subjects do not menstruate on a regular basis.

The relationship between vitamin D status and concentration of androgens and fertility was studied by both observational and interventional approaches, mainly in older subjects [6]. The European Male Ageing Study (3369 men in eight European countries) showed a positive correlation between 25(OH)D concentrations and total and free testosterone [76]. In a large cross-sectional study of 2299 men, higher total testosterone concentration and free androgen index were significantly higher in vitamin D-sufficient compared with vitamin D-insufficient or -deficient men [9]. Data from other cross-sectional studies have also shown a positive association between 25(OH)D and testosterone concentrations [7, 8, 77]. The opposite results were obtained by Sim *et al.*, who confirmed the seasonal pattern for vitamin D but not for testosterone in 1559 Korean males. Moreover, no relationship between 25(OH)D and testosterone was found [58].

Authors of all the studies mentioned above discuss the limitation of heterogeneity of subjects and multiple factors affecting the androgen level: age, BMI, smoking, alcohol, physical activity and chronic diseases. It is possible that vitamin D deficiency and low testosterone coincide as epiphenomenon in subjects with poor health condition or even the reverse causality theory could be considered [78].

Interventional studies which assessed the relationship between vitamin D supplementation and testosterone concentration also showed inconsistent results. In studies where positive results were obtained vitamin D supplementation was accompanied by body mass

reduction by diet and physical activity, which themselves are strong factors increasing the testosterone concentration [10, 79]. It is speculated that the effect of supplementation depends on the primary 25(OH)D concentration and is more pronounced in deficient subjects. In other studies no relationship was observed, either in sedentary or physically active subjects [80–82]. There is some evidence of the positive influence of vitamin D status on sperm motility [83, 84], as well as the effect of its supplementation on semen quality and male fertility [85, 86].

The two studies available in athletes also show the opposite results. In 50 young male ice hockey players examined in October at the latitude 50° N 25(OH)D concentration did not correlate with testosterone. Serum 25(OH)D concentration was also not associated with testosterone after adjusting for age, fat free mass and fat mass [30]. In Italian professional soccer players in latitude 41° to 42° N a seasonal pattern for both vitamin D and testosterone was found with a peak in August and June, respectively. Moreover, a weak but significant correlation between 25(OH)D and testosterone was detected [29]. The peak in testosterone occurred in the off-competition season. Therefore, the relationship could be accidental, as the seasonal rhythm of vitamin D depends on sun, whereas testosterone depends mostly on the training loads.

In the present study no relationship between 25(OH)D and testosterone was observed, either in athletes with adequate vitamin D status or in deficient ones, or according to the BMI or body composition. The seasonal pattern of vitamin D concentration was not reflected in testosterone and there was also no such relationship in athletes exposed to sunshine in winter and supplemented with vitamin D. Therefore, it supports the hypothesis that vitamin D status does not influence the testosterone concentration and any performance-enhancing effects of vitamin D in athletes are unlikely to be primarily mediated through testosterone. If vitamin D improves performance, it is more likely achieved by genomic and nongenomic signalling through the VDR in cardiac and skeletal muscles [30].

Concerning the current position of vitamin D in sport, primarily main health benefits should be considered, such as maintenance of immune function and musculoskeletal protection, and not its ergogenic effects. The results of the present study did not provide evidence that vitamin D influences testosterone concentration. The use of extreme doses by athletes does not improve performance; moreover, it can produce opposite effects, since there is evidence that not only low, but also high vitamin D concentrations are associated with impaired health, for example increased fall frequency, mortality, decreased sperm quality and suppression of calcitriol synthesis [87–90]. The proper understanding of the significance of the vitamin D status is a great challenge for sport medicine practitioners and nutritionists, who should provide athletes with personalized, safe supplementation protocols based on the guidelines established by real experts, not on myths and opinions of self-appointed experts. The results of the present study might be helpful to achieve that.

CONCLUSIONS

A circannual rhythm was present for vitamin D but not for testosterone. No relationship between vitamin D status and testosterone was found in track and field athletes.

Vitamin D insufficiency was present in 33% of Polish elite athletes, irrespectively from sex. The significant widespread of inadequate vitamin D status among athletes, who trained in Poland at the latitude

of 49–54°N and mostly outdoor, makes it vital to recommend to all athletes the regular monitoring of 25(OH)D concentration and use of reasonable supplementation.

Conflict of interest

The authors report no conflict of interest.

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