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ORIGINAL ARTICLE

## Circannual rhythm of plasmatic vitamin D levels and the association with markers of psychophysical stress in a cohort of Italian professional soccer players

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### ABSTRACT

Adequate plasmatic Vitamin D levels are crucial to maintain calcium homeostasis and bone metabolism both in the general population and in athletes. Correct dietary supply and a regular sun exposure are fundamental for allowing the desired and effective fitness level. Past studies highlighted a scenario of Vitamin D insufficiency among professional soccer players in several countries, especially in North Europe, whilst a real deficiency in athletes is rare. The typical seasonal fluctuations of Vitamin D are wrongly described transversally in athletes belonging to teams that play at different latitudes and a chronobiologic approach studying the Vitamin D circannual rhythm in soccer players has not been described yet. Therefore, we studied plasma vitamin D, cortisol, testosterone, and creatin kinase (CK) concentrations in three different Italian professional teams training at the same latitude during a period of two consecutive competitive seasons (2013 and 2014). In this retrospective observational study, 167 professional soccer players were recruited (mean age  $\pm$  sampling  $25.1 \pm 4.7$  years) and a total of 667 blood drawings were carried out to determine plasma 25(OH)D, serum cortisol, serum testosterone and CK levels. Testosterone to cortisol ratio (TC) was calculated based as a surrogate marker of overtraining and psychophysical stress and each athlete was drawn until a maximum of 5 times per season. Data extracted by a subgroup of players that underwent at least 4 sample drawings along a year ( $N = 45$ ) were processed with the single and population mean cosinor tests to evaluate the presence of circannual rhythms: the amplitude (A), acrophase ( $\Phi$ ) and the MESOR (M) are described. In total, 55 players (32.9%) had an insufficient level of 25(OH)D during the seasons and other 15 athletes (9.0%) showed, at least once, a deficiency status of Vitamin D. The rhythmometric analyses applied to the data of Vitamin D revealed the presence of a significant circannual rhythm ( $p < 0.001$ ) with the acrophase that occurred in August; the rhythms of Vitamin D levels were not different neither among the three soccer teams nor between competitive seasons. Cortisol, testosterone and TC showed significant circannual rhythms ( $p < 0.001$ ): cortisol registered an acrophase during winter (February) while testosterone and TC registered their peaks in the summer months (July). On the contrary, CK did not display any seasonal fluctuations. In addition, we observed weak but significant correlations between 25(OH)D versus testosterone ( $r = 0.29$  and  $p < 0.001$ ), cortisol ( $r = -0.27$  and  $p < 0.001$ ) and TC ( $r = 0.37$  and  $p < 0.001$ ). No correlation was detected between Vitamin D and CK. In conclusion, the correct chronobiologic approach in the study of annual variations of Vitamin D, cortisol and testosterone could be decisive in the development of more specific supplementation and injury prevention strategies by athletic trainers and physicians.

### ARTICLE HISTORY

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### KEYWORDS

25(OH)D; bone; chronobiology; circannual rhythm; cortisol; CK; soccer; testosterone; Vitamin D

## Introduction

Vitamin D (calcitriol) is a hormone which controls calcium and phosphate plasmatic concentration. Vitamin D induces metabolic modifications at intestinal, renal and bone levels aimed at

increasing absorption of calcium and, generally, rising the calcium and phosphate plasmatic concentrations.

In recent years, specific attention has been paid for vitamin D concentration in many conditions

and diseases (e.g., cancer, cardiovascular diseases, type 2 diabetes, autoimmune diseases, and infectious diseases) (Wacker & Holick, 2013); both deficiency and insufficiency have been claimed either to be pathogenic or to drive some symptoms in several diseases other than the classically vitamin D-associated osteomalacia and ricket (Christakos et al., 2016). The vitamin D deficiency has been definitely linked to overall mortality high rate in humans (El Hilali et al., 2015).

The general interest in medicine and biology for vitamin D was rapidly translated in specific studies on sportsmen, since the real importance of its role in physical exercise. In general, studies on athletes highlighted a surprisingly high prevalence of vitamin D insufficiency, in both outdoor and indoor disciplines, as recently reported in a review devoted to this topic (Lanteri et al., 2013). The biological reason of the prevalent insufficiency is not known. However, since the expression of the vitamin D receptor (VDR) in skeletal muscles (Bartoszewska et al., 2010; Ceglia, 2009; Hamilton, 2010) it is likely that athletes experience a higher degree of vitamin D consumption linked to the greater muscle activity.

Blood concentrations of vitamin D are generally defined on the basis of the plasma concentrations of the precursor 25-hydroxycholecalciferol, namely 25(OH)D, which can be easily measured by current laboratory methods and is sufficiently stable to be quantified. The definitive thresholds for vitamin D status are not definitely established, also due to subsequent modifications of reference range in general population. However, values commonly accepted are: <50 nmol/L deficiency, <80 nmol/L insufficiency; or <20 ng/mL deficiency, <30 ng/mL insufficiency (Larson-Meyer & Willis, 2010).

Regular and physiological concentration of vitamin D in athletes is really crucial to maintain calcium homeostasis and bone metabolism, but also to sustain the continuous skeletal and myocardial muscle contractility. Therefore, correct dietary supply and a regular sun exposure, which is crucial to trigger the production of vitamin D, are fundamental for allowing the desired and effective fitness level (Ogan & Pritchett, 2013).

Some studies have been published also in football players (Eskici, 2016; Solarz et al., 2014). They

generally showing a scenario of insufficiency, whilst a real deficiency in professional athletes is quite rare. Vitamin D levels are associated with neuromuscular performance and aerobic capacity in professional football players, and there is a linear relationship between vitamin D levels and jumping performance,  $VO_2$ max and speed. Vitamin D could also influence  $VO_2$ max through the effects on erythropoiesis, possibly affecting iron metabolism, by modulating the inflammatory response, and increasing the erythropoietin resistance (Koundourakis et al., 2014).

None of the 70 players belonging to 3 different Greek professional teams was deficient (<20 ng/mL), or severely deficient (<10 ng/mL); however, half of them were insufficient (<30 ng/mL). Interestingly, after the rest period, only 2 had insufficient concentrations: a six-weeks transition period had a boosting effect on vitamin D levels (Koundourakis et al., 2014), supporting the hypothesis that workload and psychophysical stress are associated with vitamin D consumption. Insufficiency was widely present in players belonging to Qatar (84% of 342 athletes had concentrations <30 ng/mL, despite the latitude and the observation period (July). However, the authors did not find any association with lower limb isokinetic peak torque, while there was an association with lower body mass (Hamilton et al., 2014). Similar findings were reported in Polish players during a training (83% of players with concentrations <30 ng/mL) (Kopec et al., 2013) and in English ones (65% of players with concentrations <30 ng/mL during summer) who live and trains at northern latitudes (Morton et al., 2012). Supplementation is often practised to support the regular concentrations during winter (Galan et al., 2012).

The typical seasonal fluctuations of Vitamin D are not totally confirmed and this is justified by the fact that insufficiencies are wrongly described transversally in athletes belonging to teams which play at different latitudes. The Earth follows an elliptical orbit around the sun and this astronomical characteristic leads to seasonal variations in the duration of daylight. The length of daylight varies among regions with different latitudes: athletes exercising in regions close to the equator are exposed to seasonality with longer days and higher

temperatures in summer. On the other side, athletes can also train and compete close to the polar circles where there is a constant daylight during summer and total darkness in winter (Atkinson & Drust, 2005; Rossi et al., 2015; Vitale et al., 2013). It has been shown, for instance, that young female soccer players, living in northern Sweden at 64° north of latitude and playing with low sun exposure during winter season, had low serum levels of 25(OH)D and this insufficiency was significantly correlated with time to peak torque in knee extensors (Brännström et al., 2016). In addition, in a cohort of male collegiate hockey players, living at 44° north of latitude, no deficient levels of Vitamin D were detected during the off-season (a period of rest from physical exercise between May and July with high sun exposure). The players' levels of serum 25(OH)D were also correlated with grip strength and peak power during the Wingate Anaerobic Test (Fitzgerald et al., 2015).

Not only Vitamin D levels are crucial for physical activity. Blood hormones concentrations should be deeply studied and investigated in athletes as they are strongly involved in sport performance (Urhausen et al., 1995). The serum concentration of creatine kinase, i.e., is used as an index of skeletal muscle fibre damage in sport and exercise (Clarkson et al., 2006). Testosterone is widely considered a marker of anabolism while cortisol is assumed to be a marker for catabolism and psychophysiological stress and their ratio (testosterone on cortisol) is commonly used to monitor the athletes' status of overtraining (Banfi et al., 1993). Testosterone has also large effects on strength and muscle adaptations to exercise (Bhasin et al., 2001) and, on the contrary, cortisol can exhibit an inhibition effect on the neuromuscular system. Furthermore, it has been observed that these hormones concentrations varies along the competitive season in soccer player (Banfi & Dolci, 2006; Lombardi et al., 2012; Sanchis-Gomar et al., 2015).

In accordance with the definition of circannual rhythms (Halberg et al., 1977), they are rhythms that have a period that is about a year. To the best of our knowledge, a correct chronobiologic approach studying Vitamin D and blood hormones circannual rhythm in soccer players has not been described at all.

Therefore, we retrospectively studied plasma vitamin D, serum cortisol and testosterone and creatin kinase concentrations in three different professional teams of Second Italian Division (Serie B), training at the same latitude during a period of two consecutive competitive seasons (2013 and 2014), in order to detect and describe their possible circannual rhythms. A second aim of the study was to evaluate the possible correlation between Vitamin D and cortisol, testosterone and creatin kinase.

## Materials and methods

### Study design, participants, and blood drawings

This is a retrospective observational study analysing, in consecutive samples, plasma 25(OH)D, serum cortisol, serum testosterone and creatin kinase (CK) concentrations in a cohort of professional soccer players that underwent routine medical examinations during the competitive seasons.

Blood drawings, 25(OH)D, and CK analysis were routinely performed for checking the health status of athletes and an informed consent was administered to the athletes. No additional blood was drawn for the specific measurements. Athletes belong to three football teams of the Italian Second Division (Bari Football Club – Team 1, Virtus Lanciano – Team 2, and Pescara Calcio – Team 3) were followed during two consecutive competitive seasons, from January 2013 to December 2014. For several years, the Italian Second Division of professional soccer follows the same calendar: the competitive season starts the last week of August and it ends the week before June. Therefore, the off-season typically takes place in June and the pre-season starts in July. Blood drawings were performed in January, February, March, April, August, October, and December in 2013, in January, February, March, April, July, August, October, November, and December in 2014. The teams were regularly training and playing at latitudes with high sun exposure, even during autumn and winter (between 41° and 42° N of latitude). The soccer players meeting the following inclusion criteria were then recruited to participate in the study: age 18–40 years and male sex. Exclusion

criteria were: tobacco use, use of medications, and medical conditions contraindicating physical exercise as diagnosed by a sports medicine physician. One hundred sixty-seven subjects participated in the study (mean age at sampling  $25.1 \pm 4.7$  years), supplying a total of 667 records for both 25(OH)D and creatin kinase (CK). Each athlete was drawn until a maximum of 5 times per season. Diet was controlled by the team physicians; no regular supplementation was followed by the three teams, but a sporadic intake of vitamin D by single athlete should not be excluded. Blood was obtained by standard venepuncture in plain tubes for serum and K2-EDTA tubes (Becton Dickinson, Franklin Lakes, NJ, USA) for plasma. Samples were analysed immediately after drawing. Plasma vitamin D total ECLIA (electrochemiluminescent immunoassay), serum cortisol ECLIA, and serum testosterone ECLIA (testosterone II) Elecsys were run on a Cobas E 411 (Roche Diagnostic Corp., Rotkreuz, CH). The sensitivities lower limits of detection (LLD) of the tests were 9.00 ng/mL and 4.01 ng/mL, respectively, for vitamin D, 8.5 nmol/L and 0.500 nmol/L for cortisol; 0.416 nmol/L and 0.087 nmol/L, for testosterone. Maximum intra-assay ( $CV_w$ ) and inter-assay variation ( $CV_b$ ) were 6.8% and 13.1%, respectively, for vitamin D, respectively, 1.4% and 1.6% for cortisol, 4.7% and 8.4% for testosterone. CK activity (IL Test™ CK, Werfen Life Group SA, L'Hospitalet de Llobregat, E, EU) was run on a ILab 600 (Diamond Diagnostics Inc., Holliston, MA, USA). The sensitivity of the test was 0.24 U/L and the LLD was 1 U/L. Maximum  $CV_w$  and  $CV_b$  were 1.1% and 3.5%. Testosterone to cortisol ratio (TC) was calculated based as a surrogate marker of overtraining and psychophysical stress. The study protocol is in compliance with current national and international laws and regulations governing the use of human subjects (Declaration of Helsinki II) and the guidelines required by the journal (Portaluppi et al., 2010).

### **Statistical analysis**

#### **Analysis of circannual rhythmicity**

Vitamin D, testosterone, cortisol, TC and CK data were processed with the single and population mean cosinor tests to evaluate the

presence of a circannual rhythm. These methods examine the rhythmometric characteristics of both the single subject and the whole population (Halberg et al., 1977; Nelson et al., 1979) and they allow to calculate a cosine function of which the amplitude (A), the acrophase ( $\Phi$ ) and the MESOR (M) are described. M is the Midline Estimating Statistic of Rhythm (MESOR) and it represents the rhythm-adjusted mean; A is the amplitude which is the measure of one half the extent of the rhythmic variation in a cycle;  $\Phi$  is the acrophase, it indicates the time interval within which the highest values of the variable are expected. The three parameters are reported with the relevant 95% confidence intervals (CI). To perform the cosinor tests is required to have at least 4 observations for each series (subject), therefore we included in the analysis the data of soccer players that underwent at least 4 sample drawings along the year. In total, data of 45 soccer players were grouped and analysed: 23 by the competitive season 2013 and 22 by the 2014. The statistical analyses were carried out using the Time Series Analysis Serial Cosinor 6.3 (Expert Soft Technology, Richelieu, France).

In addition, we compared the rhythmometric parameters (MESOR, amplitude and acrophase) of Vitamin D among the three professional soccer teams: after checking the normality of the distribution of each parameter using graphical methods and the Shapiro–Wilk's test, we used a one-way ANOVA followed by the Tukey–Kramer post-hoc test for the comparison.

#### **Comparisons and correlation analyses**

Data homogeneity among different teams was tested through the one-way ANOVA analysis and Bonferroni's post hoc analysis. Significance was set at  $p < 0.05$ . The existence of a correlation between vitamin D and either CK, cortisol, testosterone or TC for the whole group of players was tested by the means of the Pearson's correlation index. Correlations were considered significant when  $r > 0.25$  and  $p < 0.05$ . The same statistical analysis was also applied to the data of the subsample composed by the 45 players selected for the rhythmometric analysis.

**Table 1.** Summary of the collected data on vitamin D in professional soccer players.

	All	Team 1	Team 2	Team 3
<b>Samples</b>				
Subjects, N	167	37	58	72
Records, N	668	124	298	246
<b>Vitamin D</b>				
Median	40.0	39.6	42.0	41.0
Min, ng/mL (month/year)	7.9 (Feb)	7.9 (Feb)	18.0 (Mar)	11.0 (Mar)
Max, ng/mL (month/year)	102.0 (Jul)	70.0 (Aug)	92.0 (Jul)	102.0 (Jul)
Insufficiency, N (% records)	85 (12.7%)	27 (21.8%)	27 (9.0%)	31 (12.6%)
Insufficiency, N (% subjects <sup>a</sup> )	55 (32.9%)	16 (43.0%)	15 (25.9%)	24 (33.3%)
Deficiency, N (% records)	20 (3.0%)	8 (6.4%)	3 (1.0%)	9 (3.7%)
Deficiency, N (% subjects <sup>a</sup> )	15 (9.0%)	4 (10.8%)	3 (5.2%)	8 (11.1%)

<sup>a</sup>Subjects found at least one time vitamin D either insufficient or deficient.  
 Insufficiency: 25(OH)D concentrations comprised between 20.0 ng/mL and 30.0 ng/mL.  
 Deficiency: 25(OH)D concentrations lower than 20.0 ng/mL.

## Results

Data homogeneity among teams was verified: no differences were observed in the levels of 25(OH) D, cortisol, testosterone and CK. A general summary about the collected data of Vitamin D, both for the whole sample and for the three different professional teams, is reported in Table 1. In total, 55 players (32.9%) had an insufficient level of 25 (OH)D during the season and other 15 athletes (9.0%) showed, at least once, a deficiency status of Vitamin D levels.

### Circannual rhythmicity

The single cosinor method revealed the presence of a statistically significant rhythm in each of the 45 soccer players for Vitamin D, cortisol, testosterone and TC ( $p < 0.001$ ). Only CK did not show a significant circannual rhythm for none of the participants.

### Vitamin D

The population mean cosinor applied to the data of Vitamin D revealed the presence of a significant

circannual rhythm ( $p < 0.001$ ) and the acrophase occurred during summer season (August). Table 2 reports the rhythmometric parameters of Vitamin D and Figure 1 shows graphically the circannual rhythm of this variable. Table 3 shows the rhythmometric characteristics of Vitamin D for the three professional soccer teams: the one-way ANOVA did not show any significant differences for both MESOR, amplitude and acrophase.

### Cortisol, Testosterone, TC and CK

The population mean cosinor applied to the data of cortisol, testosterone and TC revealed the presence of significant circannual rhythms ( $p < 0.001$ ). In Table 2 are reported the rhythmometric characteristics of the three variables and in Figures 2–4 are graphically showed the rhythms. On the other side, no significant circannual rhythm was detected for CK.

### Correlation between vitamin D and the markers of muscle overuse and psychophysical stress

No correlation of Vitamin D with cortisol, testosterone, TC and CK was found for the whole

**Table 2.** Rhythmometric analysis (population mean cosinor) of Vitamin D, cortisol, testosterone, TC and CK for the subsample of soccer players with at least 4 sample drawings during the competitive season ( $N = 45$ ).

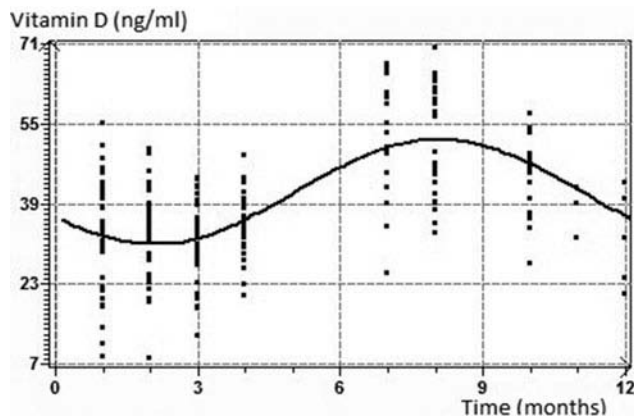
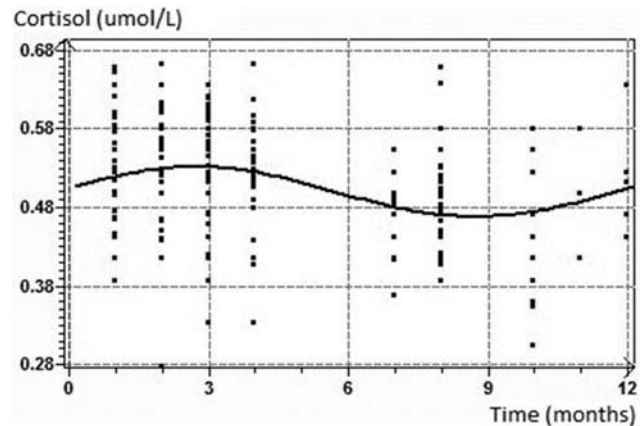
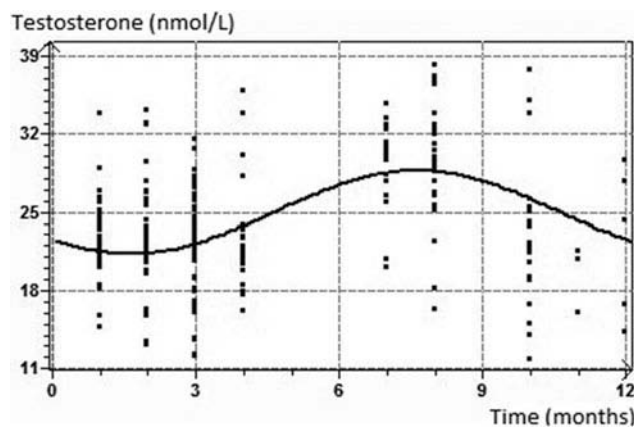
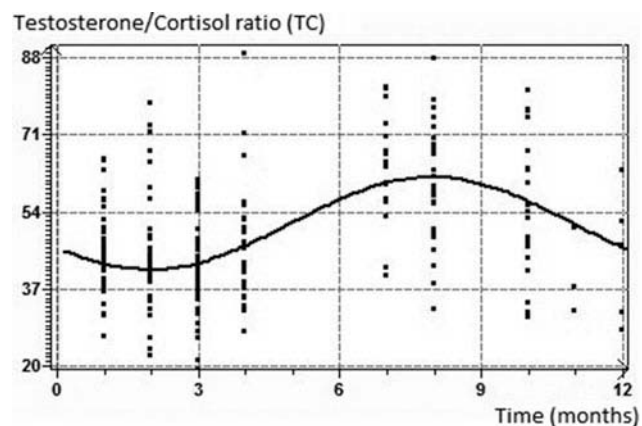
	PR (%)	p-Value	MESOR [mean and 95% CI]	Amplitude [mean and 95% CI]	Acrophase	
					Degrees [mean and 95% CI]	Month
Vitamin D (ng/ml)	88.2	<0.001	41.5 [39.5–43.6]	10.4 [9.67–11.3]	242 [232–252]	August
Cortisol (μmol/L)	58.5	<0.001	0.50 [0.48–0.52]	0.032 [0.017–0.047]	81.9 [49.5–114]	February
Testosterone (nmol/L)	71.4	<0.001	25.0 [24.0–26.0]	3.73 [3.62–3.89]	229 [213–245]	July
TC	73.6	<0.001	51.5 [49.0–53.9]	10.2 [9.28–11.6]	239 [224–253]	July
CK (U/L)			No significant circannual rhythm was detected.			

PR: percentage of rhythm. MESOR: Midline Estimating Statistic of Rhythm. Amplitude: half the difference between the highest and the lowest points of the cosine function best fitting the data. Acrophase (degrees and month) indicates the time in which the highest values occur.

**Table 3.** Circannual rhythm characteristics and population mean cosinor analysis of Vitamin D levels for the 3 professional soccer teams.

	N	Year	PR (%)	p-Value	MESOR [mean and 95% CI]	Amplitude [mean and 95% CI]	Acrophase	
							Degrees	Month
<b>Team 1</b>	23	2013	94.3	<0.001	42.0 [39.1–44.9]	12.6 [11.5–14.0]	242 [233–251]	August
<b>Team 2</b>	13	2014	83.7	<0.001	42.3 [37.6–47.0]	7.9 [6.8–9.1]	253 [234–273]	August
<b>Team 3</b>	9	2014	93.1	<0.001	41.6 [38.3–45.9]	10.3 [9.6–11.1]	241 [231–249]	August
<b>Total Group</b>	45	2013/2014	88.2	<0.001	41.8 [39.5–43.6]	10.4 [9.7–11.3]	242 [232–252]	August

PR: percentage of rhythm. MESOR: Midline Estimating Statistic of Rhythm. Amplitude: half the difference between the highest and the lowest points of the cosine function best fitting the data. Acrophase (degrees and month) indicates the time in which the highest values occurs.

**Figure 1.** 25(OH)D (ng/mL) circannual rhythm.**Figure 3.** Serum cortisol (µmol/L) circannual rhythm.**Figure 2.** Serum testosterone (nmol/L) circannual rhythm.**Figure 4.** Testosterone/cortisol ratio (TC) circannual rhythm.

sample. Conversely, taking into consideration the subsample of 45 soccer players selected for the rhythmometric analysis, we observed weak but significant correlations between 25(OH)D versus testosterone ( $r = 0.29$  and  $p < 0.001$ ), cortisol ( $r = -0.27$  and  $p < 0.001$ ) and TC ( $r = 0.37$  and  $p < 0.001$ ). No correlation was detected between Vitamin D and CK.

## Discussion

This study is highlighted by two key findings: first, we observed a relative low number of professional soccer players with insufficiency or deficiency levels of 25(OH)D during the competitive season, registered especially in winter months. Second, significant circannual rhythms for Vitamin D, cortisol and testosterone were detected. This result

could help athletic trainers and medical staff of professional teams to develop better strategies to prevent athletes' psychophysical stress.

We followed a high number of soccer players during a long period for evaluating possible deficiencies and/or insufficiencies of vitamin D, which can be deleterious in general for physical exercise and, in particular, for skeletal muscle activity and bone homeostasis. In the observed group of football players, we found insufficiency in a percentage of athletes (55 players, 32.9%) lower than that described in previous studies (Kopeck et al., 2013; Morton et al., 2012).

Two important variables could help in interpreting this discrepancy: the latitude at which the teams use to play and the different season of the year: the higher sun exposure of our sample (between 41° and 42° N of latitude) in comparison with the environmental conditions of players in North Europe partially explains these differences.

We confirm the importance of sun exposure because we observed higher concentrations of vitamin D during summer and lower levels during winter: in the present study, among all the 668 records, we observed the lowest value of 25(OH) D in February (7.9 ng/mL) and the highest one in July (102.0 ng/mL) (Table 1). Additionally, we detected the presence of a statistically significant circannual rhythm of this variable for those players that underwent at least four sample drawings during the competitive season ( $N = 45$ ) (Figure 1). The typical seasonal fluctuations over the year are maintained even in highly trained athletes, and it should be properly considered for interpreting laboratory data. We also compared the rhythmometric parameters of Vitamin D among professional teams and between competitive seasons (2013 versus 2014) but no differences for MESOR, amplitude and acrophase were observed, the circannual rhythms were extremely similar and the acrophases always occurred in August (Table 3).

Differences among sport seasons should be taken into account when vitamin D concentrations are evaluated. Previous reports on vitamin D in football players, commonly describing high or very high number of athletes characterized from insufficiency or overt deficiency, were

related to a single season (Hamilton et al., 2014). It is crucial to follow the players for a long time to obtain a real and effective background, which is fundamental for physicians' decisions, especially for a possible supplementation. In fact, a single season could not be representative of the real complexity of vitamin D metabolism (intake and consumption) of a team. The supplementation should not be globally performed in autumn to prevent possible insufficiencies but it should be personalized by player, especially in athletes who train and play in countries with lower sun exposure. Moreover, supplementation should be a decision supported by plasma concentrations data, physiological characteristics and performance levels of athletes, while the intake at pharmacological concentrations could induce, if not controlled, an unhealthy accumulation. The high turnover of athletes, typical of modern football, also recommends a long-term monitoring period, including possible ethnic, dietary, and sun exposure differences.

Data of cortisol and testosterone were also collected as these hormones' modifications in athletes, and their ratio (TC) too, are indicative in the study of overtraining adaptations, such as the imbalance between the catabolic and anabolic phases of the metabolism (Banfi & Dolci, 2006). We found significant circannual rhythms for both hormones with cortisol showing an acrophase during winter (February) while testosterone and TC registered their peaks in the summer months (July) (Table 2). These results were expected as soccer players in Italy use to suspend their professional activities in June before starting again with the pre-season conditioning program in July. The peak of their sport activity usually occurred during winter months and this is the period of the year in which the highest values of cortisol and the lowest values of Vitamin D and testosterone were recorded. No significant circannual variations were observed for CK and this was the only marker of physical stress that did not correlate with Vitamin D. On the contrary, concerning the subgroup of 45 soccer players, we observed weak but significant correlations between 25(OH) D and testosterone ( $r = 0.29$ ), cortisol ( $r = -0.27$ ) and TC ( $r = 0.37$ ).



## Limitations

This investigation has several limitations. Being Vitamin D, cortisol and testosterone strongly associated with sport performance, it would be advisable for future researches to administer, along the competitive season, a battery of sport-specific physical tests to detect the influence of seasonality in soccer players' performance. The study population was composed by a relatively small sample and the participants are not representative of athletes practicing other sports, female athletes were not recruited too. Moreover, a control group of male non-athletes could have helped in better addressing the aim of the study. Some sensible factors were not controlled: sun protection or melanin content in the skin could affect serum 25(OH)D levels. Finally, as it is largely documented that the training load significantly varies in professional soccer players during the different phases of the competitive season (Jeong et al., 2011), the training history should be recorded during each month to better understand how training load influence Vit D, cortisol, testosterone and CK concentrations.

## Practical applications

It is essential to emphasize that, especially in Italy and Southern Europe, a particular care is paid in constantly monitoring the players' health status thanks to high-quality interventions by teams' medical staffs. We are strongly convinced that vitamin D monitoring should be contemplated in routine checking in professional players all over the world: the aim of soccer teams' physicians is to balance out health and peak performance and, at this purpose, the prevention of vitamin D insufficiency, or even deficiency, is fundamental. Vitamin D insufficiency is associated with several complications for bone metabolism, skeletal muscle contractility and for the athletic performance too. Cases of deficiencies should be immediately treated and the correct chronobiologic approach in the study of seasonal variations of Vitamin D could be decisive in the development of more specific supplementation and injury prevention strategies.

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## Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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