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## Original article

# Serum 25-hydroxyvitamin D in early autumn to ensure vitamin D sufficiency in mid-winter in professional football players

Fernando Galan<sup>a,\*</sup>, Juan Ribas<sup>b</sup>, Pilar M<sup>a</sup> Sánchez-Martinez<sup>c</sup>, Tomás Calero<sup>d</sup>, Antonio Barco Sánchez<sup>c</sup>, Adolfo Muñoz<sup>d</sup>

- a Department of Medicine, Medical School, University of Seville, Internal Medicine Service, University Hospital Virgen Macarena, Avda. Doctor Fedriani s/n, Seville 41009, Spain
- <sup>b</sup> Department of Medical Physiology and Biophysics, Medical School, University of Seville, Seville 41009, Spain
- <sup>c</sup> Department of Clinical Biochemistry, University Hospital Virgen Macarena, Seville 41009, Spain

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#### SUMMARY

Background & aims: There is growing awareness that vitamin D sufficiency is required for overall optimal health. Most experts agree that 25-hydroxyvitamin D levels of at least 75 nmol/L, as sufficient vitamin D status. Our aim was to investigate the serum 25-hydroxyvitamin D concentration required in mid-October to ensure vitamin D sufficiency in early February, and to assess the rate of vitamin D insufficiency in both seasons.

*Methods*: We measured serum 25-hydroxyvitamin D, parathormone, and other related biochemical parameters, in a sample of 28 professional football players homogeneous in factors influencing serum 25-hydroxyvitamin D concentration in a sunny area of southern Spain.

*Results:* The serum 25-hydroxyvitamin D concentration of 122.7 nmol/L was required; 14.3% reached this level. Ninety-three percent had levels  $\geq$ 75 nmol/L in mid-October, and 64% had levels <75 nmol/l in early February ( $\chi^2$  test,  $\rho=0.001$ ).

Conclusions: Despite the homogeneity in sunlight exposure and vitamin D intake few football players reached the level ensuring vitamin D sufficiency in mid-winter, and two thirds had vitamin D insufficiency in early February. Given our findings, it would be advisable to assess the vitamin D levels in early autumn, although additional studies are necessary.

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### 1. Introduction

Exposure to sunlight is the main requisite for vitamin D synthesis in the skin. The skin uses ultraviolet B (UVB) light (280–315 nm wavelength) to form previtamin D3 from 7-dehydrocholesterol, which is rapidly converted to vitamin D3. Vitamin D, regardless its source and release into circulation, is metabolized in the liver to 25-hydroxyvitamin D (25 (OH) D). 25-hydroxyvitamin D is subsequently metabolized in the kidneys to its active form, 1,25-dihydroxyvitamin D which acts through specific vitamin D receptors. The major circulating form of vitamin D—25-hydroxyvitamin D—is a good reflection of cumulative effects of exposure to sunlight, dietary or supplement intake of vitamin D and is therefore used by clinicians to determine vitamin D status.

There is an ongoing debate about the cut-off or range of  $25\,(OH)\,D$  to define an optimal vitamin D status to meet all physiological needs.

What constitutes an 'optimum' level remains to be determined and may well be different for different physiological processes.<sup>2</sup> Estimation of optimal serum concentrations for multiple health outcomes begins with 75 nmol/L, and the most suitable between 90 and 100 nmol/L.<sup>3</sup> The most common cut-off or range values of serum 25 (OH) D currently used to define vitamin D status are: deficiency <50 nmol/L; insufficiency 50−74 nmol/L and sufficiency ≥75 nmol/L.<sup>1-4</sup> Defining categories of vitamin D insufficiency and deficiency will affect prevention strategies employed in clinical setting. It is self-evident that changing the threshold (cut-off) will alter the percentage of people with such condition. It will also depend upon the assays used to measure serum 25 (OH) D levels. Regardless of the definition of vitamin D sufficiency usually 50 nmol/L or 75 nmol/L, it is apparent that suboptimal 25 (OH) D levels are a worldwide phenomenon with hardly any region spared.<sup>5</sup>

In temperate climates, serum 25 (OH) D concentrations, rise and fall throughout each year, due to a corresponding variation in the amount of UVB radiation reaching the skin. At latitudes above 37 ° north during the months of November through February very little

<sup>&</sup>lt;sup>d</sup> Sport Medicine Physician, Seville 41005, Spain

<sup>\*</sup> Corresponding author. Tel.: + 34 954 556 616; fax: + 34 954 556 617. *E-mail address:* fgalan@us.es (F. Galan).

if any vitamin D3 is produced in the skin. Due to these fluctuations in 25 (OH) D concentrations, some persons may be in the target range during the summer months but perhaps these may not be sustained during the winter months, even in sunny latitudes.

Sensible sun exposure can provide an adequate amount of vitamin D3, which is stored in body fat and released during the winter, when vitamin D3 cannot be produced. Barger-Lux and Heaney reported that in a group of 26 healthy men for whom summer sun exposure was the principal source of vitamin D, a late summer 25 (OH) D level of approximately 127 nmol/L was needed to avoid levels falling under 75 nmol/L by late winter.

It is not an easy task finding a group of sun-exposed individuals<sup>9</sup> with considerable homogeneity in the factors influencing serum levels of 25 (OH) D, to study the vitamin D status properly. The included factors were: duration, time of day and body surface area exposed to the sun, latitude, season, weather conditions, skin type, place of work, type of clothing, use of sunscreens, skin type, calcium and vitamin D intake, body mass index, day and time of blood collection, use of drugs that interfere with the metabolism of vitamin D and oral vitamin D supplementation. We conducted this study in a healthy sample of professional football players that met these characteristics of homogeneity, in a highly sunny area of Southern Spain, with the aim to investigate serum 25 (OH) D concentration required at mid-October (October 15) to ensure vitamin D sufficiency equal to or greater than 75 nmol/L, in early February (February 2) and to assess the rate of vitamin D insufficiency in both seasons.

#### 2. Material and methods

#### 2.1. Study population

This study was conducted on a sample of 34 healthy professional football players, white Caucasians, from two First Division teams of Andalusia (Southern Spain), located at latitude 37 ° 23′ N and altitude 27 m above sea level with an annual average of approximately 3000 h of sunshine per year. Six participants were excluded because they did not provide a blood sample in October or February. Thus, only 28 participants were included in the analysis. All football players were informed and gave their written consent. The study received approval from the ethics committee of the University of Seville.

These professional soccer players were an appropriate group to investigate the vitamin D status properly, due to considerable homogeneity in the factors influencing serum levels of 25 (OH) D, as calcium intake and vitamin D remained constant throughout the study period. This was possible due to a schedule and daily training program was very well designed by the coach and technical team. And also because, a team of specialists in sports medicine and endocrinology and nutrition developed a balanced diet with 1000 mg of calcium and 5  $\mu g$  of vitamin D, which was followed by all football players during the pre-season, as well as in the concentration previous to the match. Of course, meals carried out at home were carefully controlled. In fact, specialists developed a diet for each player, taking into account food preferences, and compliance was monitored daily through personal interviews.

Factors influencing serum 25-hydroxyvitamin (25 (OH) D) during the study were the same for all 28 professional football players participating, are shown in Table 1.

#### 3. Methods

After overnight fasting, blood was collected, centrifuged and the serum fraction was removed after clotting and stored at -80 °C. Calcium, phosphate, creatinine, magnesium, alkaline phosphatase,

**Table 1**Factors influencing serum 25-hydroxyvitamin during the study were the same for all 28 professional football players participating.

Cloudy and/or rainy days in last half of	12 days
July, August, September and first half of October	
Length of sun exposure from July 21 to October 15	12 weeks
Sun exposure per week <sup>a</sup>	53 h
Total sun exposure	248 h
Fraction of body surface area <sup>b</sup>	45%
Monthly UV index. Year 2008	
August	9
September	7
October	5
November	3
Fitzpatrick skin type	III
Day and hour of blood collection	October 15 and
	February 2, at 8:00 am
Sunscreen use	No
Drugs that interfere with vitamin D metabolism or oral Vitamin D supplementation	No

<sup>&</sup>lt;sup>a</sup> Training schedule: from the second half of July and throughout August, the training sessions were held for 2 h in the morning (10.00–12.00 am) and for 2 h in the afternoon (6:00–8:00 pm) every day. In September, for 2 h in the morning (10.00–12.00 am) and for 1 h in the afternoon (6:00–7:00 pm), five days a week. During the first half of October, for 2 h in the morning (10.00–12.00 am), five days a week.

and total protein were measured on the automated analyzer, Architect c8000 (Abbott Diagnostic, Spain). Serum levels of intact parathyroid hormone (PTH) were measured, in the same batch, by means of a two site sandwich immunoassay using direct chemiluminescence technology (ADVIA Centaur intact PTH-serum assay, Siemens Medical Diagnostics). 10 Analytical sensitivity was 0.265 pmol/L. The intra-assay coefficient of variation (CV) was between 2.2 and 3.5%. The 25-hydroxyvitamin D (25 (OH) D) concentrations were determined by the DiaSorin "25-OH Vitamin D TOTAL" competitive chemiluminescent immunoassay (CLIA) on the automated LIAISON analyzer (Stillwater, MN). This method has 100% specificity for both 25 (OH) vitamin D2 and 25 (OH) vitamin D3. This assay has a limit of detection of 10 nmol/L. The intra-assay CV was between 1,9 and 2.9%, and the inter-assay CV between 5.3 and 7.7%. Samples were analyzed with quality control samples inserted at periodic intervals.<sup>11</sup> The method used in our laboratory was quarterly monitored by participating in the vitamin D External Quality Assessment Scheme (DEQAS) for 25-hydroxyvitamin. Validity is supported by our compliance with the accuracy objectives of the DEOAS. In addition, we have the proficiency certificate issued annually to our laboratory for having met the performance target set by the DEQAS Advisory Panel for 25 (OH)D assays.

Anthropometric parameters were measured (height, weight, and body mass index). BMI was calculated as weight (kg) divided by height (m<sup>2</sup>).

#### 3.1. Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Science (SPSS version 15.0) for Windows. Continuous variables were expressed as mean  $\pm$  standard deviation (SD) and categorical variables as numbers and percentages. Paired samples t-test was used to compare means of parameters analyzed in the serum samples collected twice in the same participants. The chisquare test of McNemar-Bowker ( $\chi^2$  test) was used to compare changes for number and percentage of players, according to serum 25 (OH) D in October and February. Pearson coefficient was used to detect any cross-correlation among other biochemical data. A p-value less than 0.05 was considered statistically significant.

a week.

<sup>b</sup> Fraction of body [surface area following the adaptation of the "rule of nines" made by Barger-Lux and Heaney <sup>8</sup>].

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