Journal of Steroid Biochemistry & Molecular Biology 175 (2018) 97–101

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry & Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Serum 25-hydroxyvitamin D levels in a healthy population from the North of Portugal

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ARTICLE INFO

Article history:
Received 26 July 2016
Received in revised form 26 September 2016
Accepted 3 November 2016
Available online 5 November 2016

Keywords:
Portugal
25(OH)D levels
Vitamin D status
Healthy adult population

ABSTRACT

Vitamin D *status* in human populations has become a matter of great concern, in the wake of a multitude of published works that document widespread vitamin D deficiency across Europe, even in countries with abundant sunlight. In Portugal there are no measures of 25-hydroxyvitamin D – 25(OH)D – levels in the general adult population. The purpose of this study was to measure 25(OH)D levels in a healthy population cohort and investigate the possible association with season and selected demographic and laboratory measurements.

A cohort of 198 participants (18–67 years) living in the north of Portugal, Porto, conducted in July and August 2015 (summer time) and April 2016 (winter time) was studied to evaluate serum 25(OH)D levels. Sociodemographic characteristics (age, sex and body mass index) and season of the year were taken into account as possible 25(OH)D levels codeterminants.

In the whole group, the mean level of serum 25(OH)D was 55.4 ± 23.4 nmol/L, with 48% of the population presenting levels compatible with vitamin D deficiency (below 50 nmol/L). In the winter period, this value reaches 74%. No statistically significant differences were observed between genders (57.4 ± 23.9 vs. 53.3 ± 22.8 nmol/L, $p = 0.219$) as well as no statistically significant correlation was found between age and 25(OH)D levels ($p = 0.349$). As expected higher levels of 25(OH)D were observed in summer than in winter (68.2 ± 21.5 *vs.* 42.2 ± 16.9 nmol/L; $p < 0.0001$). Serum 25(OH)D levels were significantly lower in obese compared to non-obese subjects (46.6 ± 17.6 vs. 57.7 ± 24.2 nmol/L, $p = 0.012$).

Vitamin D deficiency is prevalent in this area, affecting almost half of the population. Body mass index and season are predictors for lower 25-hydroxyvitamin D levels and vitamin D *status*. An effective strategy to prevent vitamin D deficiency and insufficiency should be envisaged and implemented in our population.

1. Introduction

Vitamin D is unique among vitamins, since it works as a hormone and can be synthesized on the skin as a result of exposure to sunlight. It is acquired both through nutrition (10–20%) and by cutaneous synthesis under the action of sunlight [1]. Dietary sources of vitamin D include fish oils and, in some countries (USA and Northern Europe) fortified food products (dairy and bread products). In Portugal, vitamin supplements containing vitamin D exist in the market. However, the main source of vitamin D results from cutaneous synthesis on sun exposure and is dependent on various factors such as the geographical area latitude, altitude, season, time of day, the exposed body surface and exposure duration, use of sunscreens, skin pigmentation, obesity and age [2].

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http://dx.doi.org/10.1016/j.jsbmb.2016.11.005
0960-0760/

Vitamin D_3 or cholecalciferol, after formation in the skin, and vitamin D_2 or D_3, from dietary sources, are hydroxylated in the liver, resulting in the formation of 25-hydroxyvitamin D [25(OH) D], the main circulating form. This form subsequently undergoes hydroxylation in the kidney and other organs to generate the biologically active, dihydroxylated form of vitamin D, calcitriol or $1,25(OH)_2D$, which acts through specific vitamin D receptors [1]. The vitamin D role on the maintenance of calcium serum levels, by promoting calcium and phosphorus absorption from the intestine and calcium bone reabsorption, is well known [3]. Recent evidences correlate insufficient vitamin D levels with an increased risk of developing other non-bone-related disorders: cardiovascular diseases, hypertension, malignant neoplasia, type I diabetes mellitus, multiple sclerosis, dementia, rheumatoid arthritis, and infectious disease [2–4].The identification of vitamin D receptors in immune system cells, and the discovery that dendritic cells can produce the metabolically active form of vitamin D, have led to the suggestion that vitamin D is also an important immune modulator [5].

The high prevalence of inadequate vitamin D is nowadays seen as a public health problem affecting several countries in Europe and the USA, particularly in those people at risk for osteoporosis and its consequences [2]. Vitamin D deficiency screening is accomplished through measurement of 25(OH)D, which is the best index for assessing vitamin D reserve in the body [2], due to its greater half-life comparing with the metabolically active form. Only at-risk populations are routinely tracked for vitamin D deficiency, including the elderly, the institutionalized, pregnant women and post-menopausal women (increased risk of fractures) [3]. Much debate has taken place over the definition of vitamin D deficiency. Most agree that a 25(OH)D concentration <50nmol/L, or 20 ng/mL, is an indication of vitamin D deficiency, whereas a 25 (OH)D concentration of 51–74 nmol/L, or 21–29 ng/mL, is considered to indicate insufficiency; concentrations >75 nmol/L, or 30 ng/mL, are considered to be adequate [6–9]. The optimal serum 25 (OH)D levels are those for which calcium absorption is optimized, parathyroid hormone (PTH) levels reduced and the greatest benefit to the bone and muscle function are obtained; currently levels above 75 nmol/L (30 ng/mL) are recommended.

Several studies have described inadequacy of 25-hydroxyvitamin D all over Europe, although vitamin D *status* within different European countries shows a high variation [10–12]. In Portugal, the prevalence of vitamin D deficiency is unknown because there are no epidemiological studies in adult healthy individuals; however,

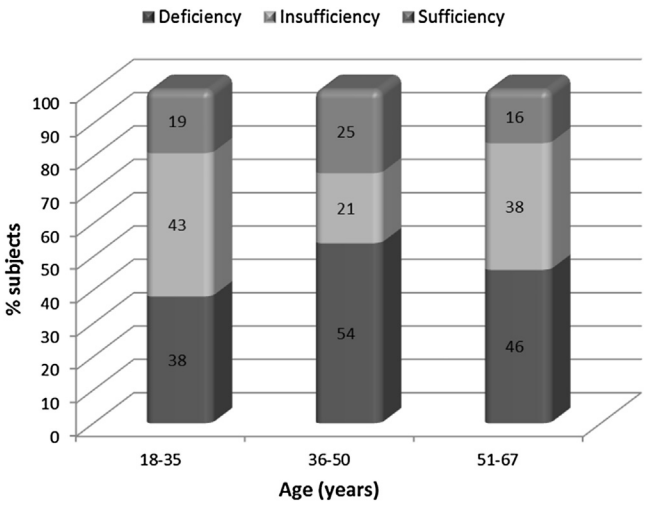

Fig. 1. Global prevalence of vitamin D deficiency and insufficiency by age.

several studies in healthy pediatric populations and in specific hospital populations have been published [3,13–22]. In 2009 a study in a healthy pediatric population from Porto was published. A group of 45 children (33F, 12 M; 2.5–16 years) were evaluated in winter and spring. None of them was supplemented after the first year of life. Values above 100 nmol/L were considered optimal, 75–100 nmol/L sufficient, 50–74 nmol/L relative insufficient and <50 nmol/L deficient. Vitamin D deficiency was found in 26% of the studied population during the months with less sunlight. According to these cut off values, 80% of the children did not achieve optimal levels [23]. In another pediatric study, 73 children (37F, 36 M), aged 12 months to 17 years, from the outpatient clinic of Centro Hospitalar do Porto, were studied. The study occurred between March 2008 and July 2010. The children were divided in to pre-school age (12 months to 5 years; 23.3% (17/73)) and school age (6 to 17 years; 76.7% (56/73)). Normal 25(OH)D levels (>75 nmol/L) were observed in 17.8% of the children (11% with optimal values, >100 nmol/L; and 6.8% with sufficient values, 75–100 nmol/L). On the other hand, 82.2% had low 25(OH)D levels (42.5% with relative insufficiency, 50–74 nmol/L; and 39.7% with deficiency, <50 nmol/L). Gender, residential area, BMI and season were not related to 25(OH)D levels. It was observed that school age children had higher vitamin D deficiency ($p = 0.013$), thus establishing a relation with age [24]. Another cohort of 122

Table 1
Characteristics of the study population.

Characteristics	Total (n = 198)	Women (n = 95)	Men (n = 103)
Sociodemographics			
Age, years, mean ± SD	43.1 ± 12.1	41.9 ± 12.5	44.2 ± 11.7
Season			
Summer, n (%)	101 (51.1)	44 (46.3)	57 (55.3)
Winter, n (%)	97 (49.0)	51 (53.7)	46 (44.7)
BMI, mean ± SD	27.0 ± 4.3	26.9 ± 4.4	27.2 ± 4.2
Laboratory measurements			
PTH levels (pg/mL), mean ± SD	44.9 ± 14.7	45.6 ± 12.6	44.3 ± 16.5
Creatinine levels (mg/dL), mean ± SD	0.8 ± 0.2	0.8 ± 0.1	0.8 ± 0.2
Total calcium levels (mmol/L), mean ± SD	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1
Phosphorus levels (mmol/L), mean ± SD	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.2
25(OH)D levels (nmol/L)			
Mean ± SD	55.4 ± 23.4	53.3 ± 22.8	57.4 ± 23.9
<50 nmol/L (deficiency), n (%)	95 (48.0)	50 (52.6)	45 (43.7)
50–75 nmol/L (insufficiency), n (%)	60 (30.3)	27 (28.4)	33 (32.0)
>75 nmol/L (optimal), n (%)	43 (21.7)	18 (18.9)	25 (24.3)

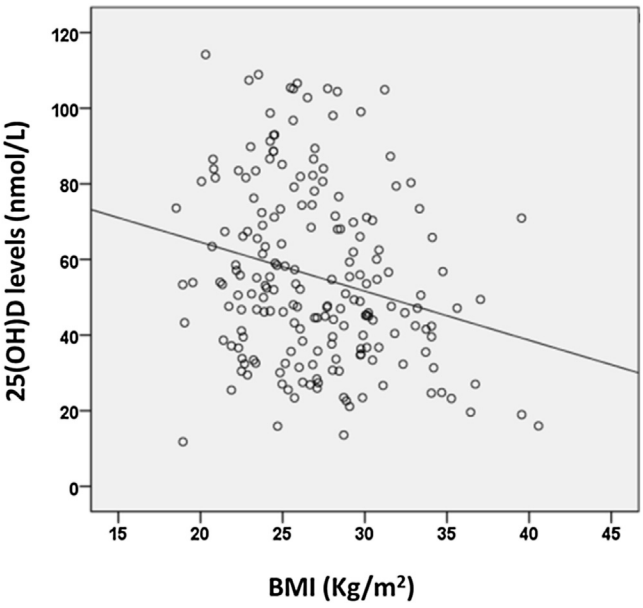

Fig. 2. Correlation between 25(OH)D and BMI.

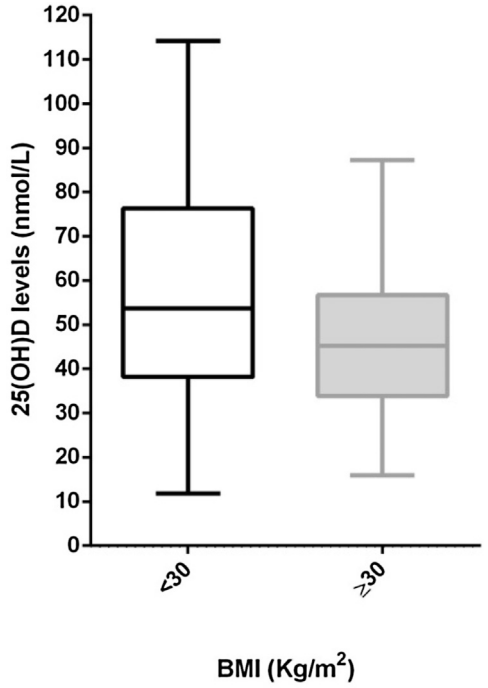

Fig. 3. Comparison of serum 25 (OH)D levels between obese (BMI ≥ 30) and non-obese (BMI < 30) individuals.

healthy children and adolescents (5–18 years) from Porto was studied. They were observed in the pediatric outpatient clinic during the winter and spring of 2011/2012. Vitamin D *status* was observed to be insufficiency (≥20 and <30 ng/mL) in 92.5% of the cases, from which 47.8% presented deficiency (≥10 and <20 ng/mL) and 6% severe deficiency (< 10 ng/mL). Only 7.5% of the sample had an adequate vitamin D *status* (≥30 ng/mL) [25].

As already stated, vitamin D *status* is often studied in specific groups that have increased risk of vitamin D deficiency or osteoporosis, such as hospitalized or elderly people. In such groups, confounding of variables makes it difficult to translate findings to the general population [26]. Thus, the aim of the current study is to evaluate vitamin D *status* in non-supplemented healthy adults living in Porto, north of Portugal.

2. Subjects and methods

2.1. Subjects

The study was conducted in Porto (~41° N; elevation: 104 m), in July and August 2015 (summer time) and April 2016 (winter time). Two hundred healthy blood donors voluntarily participated in this study. Two men were excluded, because they were taking multivitamin supplementation. The average age of these individuals was 43.1 ± 12.1 years. Subjects were stratified in three groups according to age [27].

A questionnaire about age, gender, weight, height, ethnicity, nationality, place of birth, occupation, sun exposure, sunscreens use, eating habits, smoking habits, physical activity, diagnosed pathologies, use of medicines and food supplements, was answered during blood donation. In order to analyze the influence of BMI on the 25(OH)D levels, the subjects were divided into two groups based on BMI values: BMI < 30 kg/m^2 (non-obese) or BMI ≥ 30 kg/m^2 (obese). Written informed consent was obtained for each volunteer, and the study was approved by the Ethics Committee of Centro Hospitalar do Porto, according to the Declaration of Helsinki.

2.2. Laboratory measurements

Blood was collected in Vacuette® Z Serum Clot Activator tubes for the measurement of PTH and in Vacuette® Z Serum Separator Clot Activator tubes for the other measurements. Serum was obtained by centrifugation and stored in several aliquots at −20 °C until analyzed. Serum 25(OH)D was chosen as a reliable marker of individual vitamin D *status* as it reflects vitamin D obtained from food sources and cutaneous synthesis, and it is not prone to diurnal variation.

Serum 25(OH)D was measured using an electro-chemiluminescence binding assay (ECLIA) for the in-vitro determination of total 25-hydroxyvitamin D (Elecsys® Vitamin D total, Cobas, Roche©). The reference range for 25(OH)D was >75 nmol/L (measurement range: 7.50-175 nmol/L). The serum PTH concentration was assessed using an electro-chemiluminescence assay with 15–65 pg/mL as a reference range. Serum total calcium,

Table 2
Differences in 25(OH)D concentration according to season.

25(OH)D levels (nmol/L)	Winter (n = 97)	Summer (n = 101)	p
Mean ± SD	42.2 ± 16.9	68.2 ± 21.5	<0.0001
<50 nmol/L (deficiency), n (%)	72 (74.2)	23 (22.8)	<0.0001
50–75 nmol/L (insufficiency), n (%)	20 (20.6)	40 (39.6)	
>75 nmol/L (optimal), n (%)	5 (5.2)	38 (37.6)	

Table 3
Results of a multiple linear regression analysis on determinants of 25(OH)D levels.

Variable	B	SE	p
Intercept	4.224	0.189	<0.0001
Age	−0.002	0.002	0.499
Season	0.482	0.054	<0.0001
Gender	−0.40	0.054	0.456
Body Mass Index (kg/m^2)	−0.17	0.007	0.010
Corr .r^2 = 0.341			

phosphate and creatinine concentrations were measured by routine laboratory methods in a Cobas Integra 800.

2.3. Statistical analyses

Continuous data were checked for normality using the Kolmogorov-Smirnov test and natural logarithm (ln) transformations were used for skewed variables previous to the statistical analysis. Differences between groups were tested using the Student's *t*-test or one-way ANOVA (continuous variables) and χ^2 test (dichotomous variables). Pearson's or Spearman's correlation coefficients were calculated to test relationships between continuous variables.

Multiple linear regression analysis was used to consider potential determinants of 25(OH)D levels (dependent variable). The following independent variables: age and BMI (as continuous variables), season and gender (as categorical variables) were included in the model.

A *p*-value below 0.05 was considered to be statistically significant. Statistical analyses were performed using *Statistical Package for the Social Sciences* software (version 23, IBM SPSS Statistics, NY, USA).

3. Results

The characteristics of the study population are shown in Table 1. Approximately 48% were women, and the mean age (±SD) of the study population was 43.1 ± 12.1 years. No statistically significant differences were observed between genders. The frequency of obesity was significantly higher in this population compared with the general Portuguese population (22.7% vs. 14.2%, p = 0.001, OR = 1.77, 95%CI = 1.26–2.50) [28].

The mean serum 25(OH)D concentration was 55.4 ± 23.4 nmol/L in all participants (median 50.9 nmol/L) with no significant differences between men and women (57.4 ± 23.9 vs. 53.3 ± 22.8 nmol/L; p = 0.219). Fifty women (52.6%) and 45 men (43.7%) were deficient in 25(OH)D but the gender difference was not statistically significant (Table 1).

No statistically significant correlation was found between age and 25(OH)D levels (p = 0.349). When subjects were categorized in groups according to age (Fig. 1), no differences in 25(OH)D levels between the 3 groups (p = 0.311) were found.

Body mass index was negatively correlated with 25(OH)D levels (p = 0.001, r = −0.237) (Fig. 2). In conformity, 25(OH)D levels were significantly lower in obese compared to non-obese subjects (46.6 ± 17.6 vs. 57.7 ± 24.2 nmol/L, p = 0.012) (Fig. 3).

In the winter period, 74.2% of the studied population had a 25(OH)D concentration below 50.0 nmol/L compared with 22.8% in the summer period (p < 0.0001). Only 5 individuals (5.2%) presented optimal levels of 25(OH)D in winter, and 38 (37.6%) in summer (Table 2).

In multiple linear regression analysis, controlling for age and gender, significant associations between 25(OH)D levels and season and BMI were found. Winter and higher BMI were significantly associated with lower serum 25(OH)D levels (Table 3).

4. Discussion

There are many studies on vitamin D *status* of the general population in the USA, Canada, Asia Pacific, Middle East, Africa and across Europe [29], but to the best of our knowledge this is the first conducted in a Portuguese healthy adult population.

Globally, vitamin D deficiency is more prevalent in winter, women, older age groups, individuals with darker skin, and higher latitudes [7,30,31]. In the present study, the frequency of 25(OH)D deficiency was significantly higher in winter, confirming the well-known seasonal fluctuation in 25(OH)D concentration. No association between 25(OH)D levels and gender was observed in our study, although women presented slightly lower levels of 25(OH)D but the difference was not statistically significant. It has been established that the ageing skin produces less vitamin D [32]. However, in our study, we did not find any association between vitamin D *status* and age.

The negative association of vitamin D *status* with obesity is well documented in different studies [33]. This is probably due to the decreased bioavailability of vitamin D from cutaneous and dietary sources because of its sequestration in body fat compartments [34]. Our observations confirm the association of vitamin D *status* with BMI. Furthermore, an inverse correlation between vitamin D *status* and BMI was found.

There is ongoing debate related to the optimal levels of 25(OH)D. All available evidence suggest that children and adults should maintain a blood level of 25(OH)D above 50 nmol/L to prevent rickets and osteomalacia, respectively. However, to maximize vitamin D effect on calcium, bone, and muscle metabolism, the 25(OH)D blood level should be above 75 nmol/L. Numerous epidemiological studies have suggested that a 25(OH)D blood level above 75 nmol/L may have additional health benefits in reducing the risk of common cancers, autoimmune diseases, type 2 diabetes, cardiovascular disease, and infectious diseases [35].

In this study we observed that almost half of the studied population presented serum 25(OH)D values suggestive of vitamin D deficiency, reaching 74% in winter. This observation is in line with a recent study that suggests that vitamin D deficiency is widespread across Europe, even in countries with abundant sunlight, and at prevalence rates that meet the criteria of a pandemic [10]. An effective strategy to prevent vitamin D deficiency and insufficiency should be envisaged. For specific high-risk groups use of vitamin D supplements would be an effective measure. For the general population fortification of widely used foods could be considered, especially in winter.

5. Conclusions

The present study analyzed serum 25-hydroxyvitamin D levels in healthy adults between 18 and 67 years of age. BMI and season are predictors for lower 25(OH)D levels and vitamin D *status* in this population. The strengths of this work comprise a detailed questionnaire documenting demographic data and blood sampling taking place through summer as well as wintertime. On the other hand, although the questionnaire included data about sun exposure, sunscreens use, eating habits, smoking habits, physical activity, diagnosed pathologies, and use of medicines and/or food supplements, these parameters were only used to exclude confounding factors that could bias our results, and were not used in the analysis of the results, as that was beyond the aim of the present study.

Conflict of interest

The authors have declared no conflict of interests.

Funding source

This study was supported by Merck S.A.

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