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ORIGINAL ARTICLE

Prevalence of vitamin D deficiency and its predictors in the Portuguese population: a nationwide population-based study

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Abstract

Summary Vitamin D deficiency is prevalent worldwide, but its prevalence is unknown in adult Portuguese population. In Portugal, 66% of adults present Vitamin D insufficiency/deficiency. Winter, living in Azores, older age, and obesity were the most important risk factors. It highlights the need of strategies to prevent vitamin D deficiency in Portugal.

Objective To estimate the prevalence and risk factors of vitamin D deficiency in the adult Portuguese population.

Methods Adults (≥ 18 years old) from the EpiReumaPt Study (2011–2013) were included. Standardized questionnaires on socio-demographic and lifestyle features were obtained. Serum 25-hydroxyvitamin D [25(OH)D] concentrations were evaluated using ADVIA Centaur VitD competitive immunoassay (Siemens Healthineers) in 2015–2017 as 25 (OH)D Level 0: ≤ 10 ng/mL; Level 1: 11–19 ng/mL; Level 2: 20–29 ng/mL, and Level 3: ≥ 30 ng/mL. Weighted multinomial regression analysis was conducted to evaluate the association between socio-demographic and lifestyle variables and vitamin D status.

Results Based on weighted analysis, the estimated prevalence of levels of 25(OH)D ≤ 10, < 20, and < 30 ng/mL was 21.2, 66.6, and 96.4%, respectively. The strongest independent predictors of serum 25 (OH)D ≤ 10 ng/mL were living in the Azores archipelagos (OR 9.39; 95%CI 1.27–69.6) and having the blood sample collection in winter (OR 18.53; 95%CI 7.83–43.87) or spring (11.55; 95%CI 5.18–25.74). Other significant predictors included older age (OR 5.65, 95%CI 2.08–15.35), obesity (OR 2.61; 95%CI 1.35–5.08), current smoking (OR 2.33; 95%CI 1.23–4.43), and female gender (OR 1.9, 95%CI 1.1–3.28). Conversely, physical exercise (OR 0.48, 95%CI 0.28–0.81) and occasional alcohol intake (OR 0.48, 95%CI 0.29–0.81) were associated with a lower risk of 25(OH)D ≤ 10 ng/mL.

Conclusion Vitamin D deficiency/insufficiency [25(OH)D < 20 ng/ml] is highly prevalent in Portugal, affecting > 60% of all Portuguese adults, with strong geographical and seasonal variation. This study highlights the need to critically assess the relevance of vitamin D deficiency as a public health problem and the urgent need for a wide and scientifically robust debate about the most appropriate interventions at the individual and societal levels.

Keywords Vitamin D, prevalence, Portugal · 25-hydroxyvitamin D · Vitamin D deficiency · Vitamin D insufficiency

Introduction

Vitamin D deficiency and insufficiency is a global health issue that affects more than one billion of children and adults worldwide and is now recognized as a pandemic and a major public health concern [1].

Vitamin D in humans is spontaneously obtained mainly through skin exposure to UVB radiation and, in a small proportion, from dietary intake. The liver and other body tissues metabolize vitamin D3 synthesized in the skin and provided from the diet (which includes a small amount of vitamin D2) to 25-hydroxyvitamin D [25(OH)D], the main circulating form. 25(OH)D is then further metabolized, by the kidneys and other body tissues, to the active form 1,25-dihydroxyvitamin D [1.25$(OH)_2$D] [2]. The total serum concentration of 25(OH)D is used to define vitamin D status, as it is regarded as the most reliable indicator of its storage in the human body [3].

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Over recent years, vitamin D has become increasingly recognized as a pluripotent regulator of a multitude of biological functions above and beyond its classical effects on bone and calcium homeostasis [4]. Vitamin D deficiency has been associated with the incidence and severity of a myriad of different diseases such as sarcopenia, multiple sclerosis, cardiovascular disease, diabetes, infections, auto-immune diseases, and cancer, besides osteoporosis and rickets [1, 5]. Moreover, several studies have demonstrated an association between vitamin D deficiency and increased mortality, both overall and mortality associated with specific causes, such as cardiovascular disease and cancer [6, 7]. Such observations, together with the high prevalence of vitamin D deficiency worldwide, support the view that this is a widespread health issue of potentially overwhelming importance [8, 9].

Despite this recognition, there is still debate on the exact cut-off definition of vitamin D deficiency and the individual cut-off for clinical significance [10, 11]. The US Institute of Medicine (IOM) considers that people with a serum value of 25(OH)D < 12 ng/mL (30 nmol/L) are at risk of vitamin D deficiency, a cut-off established taking into account the risk of metabolic bone disease [12]. The Endocrine Society, together with other Scientific Societies as European Calcified Tissues Society (ECTS), International Osteoporosis Foundation (IOF) or American Geriatrics Society (AGS) established the threshold of deficiency at ≤ 20 ng/mL (50 nmol/L), based on the demonstration that this corresponds to increasing levels of circulating parathormone and designates levels ≤ 10 as severe deficiency [3, 10, 11]. The IOM considers 25(OH)D serum levels between 12 and 20 ng/mL as corresponding to a risk of insufficiency, and levels above (≥) 20 as being sufficient, similarly to ECTS. The Endocrine Society determines that only levels above (≥) 30 ng/mL are to be considered normal, designating levels between 21 and 29 ng/mL as insufficient. This lack of consensus remains unresolved, hindering the interpretation of epidemiological data and causing confusion in clinical practice.

Over the past two decades, several population-based epidemiological studies have identified a high prevalence of "vitamin D deficiency" in different parts of the world [13–19]. In a recent study, applying the Vitamin D Standardization Program (VDSP) on data from different European populations ($n = 55{,}844$), Cashman and colleagues reported an overall prevalence of vitamin D deficiency (< 20 ng/mL) of 40.4%, which was significantly higher in some sub-groups [20]. Socio-demographic, lifestyle, and clinical factors were found to associate with vitamin D deficiency in these different studies.

In Portugal, some studies have been conducted to evaluate the vitamin D status among specific groups of individuals. A retrospective study including a total of 5439 of spontaneously requested measurements of serum 25(OH)D in 3257 patients from a single hospital showed that 60% had levels ≤ 20 ng/mL [21]. More recently, a study including 198 blood doners, ages 18 to 65 years, from the North of Portugal found that 48% of the subjects had 25(OH)D levels below 20 ng/mL [22]. In a nationwide study of older adults (> 65 years old), 39.6% of the subjects had levels < 12 ng/mL and 69% below 20 ng/mL [23]. In 500 subjects randomly selected from PORMETS, a national cross-sectional study that includes a sample of adults registered in primary health care centers of the Portuguese mainland, 37.7% were at risk for deficiency of vitamin D (< 12 ng/mL) and only 14.4% had normal levels of 25(OH)D (>20 ng/mL) [24].

Because these studies are limited to a geographical area or a specific age group, they fail to provide an overall epidemiological picture of vitamin D status in the adult Portuguese population. The present study was planned to address this need.

Material and methods

Data and sera samples were collected in the context of EpiReumaPt, a nationwide health survey conducted between September 2011 and December 2013 to assess the prevalence of Rheumatic Diseases in Portuguese adults [25, 26]. A detailed description of the study protocol has been previously published elsewhere [27]. A random sample of non-institutionalized and living in private houses subjects with ≥ 18 years of age was drawn. The participants were selected through a process of multistage random sampling. The sample was stratified according to the Portuguese statistic regions NUTS II in the 2001 Census and the size of the population (less than 2000, 2000–9999, 10,000–19,999, 20,000–99,999, and ≥ 100,000 inhabitants). The number of participants of each stratum was proportional to the actual distribution of the population. In Madeira and the Azores, we increased the sample size (oversampling) to allow separate analyses in these regions. Candidate households were selected through a random route process: sampling points were randomly selected on the maps of each locality, where the interviewer began a systematic step count (defined for each locality according to its size), granting each household and each individual an equal probability of being chosen. Dwellings with commercial or industrial purposes, private or public institutions, and visibly unoccupied buildings were considered ineligible. In the household, the individual over 18 years old with permanent residence and with the most recently completed birthday was selected [28]. The selected candidates were invited to participate in EpiReumaPt survey and signed an informed consent.

EpiReumaPt's participants answered a structured face-to-face interview about their socio-demographic data (age, gender and ethnicity), socio-economic profile (including measures of wealth and household income), anthropometric data (self-reported weight and height), and lifestyle habits, and

screening questionnaire for rheumatic diseases was applied. All participants who screened positive for at least one rheumatic disease plus 20% of all individuals with no rheumatic symptoms in the screening were invited to for a structured evaluation by a rheumatologist at the local primary care centre. The visit included not only a structured evaluation by a rheumatologist but also a blood sample collection for biobanking, after signed informed consent [25, 27]. Patients with known hepatitis C, HIV infection, or debilitating conditions were excluded. A 15–25 mL blood sample was obtained and half was immediately centrifuged (800 g, 10 min) for serum separation. Samples were kept at 4 °C and sent in a cooler on the same day or within 2 days to Biobanco-IMM in Lisbon. Serum and whole blood samples were divided into 250 μL and 2 mL aliquots, respectively, and stored at − 80 °C until use [25].

For the purpose of this study, we included data from all EpiReumaPt participants who had an available serum sample in the biobank.

Measurements

Demographic and socioeconomic measures

Socio-demographic measures included gender (female/male), age (categorized in eight categories ≥ 18–29, 30–39, 40–49, 50–59, 60–69, 70–74, and ≥ 75 years old), ethnicity (Caucasian, "Other"), educational level (> 12, 10–12, 5–10, 0–4 years), and household income in the last month (< 750€, 751–1500€, 1501–2500€, ≥ 2501€).

Geographical area

Geographical area was classified according to Portuguese Nomenclature of Territorial Units for Statistics: *Norte*, *Centro*, *Alentejo*, *Algarve*, *Lisboa e Vale do Tejo*, *Madeira and Azores* [28].

Seasonality

Seasonality was recorded based on the date of blood sample collection. Winter was defined as January through to March, spring as April through to June, summer as July through to September, and autumn as October through to December.

Lifestyle and anthropometric measures

Lifestyle habits, registered based on participants' self-report, included alcohol consumption (never, occasionally, daily), smoking status (never smoker, current smoker, past smoker), and practice of recreational physical exercise (yes/no).

Anthropometric data, [weight (kg) and height (cm)], was collected by self-report in the first-phase. Body mass index (BMI) was calculated through standard formula and classified according to World Health Organization criteria (underweight < 18.5 kg/m^2; normal weight 18.5–24.9 kg/m^2; overweight 25–29.9 kg/m^2 and obese ≥ 30.0 kg/m^2) [29].

Determination of serum 25(OH)vitamin D levels

Total serum 25(OH)D levels were determined in the stored serum samples between August 2015 and March 2016, using the ADVIA Centaur® VitD competitive immunoassay (Siemens Healthineers, Germany) performed by a single technician (HC) following the manufacturer's instructions. ADVIA Centaur VitD is a competitive chemiluminescent immunoassay, traceable to the Ghent University 25(OH)D Reference Measurement Procedure (RMP) and certified by the CDC-VDSCP [30]. The standardized assay is reported to demonstrate equimolar cross-reactivity with 25(OH) D_2 (104.5%) and 25(OH)D_3 (100.7%), minimal cross-reactivity with 3-epimer of 25(OH)D_3 (3-epi-25(OH)D_3) (1.1%), and a broad assay range of 10.5–375 nmol/L (4.2–150.0 ng/mL). The limit of quantitation (LoQ) of the assay is 10.5 nmol/L (4.2 ng/mL). Precision analysis involved assaying six samples twice a day in replicates of 2, over 20 days ($n = 80$ replicates per sample) according to the Clinical and Laboratory Standards Institute (CLSI) protocol EP5-A2; the run-to-run CVs were in the range of 4.2% and 11.9%. The ADVIA CENTAUR VitD presents a − 0.09% bias versus NIST, which is in accordance with the performance criterion of ± 5% mean bias, attesting the accuracy of the method. Our laboratory has participated in the international 25-hydroxivitamin D EQAS and met the performance target, with 75% or more results fell within the ± 25% of the target value. All samples were run in singlicate on both the ID-LC-MS/MS and a single ADVIA Centaur system [31].

As no consensus exist in the definition of vitamin D states, in this study, vitamin D levels were categorized as 25(OH)D Level 0 ≤ 10 ng/mL, 25(OH)D Level 1 11–19 ng/mL, 25(OH)D Level 2 20–29 ng/mL, and 25 (OH)D Level 3 ≥ 30 ng/mL.

Statistical analysis

EpiReumaPt was designed to obtain a representative sample of the Portuguese population. Exactly in order to guarantee its representatively, the design effect namely the existence of two survey phases was considered. In order to allow appropriated generalization of observed data to the overall adult Portuguese population, data from observed individuals in EpiReumaPt phase 2 were weighted taking into account the study design, namely the effect of the screening in phase one, and also matching age, gender, and geographical distribution of the

adult Portuguese population as determined by Census 2011 as described elsewhere [25]. Accordingly, prevalence estimates for vitamin D levels were computed as weighted proportions.

Descriptive characteristics are presented as means (±SD), as adequate, for continuous variables and as proportions (%) for categorical variables. There was no imputation of missing data.

Due to the small proportion of subjects with vitamin D levels ≥ 30 ng/ml, 25 (OH)D Levels 2 and 3 described above were combined for statistical analysis. A univariable multinomial logistic regression analysis was performed to look for potential predictors of vitamin D levels. Variables with a p value < 0.05 in univariate analysis were re-tested in multivariable multinomial logistic regression to identify independent predictors of vitamin D levels. Income was excluded from the multivariable regression due to the large proportion of missing data. The final model was adjusted for socio-demographic and lifestyles variables.

Statistical analysis was carried out using Stata IC version 12® (StataCorp, 2011, Stata Statistical Software: Release 12, College Station, TX, USA). $p < 0.05$ was considered as statistically significant.

Ethical issues

This study was conducted respecting the Declaration of Helsinki and was approved by Coimbra Medical School Ethics Committee and EpiReumaPt Scientific Board.

EpiReumaPt was performed according to the principles established by the Declaration of Helsinki, revised in Fortaleza [32]. EpiReumaPt was approved by the National Committee for Data Protection (Comissão Nacional de Proteção de Dados) and by the NOVA Medical School Ethics Committee. Written informed consent was obtained in all phases for all participants, including a separated informed consent for biobank. Further details of ethical issues of EpiReumaPt have been described elsewhere [27].

Results

Sample characterization

This ancillary study included data from 3092 (79.8%) of the 3877 participants entered in phase II of EpiReumaPt. Sociodemographic characteristics of participants of the vitamin D and EpiReumaPt studies and of the Portuguese population (Census 2011) are summarized in Table 1.

25(OH) D levels

The distribution of serum 25(OH)D concentrations according to the categories described above is presented in Table 2. The mean serum 25(OH)D concentration was 16.86 ng/mL (± 6.84). Almost half of the subjects had 25(OH)D levels below 20 ng/mL, and 21.2% had ≤ 10 ng/mL. Toxic levels (> 150 ng/mL) were not observed, (the highest level determined of 48 ng/mL).

Applying the exact IOM cut-offs to these estimates, 34.5% of the Portuguese adult population are classified as having normal levels of vitamin D, 41.4% as being at risk of inadequacy, and 25.1% as being at risk of deficiency.

Gender and age

When compared to men, women presented a lower proportion of values > 20 ng/mL (25.5 vs. 42.1%). The proportion of people with levels of 25(OH)D > 20 ng/mL decreases with age, with only 17% of the participants older than 75 years in this category which contrasts with 44.4% in those ages 18–29 (Fig. 1).

Seasonal variation

Significant differences in the prevalence of 25(OH)D level categories were observed according to the time of the year when blood was collected. According to our estimates, 56.8% of the Portuguese adult population will have 25(OH)D levels above 20 ng/mL in the summer, in contrast with only 24% in winter (Fig. 2).

Geographical variation

Deficient or inadequate levels of 25(OH)D (< 20 ng/ml) were less prevalent in the Algarve (45%) and rather similar in the rest of the continental territory of Portugal and Madeira Islands (58.7–69.4). The prevalence of low levels was highest in the Azores archipelagos, where 82% of the population are estimated to present 25(OD)D levels < 20 ng/mL (Fig. 3).

The results of the univariable and multivariable multinomial logistic regression analysis are presented in Table 3.

The strongest independent (adjusted) predictors of 25(OH)D ≤ 10 ng/mL are living in the Azores and blood sample collection in winter. Being female, age ≥ 75, obesity, and current smoking are also associated with higher probabilities of 25(OH)D ≤ 10 ng/mL. Recreational physical exercise and occasional alcohol intake were associated with lower risks of 25(OH)D ≤ 10 ng/mL.

Discussion

The estimated national prevalence of 25(OH)D ≤ 10 ng/mL and < 20 ng/mL is 21.2% and 45.4% respectively, based on EpiReumaPt (a representative sample of the adult Portuguese population). Only 33.4% of the adult population presents

Table 1 Socio-demographic characteristics of participants included in this vitamin D study, in EpiReumaPt and of the Portuguese adult population according to Census 2011 [25, 33, 34]

	Vitamin D study *N* (%[a]) (Total = 3092)	EpiReumaPt *N* (%[a]) (Total = 3877)	Census 2011 *N* (%[b]) (Total = 8,657,240)
Gender			
Female *n* (%)	1995 (52.6%)	2630 (52.5%)	4,585,118 (53.0%)
Age*			
≥ 18–29	155 (20.2%)	190 (21%)	1,470,782 (17%)
30–39	355 (18.7%)	403 (19.3%)	1,598,250 (18.5%)
40–49	608 (18.5%)	680 (18.2%)	1,543,392 (17.8%)
50–59	723 (14.8%)	818 (14.7%)	1,400,011(16.2%)
60–69	698 (13.7%)	914 (13.4%)	1,186,442 (13.7%)
60–74	249 (5.7%)	376 (5.3%)	496,438 (5.7%)
≥ 75	304 (8.5%)	496 (8.0%)	961,925 (11.1%)
Ethnicity			
Caucasian	3022 (94.9%)	3876 (93.3%)	Not applicable
Black	52 (4.4%)	64 (6.1%)	
Asian	2(0%)	2 (0.0%)	
Gipsy	2 (0%)	3 (0.1%)	
Other	9 (0.5%)	13 (0.5%)	
Education Level			
> 12	387 (18.6%)	508 (21.1%)	1,741,567 (20.1%)
10–12	517 (24.2%)	575 (23.2%)	1,560,958 (18.0%)
5–10	796 (27.4%)	775 (22.4%)	2,134,401 (24.6%)
0–4	1392 (29.8%)	1997 (33.4%)	3,239,724 (37.4%)
NUTS II			
Norte	847 (38.4%)	1050 (37.2%)	3,007,823 (34.7%)
Centro	682 (20.2%)	856 (19.8%)	1,938,815 (22.4%)
Alentejo	198 (5.7%)	273 (5.8%)	633,691 (7.3%)
Algarve	101 (2.9%)	144 (3.1%)	370,704 (4.3%)
Lisboa e Vale do Tejo	542 (28%)	708 (29.6%)	2,300,053 (26.6%)
Azores	344 (2.3%)	420 (2.3%)	192,357 (2.2%)
Madeira	378 (2.5%)	426 (2.2%)	213,797 (2.5%)

[a] Percentages represent the proportion of the respective category after weighing to correct for the imbalances between the socio-demographic characteristics of the sample and of the Portuguese adult population in 2011 [33]

[b] Percentages represent the actual proportion of the respective category in the adult Portuguese population 2011

normal values according to the IOM (≥ 20 ng/mL). Surprisingly, no more than 3.6% present normal values according to the Endocrine society (≥ 30 ng/mL). These data demonstrate that low levels of vitamin D are highly prevalent among the Portuguese adult population, raising a public health concern that cannot be ignored.

Our results are in agreement with the results reported by a large number of studies in different countries. In a recent systematic review, Manios et al. reported that in southern European countries, more than one-third of the population had 25(OH)D levels < 20 ng/mL and 10% < 10 ng/mL. Countries with a latitude similar to Portugal have reported prevalences of 25(OH)D < 10 ng/mL ranging from 2% in Spain to 36% in Turkey [35]. Cashman et al. using the Vitamin D Standardization Program (VDSP) protocol to standardize serum 25(OH)D data from past European surveys ($n = 55,844$), reported that, overall, 13% of individuals had concentrations below 12 ng/mL and 40% below 20 ng/mL [20], being lower than the prevalence found in our study. A recent meta-analysis including 21 474 participants from 23 African countries found a prevalence of vitamin D deficiency (< 12 ng/mL) of 18.46% [36] very similar to ours. Our results set Portugal among the countries with higher prevalence of vitanmin D deficiency, but they are paralleled by other Portuguese reports addressing limited age or geographical groups. A recent study including 1500 non-institutionalized individuals aged 65+ found that 25(OH)D levels were < 12 ng/mL in 39.6% of the

Table 2 Estimated distribution of study population through vitamin D levels

25(OH)D level	% (*N*) (Total 3092)	IOM classification[12]	ES classification[3]
0 (≤ 10 ng/mL)	21.2 (706)	At risk of deficiency[a]	Severe deficiency
1 (> 10; < 20 ng/mL)	45.4 (1464)	At risk of inadequacy[a]	Moderate deficiency
2 (≥ 20; < 30 ng/mL)	29.8 (830)	Normal	Insufficiency
3 (≥ 30 ng/mL)	3.6 (92)	Normal	Normal

The percentages (%) represent weighted values to the the Portuguese adult population in 2011, considering the study design

IOM Institute of Medicine, *ES* Endocrine Society

[a] The cut-off between risk of inadequacy and risk of deficiency proposed by the IOM IS 12 ng/mL

participants and between 12 and 20 ng/mL in 29.4% [23]. A cross-sectional study with 198 healthy adult subjects (18–65 years old) from the north of Portugal reported that 48% of the subjects were deficient in vitamin D (< 20 ng/mL) [22].

In our study, several factors were associated with increased probability of low levels of vitamin D. Area of residence (NUTS II) was a strong predictor of vitamin D deficiency which can be related with the different latitudes across the country (~ 32°N in Madeira to ~ 42°N in the north). The special case of the Azores, an archipelagos situated at an average latitude of ~ 38.3°N, could be associated with relatively intense cloudiness of the region. The importance of UV radiation is supported also by the seasonal differences observed, with a significantly higher 25(OH)D level in the summer and lower level in winter and spring. However, many other factors could underly the geographical disparity observed, including life-style and penetration of vitamin D supplementation, which cannot be accounted for in this study.

Consistent with other studies [8, 15, 17, 19], we found that older adults (≥ 75 years old) have higher odds of low levels of vitamin D, which have been attributed to a variety of causes, including decreased cutaneous production of vitamin D3 [37], impaired vitamin D absorption [38], lower dietary vitamin D intake [39], and less sun exposure [23].

Also in agreement with previous studies [15, 19, 23, 40–42], we have found that obesity, physical inactivity, and current smoking are associated with an increased probability of 25(OH)D levels ≤ 10 ng/mL. The association of occasional consumption of alcoholic beverages with a lower risk of vitamin D deficiency has also been previously reported [15, 23, 43]. The mechanisms underlying these associations have been extensively discussed [15, 23, 41, 42, 44, 45] and are outside the scope of this publication.

These observations provide very important clues for physicians and health authorities regarding the groups that require special attention for prevention, identification, and correction of vitamin D deficiency. In fact, they confirm international recommendations that the aged, obese, physically inactive, and current smokers are the highest risk groups and should be primordial targets of intervention, namely through correction of these modifiable risk factors. These efforts merit rigorous attention all over the country, but most especially in the Azores Islands and for seasons other than summer. Encouraging people to adopt healthy lifestyles, including exercise, smoke cessation, sensible sun exposure, and ideal body weight are confirmed as important tools in this endeavor.

This study has many strengths, with emphasis on the large sample size, the nationwide coverage, and the statistical adjustment to the national population. This is the

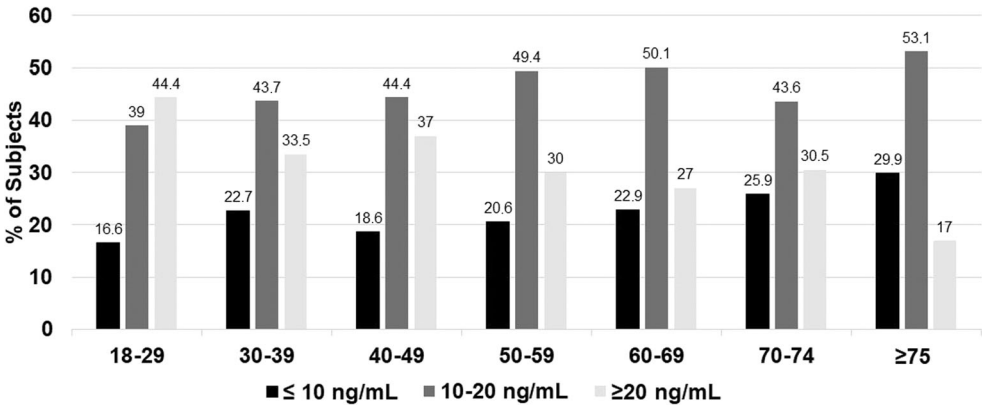

Fig. 1 Prevalence of vitamin D level categories by age. Values are estimated for the overall Portuguese adult population based on observed values corrected for the small imbalances between the socio-demographic characteristics of the sample and of the Portuguese adult population in 2011 [33]

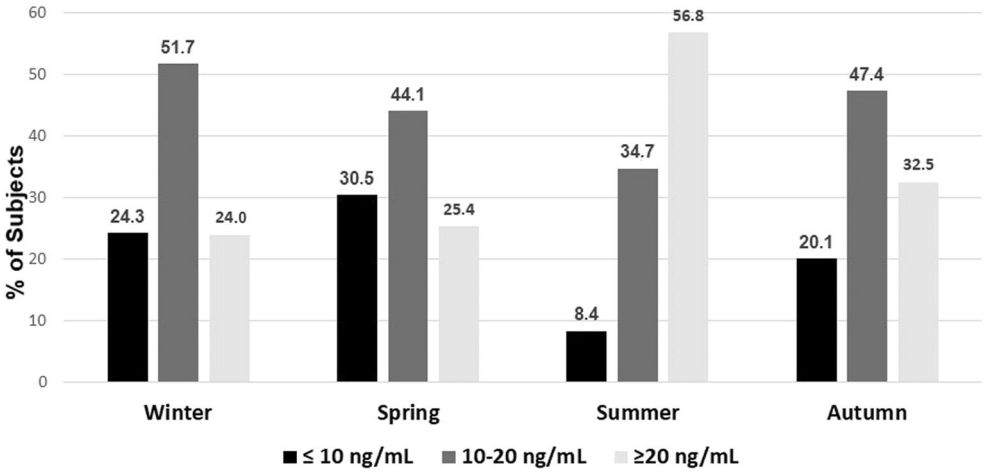

Fig. 2 Prevalence of vitamin D level categories by season. Values are estimated to the overall Portuguese adult population based on observed values corrected for the small imbalances between the socio-demographic characteristics of the sample and of the Portuguese adult population in 2011 [33]

first nationwide study statistically adjusted covering the overall Portuguese adult population. Another strength is the minimization of variability in laboratory measurement by using the same certified assay, performed at the same laboratory, by the same technician, within a short period of time, under strict quality control.

The laboratory method used to assess 25(OH)D levels deserves consideration. Previous reports comparing automated immunoassays with HPLC have showed heterogeneous results [46–49]. Despite a good correlation between ADVIA Centaur and HPLC, the former has been attributed a rather consistent negative bias, especially in the range bellow < 50 nmol/L, leading to a significant disagreement at the categorization of patients around the diagnostic threshold and an overdiagnosis of vitamin D insuff iciency/deficiency [46–49]. Although, such differences may impact significantly in the classification of individuals, they are probably of minor clinical relevance. It is worth noting, in this respect, that the ADVIA Centaur VitD assay was already certified by CDC at the time of our sample analysis [30]. Moreover, other Portuguese studies described above using the the Liason® [21, 24] or the Roche Cobas® [22, 23] method of radioimmunoassay obtained results very similar to ours.

Several limitations need also to be considered. This is a cross-sectional study which precludes the interpretation of cause and effect relationships. Some important factors that can affect vitamin D levels, such as hours of sun exposure, time spent outdoors, or use of sunscreen or vitamin D supplements were not collected. This hinders our ability to explore the relevance of these factors, but it does not question the validity of our results in reporting the actual prevalence of vitamin D deficiency in our adult population at the time of blood collection. These were performed in 2011–2013. It seems unlikely, however, that relevant changes may have occurred since then, in the absence of vitamin D supplementation. One potential limitation of this study is the measurement of 25(OH)D using frozen samples. However, previous work did not find an impact of storage conditions, refrigeration, or use of plasma or serum in vitamin D measurement [50–52].

In conclusion, vitamin D inadequacy and deficiency are highly prevalent among the adult Portuguese population. A large number of important factors associated with an increased

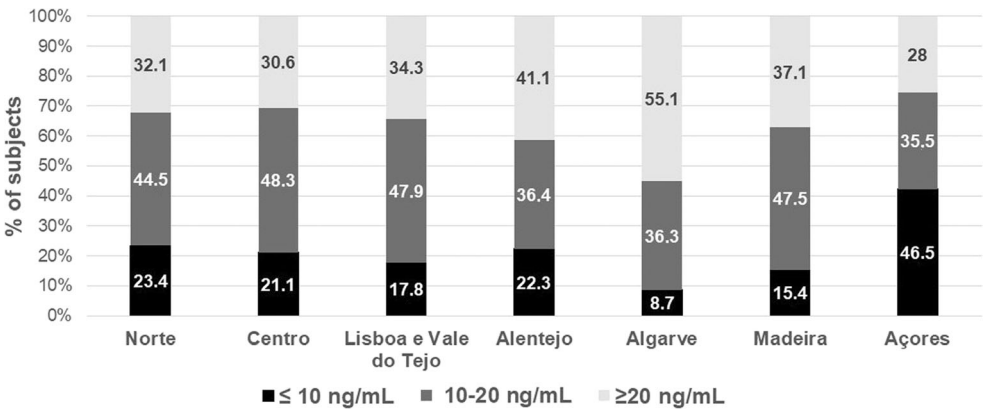

Fig. 3 Prevalence of vitamin D levels by NUTS II. The prevalence estimated values represent weighted values to the Portuguese Adult Population in 2011 [33], considering the study design

Table 3 Crude and adjusted estimated odds ratio for vitamin D deficiency by demographic, economic and life-style variables

		At risk of level > 10 < 20				At risk of level ≤ 10			
Variable	Categories	Unadjusted OR (95%IC)	*p*	Adjusted OR (95%IC)	*p*	Unadjusted OR (95%IC)	*p*	Adjusted OR (95%IC)	*p*
Gender (vs. male)	Female	1.97 (1.32–2.93)	*0.001*	1.64 (1.10–2.54)	*0.028*	2.51 (1.54–4.09)	*<0.001*	1.90 (1.10–3.28)	*0.021*
Age (vs. 18–29 years)	30–39	1.48 (0.62–3.54)	0.375	1.26 (0.59–2.68)	0.554	1.81 (0.64–5.07)	0.261	1.48 (0.57–3.85)	0.418
	40–49	1.37 (0.63–2.95)	0.428	1.24 (0.59–2.57)	0.571	1.34 (0.54–3.34)	0.524	1.35 (0.54–3.42)	0.521
	50–59	1.84 (0.88–4.00)	0.104	1.69 (0.82–3.48)	0.155	1.84 (0.73–4.64)	0.198	1.91 (0.76–4.77)	0.168
	60–69	2.11 (1.00–4.49)	0.051	1.69 (0.77–3.72)	0.189	2.28 (0.95–5.45)	0.065	2.14 (0.86–5.30)	0.101
	70–74	1.62 (0.73–3.62)	0.233	1.41 (0.61–3.27)	0.417	2.27 (0.88–5.84)	0.089	2.67 (1.01–7.6)	*0.048*
	≥75	3.55 (1.62–7.76)	*0.002*	3.37 (1.38–8.24)	*0.008*	4.70 (1.83–12.03)	*0.001*	5.65 (2.08–15.4)	*0.001*
Ethnicity (vs. Caucasian)	Other	1.42 (0.38–5.36)	0.603	NA	NA	0.60 (0.20–1.78)	0.356	NA	NA
Education level (vs. > 12 years)	0–4	1.85 (1.01–3.38)	*0.044*	1.04 (0.53–2.02)	0.911	1.95 (0.92–4.11)	0.081	0.90 (0.41–1.96)	0.784
	5–9	1.18 (0.59–2.36)	0.650	1.02 (0.56–1.88)	0.943	1.07 (0.46–2.50)	0.883	0.78 (0.37–1.68)	0.531
	10–12	1.10 (0.54–2.22)	0.794	1.10 (0.57–1.34)	0.773	1.04 (0.44–2.46)	0.925	1.22 (0.56–2.67)	0.618
Income (vs. ≥2500€)	≤750	2.44 (0.80–7.42)	0.116	NA	NA	10.30 (3.31–31.9)	*< 0.001*	NA	NA
	751–1500€	2.12 (0.70–6.56)	0.189	NA	NA	6.89 (2.12–22.3)	*0.001*	NA	NA
	1501–2500€	2.21 (0.67–7.38)	0.194	NA	NA	6.46 (1.84–22.6)	*0.004*	NA	NA
NUTS II (vs. Algarve)	*Norte*	2.11 (0.95–4.66)	0.066	2.19 (0.85–5.6)	0.107	4.64 (1.27–16.9)	*0.020*	5.09 (0.79–32.7)	0.086
	Centro	2.40 (1.11–5.18)	*0.026*	1.43 (0.54–3.81)	0.476	4.40 (1.99–16.2)	*0.026*	2.89 (0.43–19.6)	0.276
	Lisboa e Vale do Tejo	2.13 (0.91–4.94)	0.080	1.04 (0.37–2.93)	0.938	3.31(0.88–12.5)	0.077	1.71 (0.24–12.12)	0.586
	Alentejo	1.34 (0.44–4.06)	0.606	0.95 (0.31–2.87)	0.927	3.44 (0.74–16.1)	0.117	2.27 (0.32–16.0)	0.411
	Madeira	1.95 (0.84–4.50)	0.120	1.09 (0.36–3.33)	0.877	2.63 (0.71–9.7)	0.146	1.69 (0.24–11.8)	0.595
	Azores	3.00 (1.10–8.15)	*0.031*	1.74 (0.51–5.90)	0.374	16.45 (3.93–68.8)	*< 0.001*	9.39 (1.27–69.6)	*0.028*
Season (vs. summer)	Winter	3.54 (1.79–6.99)	*< 0.001*	6.04 (3.11–11.7)	*< 0.001*	6.83 (3.01–15.5)	*< 0.001*	18.53 (7.8–43.9)	*< 0.001*
	Spring	2.84 (1.50–5.36)	*0.001*	3.70 (2.05–6.6)	*< 0.001*	8.07 (3.69–17.8)	*< 0.001*	11.55 (5.2–25.7)	*< 0.001*
	Autumn	2.39 (1.38–4.15)	*0.002*	3.23 (1.81–5.8)	*< 0.001*	4.16 (1.96–8.9)	*< 0.001*	7.21 (3.18–16.3)	*< 0.001*
Alcohol consumption (vs. never)	Occasionally	0.64 (0.42–0.99)	*0.046*	0.87 (0.56–1.37)	0.561	0.33 (0.20–0.57)	*< 0.001*	0.48 (0.29–0.81)	*0.005*
	Daily	0.58 (0.36–0.94)	*0.027*	0.61 (0.36–1.04)	0.067	0.48 (0.27–0.88)	*0.017*	0.53 (1.22–1.02)	0.059
Smoking status (vs. non-smoker)	Past smoker	0.33 (0.10–1.02)	0.056	0.55 (0.23–1.34)	0.191	0.29(0.061–1.33)	0.111	0.48 (0.11–2.02)	0.317
	Current	0.64 (0.37–1.14)	0.130	0.96 (0.58–1.59)	0.872	1.23 (0.64–2.34)	0.53	2.33 (1.22–4.43)	*0.01*
BMI (vs. normal)	Underweight	1.18 (0.47–2.93)	0.724	0.85 (0.31–2.38)	0.760	0.79 (0.31–2.03)	0.625	0.40 (0.14–1.12)	0.082
	Overweight	1.47 (0.94–2.31)	0.093	1.45 (0.93–2.29)	0.105	1.28 (0.74–2.22)	0.372	1.36 (0.78–2.39)	0.282
	Obese	2.41 (1.26–4.60)	*0.08*	2.29 (1.35–3.87)	*0.002*	2.41 (1.24–4.68)	*0.009*	2.61 (1.35–5.08)	*0.004*
Exercise (vs. no)	Yes	0.51 (0.33–0.78)	*0.002*	0.53 (0.35–0.80)	*0.003*	0.49 (0.29–0.83)	*0.008*	0.48 (0.28–0.81)	*0.006*

Numbers in italics highlight statistically significant differences. OR adjustment considered all variables shown. Values are estimated for the overall Portuguese adult population based on observed values corrected for the small imbalances between the socio-demographic characteristics of the sample and of the Portuguese adult population in 2011

IC interval confidence, *vs.* versus, odds ratio, *NUTTS II* Nomenclature of Territorial Units for Statistics, *BMI* body mass index. *NA* not applicable

or decreased risk of vitamin D deficiency have been identified that can support the design of rational strategies to prevent and correct this condition both at the level of clinical practice and national policy making.

These observations underline the urgent need for a wide and scientifically robust debate about the actual relevance of vitamin D deficiency and the most appropriate interventions at the individual and societal levels.

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Author contributions CD wrote the article. All the authors were involved in revising the manuscript critically and approved the final version to be submitted for publication. CD had full access to all of the data in the study and takes responsibility for its integrity and for the accuracy of the data analysis.

Study conception and design: CD, AM, JAPS.
Acquisition of data: AR, HC, JB, CD, HC.
Analysis and interpretation of data: CD, AR, SD, JAPS.
Final report writing: CD and JAPS.
Report revision and approval: all authors.

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Compliance with ethical standards

Competing interests Da Silva has received honoraria as speaker on Vitamina D from Tecnimed, Jaba-Recordati, Azevedos, Merck S.A, Laboratórios Vitoria and Siemens. da Silva has received as Scientific Director of Forum D (http://forumd.org/forumd/), which receive unrestricted financial support of pharmaceutical companies. Duarte has received Scientific Redactor of Forum D (http://forumd.org/forumd/) which received unrestricted financial support of pharmaceutical companies.

Ethics approval and consent to participate This study was conducted respecting the Declaration of Helsinki and was approved by Coimbra Medical School Ethics Committee and EpiReumaPt Scientific Board.

EpiReumaPt was performed according to the principles established by the Declaration of Helsinki, revised in Fortaleza [32]. EpiReumaPt was approved by the National Committee for Data Protection (Comissão Nacional de Proteção de Dados) and by the NOVA Medical School Ethics Committee. Written informed consent was obtained in all phases for all participants, including a separated informed consent for biobank. Further details of ethical issues of EpiReumaPt have been described elsewhere [27].

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