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Vitamin D status and associated factors among Portuguese older adults: results from the Nutrition UP 65 cross-sectional study

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ABSTRACT

Objectives To evaluate vitamin D status and its associated factors in Portuguese older adults from the Nutrition UP 65 study.

Design Cross-sectional observational study.

Participants and methods Nationwide cluster sample of 1500 Portuguese subjects ≥65 years old. Participants were classified, according to US Institute of Medicine cut-offs, as presenting normal 25-hydroxyvitamin D (25(OH)D) levels (≥50.0 nmol/L), at risk of inadequacy (30.0–49.9 nmol/L) or at risk of deficiency (<30 nmol/L). The association between individuals' characteristics and 25(OH)D levels was analysed through multinomial logistic regression analysis.

Results Median 25(OH)D serum value was 36.1 (interquartile range (IQR): 35.5) nmol/L. According to the used cut-offs, 39.6% of participants were at risk of 25(OH)D deficiency and 29.4% were at risk of 25(OH) D inadequacy. In the adjusted model, having higher skin pigmentation and waist circumference >88 cm for women and >102 cm for men were associated with higher odds of 25(OH)D deficiency. Otherwise, living in Lisbon Metropolitan Area and in Madeira, 1–12 years of schooling, being married or in a common-law marriage, monthly income ≥€1000, alcohol consumption, medication or supplements with vitamin D supplement use, and blood samples collected in spring or summer were associated with lower odds of being at risk of 25(OH)D deficiency. In this model, season of blood sample collection, medication or supplements use, and waist circumference were the factors more strongly associated with 25(OH)D levels.

Conclusions Despite using the conservative Institute of Medicine cut-offs, over two-thirds of these study participants presented inadequate 25(OH)D levels, warranting the implementation of corrective measures. Potentially modifiable factors were strongly associated with 25(OH)D levels in this study. These findings may be particularly relevant to the development of public health policies in southern European countries.

Strengths and limitations of this study

- ► The large number of studied subjects.
- ► The population-based recruitment of a nationally representative sample of Portuguese older adults.
- ► The centralisation of 25-hydroxyvitamin D assays in the same laboratory.
- ► The low proportion of institutionalised older adults.
- ► Data on parathyroid hormone were not obtained within this study.

INTRODUCTION

In the 28 member states of the European Union, the proportion of young people (aged 0–14) is projected to remain fairly constant by 2060 (circa 15%), while the share of those aged 15–64 will decline from 66% to 57%. The proportion of persons aged 65 and over will be much larger (rising from 18% to 28% of the population), and those aged 80 and over (rising from 5% to 12%) will become by 2060 nearly as numerous as the young population.[1] In Portugal, data from the most recent national census in 2011 revealed that 19% of the population was aged 65 and over, and that there was an increase of 18.7% in those aged 65 and over between 2001 and 2011.[2]

Serum total 25-hydroxyvitamin D (25(OH) D) concentration is the generally accepted marker of vitamin D status.[3] Vitamin D is crucial for preserving normal bone metabolism. Its deficiency causes skeletal mineralisation defects linked with osteomalacia and its insufficiency increases the risk of osteoporosis.[4] Adequate vitamin D status appears to be protective against musculoskeletal disorders (muscle weakness, falls, fractures), sarcopenia, frailty, infectious diseases, autoimmune diseases, cardiovascular disease, type 1 and type 2 diabetes mellitus, several types of cancer, neurocognitive dysfunction and mental illness, as well as other diseases. Moreover, vitamin D insufficiency or deficiency is linked with all-cause mortality.[5 6]

Nutritional status of fat-soluble vitamins in subjects aged 65 and over is highly variable and determined by season, nutritional status, inflammation, renal function and hospitalisation.[7] Older adults have a higher risk of vitamin D insufficiency and deficiency for a number of reasons. It has been established that the level of ingestion of vitamin D is below the estimated average requirement in 84% of older adults; therefore, inadequate dietary vitamin D intake appears to be highly prevalent.[8] Moreover, the skin of elderly people produces less vitamin D than the skin of younger people. Also, older adults spend less time in the sun, and this increases the risk of 25(OH)D deficiency.[9] Additionally, at the latitude of the Portuguese mainland (37°–42° N), little or no cutaneous vitamin D synthesis occurs during the winter months.[10]

Data from a European report suggest that 25(OH)D deficiency is widespread across Europe, reaching prevalence rates that meet the criteria of a pandemic.[11] The prevalence of 25(OH)D deficiency in Northern Europe is high, affecting upwards to 50% of older adults.[12 13] However, there is a lack of knowledge regarding the burden of 25(OH)D deficiency in Portugal, particularly in the elderly.

The Nutrition UP 65 project was designed with the aim of identifying and reducing nutritional inequalities among older adults in Portugal.[14] In this particular paper our aim was to evaluate 25(OH)D status and its associated factors in a nationally representative sample of Portuguese older adults from the Nutrition UP 65 study. These data may help identify those at risk of vitamin D inadequacy, being of the utmost importance for planning and implementing strategies designed to improve 25(OH)D status in this population.

METHODS

Study design and sampling

A cross-sectional observational study was conducted in Portugal in a sample of 1500 Portuguese subjects ≥65 years old. To achieve a nationally representative sample of Portuguese older adults, a quota sampling approach was adopted using data from Census 2011, regarding sex, age, educational level and regional area defined in the Nomenclature of Territorial Units for Statistical purposes (NUTS II). Detailed information about the Nutrition UP 65 project methodology was previously published.[14]

The potential participants were contacted by the interviewers who provided information about the study purposes and the methodology and invited them to participate. Individuals presenting any condition that precluded the collection of venous blood samples or urine were excluded from the study.

Data were collected between December 2015 and June 2016.

Ethics

This research was conducted according to the guidelines established by the Declaration of Helsinki and the study protocol was approved by the Ethics Committee of the department of 'Ciências Sociais e Saúde' (Social Sciences and Health) from the 'Faculdade de Medicina da Universidade do Porto' (n° PCEDCSS – FMUP 15/2015) and by the Portuguese National Commission of Data Protection (n° 9427/2015). All participants, or two representatives per participant, were asked to read and to sign a duplicated 'informed consent' form.

Data collection

Sociodemographic, lifestyle, clinical and nutritional status, skin phenotype, cognitive performance and household income data were collected using a structured questionnaire. The interview was conducted by eight previously trained registered nutritionists, who also carried out anthropometric data collection.

Sociodemographic data included information on sex, date of birth, regional area, residence type, marital status, education and professional activity. The regional areas used are defined in NUTS II: Alentejo, Algarve, Azores, Lisbon Metropolitan Area, Centre, Madeira and North.[15] Marital status was categorised as single, divorced, widowed, married or in a common-law marriage. Educational level was determined by the number of completed school years and the following categories were used: no formal education, 1–4, 5–12 and >12 years of schooling. Residence type was defined as home or institution, and professional activity as active or non-active. Cognitive performance was assessed by the Portuguese version of the Mini Mental State Examination (MMSE),[16] and the participants were classified as presenting normal cognition or cognitive impairment. The MMSE consists of 30 questions, each scored 1 point if correct. The previously validated cut-off scores used to identify cognitive impairment in the Portuguese population are as follows: individuals with no education, ≤15 points; 1–11 years of school completed, ≤22 points; and >11 years of school completed, ≤27 points.[16]

Lifestyle data included information on physical activity, smoking habits and alcoholic beverages consumption. Physical activity was assessed by the short form of the International Physical Activity Questionnaire (IPAQ),[17] which concerns activities performed during the 7 days before the interview. Participants were asked if they were smokers or non-smokers, and alcoholic beverages consumption was assessed by the number of drinks per day.

Skin phenotype was evaluated by the Fitzpatrick classification: red-haired with freckles, fair-haired, dark-haired, Latin, Arabic, Asian or Black.[18]

Clinical data included medication and supplements use and subject's self-perception of health: participants classified their health status as very good, good, moderate, bad or very bad. Undernutrition status was evaluated by the Portuguese version of the Mini-Nutritional Assessment–Short-Form (MNA-SF).[19 20] Body weight, standing height, or alternatively calf circumference was used to complete the MNA-SF. Waist circumference was assessed as part of nutritional status evaluation.

Anthropometric measurements were collected following standard procedures.[21] Standing height was obtained with a calibrated stadiometer (Seca 213, Hamburg, Germany) with 0.1 cm resolution. For participants with visible kyphosis or when it was impossible to measure standing height due to participant's paralysis or due to mobility or balance limitations, height was obtained indirectly from non-dominant hand length (in centimetres), measured with a calibrated paquimeter from Fervi Equipment (Vignola, Italy), with 0.1 cm resolution (n=37).[22] Body weight (in kilograms) was measured with a calibrated portable electronic scale (Seca 803) with 0.1 kg resolution, with the participants wearing light clothes. When it was not possible to weigh a patient, because of the previously presented factors that prevented standing height measurement, body weight was estimated from mid-upper arm and calf circumferences (n=17).[23] Mid-upper arm, calf and waist circumferences were measured with a metal tape from Lufkin (Sparks, Maryland, USA), with 0.1 cm resolution. Body mass index (BMI) was calculated using the standard formula: [body weight (kg)/stature^2 (m)]. Within this sample, it was not possible either to measure or to estimate weight for four participants, and thus calf circumference was used in order to complete the MNA-SF evaluation.

A sample of blood was collected for each participant and date of sample collection was registered. A certified laboratory, Labco Portugal, was responsible for blood samples collection and analyses. Vitamin D status was evaluated by dosing the serum levels of 25(OH)D through electrochemiluminescence immunoassay using Roche Cobas Vitamin D total assay reagent (Roche Diagnostics, Mannheim, Germany). All analyses were done in the same equipment. Blood samples that enabled this analysis were collected by qualified nurses within a few days after the application of the questionnaire. Participants were classified as presenting adequate 25(OH)D levels (≥50.0 nmol/L), as being at risk of inadequacy (30.0–49.9 nmol/L) or as being at risk of deficiency (<30.0 nmol/L).[24]

Statistical analysis

Categorical variables were reported as frequencies. The normality of the distribution regarding quantitative variables was evaluated through Kolmogorov-Smirnov test, and results were described as mean and SD or as median and interquartile range (IQR), according to whether variables presented normal distribution or not.

Age was dichotomised into two categories: 65–79 years and ≥80 years. Household income was summarised using the following cut-offs: <€500, €500–999 and ≥€1000. From all the included participants, 772 (51.5%) did not know or preferred not to declare their income, and thus they were allocated in a separate category. Data collected with IPAQ was converted to metabolic equivalent of task per minute (MET-min). Median values were calculated for walking, moderate-intensity activities and vigorous-intensity activities using established formulas. Total physical activity MET-min/week was defined as the sum of walking + moderate + vigorous MET-min/week scores.[25] Kilocalories were computed from MET-min/week scores,[17] and individuals were classified as either presenting low physical activity levels, <383 kcal/week for men and <270 kcal/week for women, or as presenting normal physical activity levels, ≥383 kcal/week and ≥270 kcal/week, respectively, for men and women.[26] Moderate alcoholic consumption was defined as one drink/day for women and as 1 or 2 drinks/day for men, whereas excessive consumption was defined as ≥2 drinks/day for women and as ≥3 drinks/day for men.[27] Due to the small number of participants in some categories, marital status was dichotomised as single, divorced or widowed and as married or in a common-law marriage; according to skin phenotype, subjects were grouped as the following: red-haired with freckles, fair-haired, dark-haired, Latin, Arabic, Asian and Black people. The self-perception of health categories used were very good, good, moderate bad, and very bad.

Participants were grouped according to their BMI in the following categories: underweight/normal range (≤24.99 kg/m^2), preobese (25.00–29.99 kg/m^2) and obese (≥30.00 kg/m^2).[28] Three categories of waist circumference were created: ≤80 cm (women) and ≤94 cm (men); 81–88 cm (women) and 95–102 cm (men); and >88 cm (women) and >102 cm (men).[29] According to date of blood sample collection, participants were grouped into the following two categories: late autumn or winter, and spring or summer.

In order to study a possible seasonal variation in vitamin D levels, a plot was charted representing vitamin D serum concentration (nmol/L) by the four seasons of blood sample collection (autumn, winter, spring and summer).

According to 25(OH)D levels, adequate (≥50.0 nmol/L), risk of inadequacy (30.0–49.9 nmol/L) and risk of deficiency (<30.0 nmol/L),[24] participants were compared for several sociodemographic, lifestyle, and clinical and nutritional characteristics using Kruskal-Wallis test or one-way analysis of variance for continuous variables, and Pearson χ^2 test for categorical variables.

Before conducting the regression procedures, missing data for independent variables were imputed (occurring in 1.9% of participants for a total of 0.12% of data points — footnote table 1) using a Markov Chain Monte Carlo approach (5 imputation data sets, 10 iterations). Bivariable and multivariable, multinomial logistic regression models were conducted to identify the independent factors associated with risk of 25(OH)D inadequacy or risk of 25(OH)D deficiency (dependent variable). ORs and respective 95% CIs were calculated. The following characteristics were considered in the multivariable procedure: sex (dichotomous), age (dichotomous), regional area (categorical), residence (dichotomous), education (categorical), marital status (dichotomous), professional activity (dichotomous), skin phenotype (categorical), household income (categorical), physical activity (dichotomous), cognitive performance (dichotomous), smoking habits (dichotomous), alcoholic beverages consumption (categorical), self-perception of health (categorical), medication and supplements use (categorical), MNA-SF (dichotomous), waist circumference (categorical), and season of blood sample collection (dichotomous).

Results were considered significant when p<0.05. Statistical analyses were conducted using the Software Package for Social Sciences for Windows V.23.0.

RESULTS

The characteristics of the 1500 subjects, aged between 65 and 100 years, according to 25(OH)D status (adequacy, at risk of inadequacy and at risk of deficiency) are presented in table 1. The mean 25(OH)D serum value was 42.3 nmol/L (SD: 29.7 nmol/L). The median 25(OH) D serum value was 36.1 nmol/L (IQR: 35.5 nmol/L). Within this sample, using the US Institute of Medicine (IOM) cut-off points, 39.6% of participants were at risk of 25(OH)D deficiency and 29.4% were at risk of 25(OH) D inadequacy. When applying the Endocrine Society's practice guidelines' cut-offs, 1050 participants (70.0%) presented vitamin D levels ≤50 nmol/L, 273 (18.2%) presented values within the range 51–74 nmol/L, and for 177 (11.8%) of the included participants the levels were ≥75 nmol/L. Seven participants (0.5%) presented 25(OH)D levels >125.0 nmol/L and eight participants had 25(OH)D levels >150.0 nmol/L. From the included female participants, 15.1% presented waist circumference values between 81 and 88 cm and 77.7% had waist circumference values >88 cm. Regarding male participants, 29.3% presented waist circumference values between 95 and 102 cm and 50.2% had values >102 cm. Blood samples collection seasonal distribution was as follows: 51.2% in late autumn or winter, 43.1% in spring and 5.7% in summer. A seasonal variation in 25(OH)D median values can be observed in figure 1.

Compared with men's group, a higher proportion of women were found to be at risk of 25(OH)D deficiency and at risk of inadequacy. Regarding sample distribution through the national territory, subjects living in the North, Centre and Alentejo were more likely to be at risk of 25(OH)D deficiency. Higher proportions of institutionalised subjects, in the lower educational level, single, divorced or widowed, and with darker skin pigmentation phenotypes reporting low levels of physical activity and presenting cognitive impairment were also found to be at risk of 25(OH)D deficiency. Regarding clinical data, the majority of subjects who rated their health as bad or very bad were found to be at risk of 25(OH)D deficiency. Otherwise, a higher proportion of subjects who used medication or supplements with vitamin D in their composition presented adequate 25(OH)D levels. Higher proportions of subjects presenting undernutrition risk and waist circumference values >88 cm (women) or >102 cm (men) were found to be at risk of 25(OH) D deficiency. In fact, women and men who were at risk of 25(OH)D deficiency had higher mean waist circumference values than participants at risk of inadequacy or presenting adequate levels of 25(OH)D. Higher proportions of obese participants were found in the risk of inadequacy and in the risk of deficiency categories. For both men and women, as vitamin D levels decreased, increases in BMI were observed. Moreover, participants who were at risk of 25(OH)D deficiency were older and reported lower median income values compared with participants at risk of inadequacy or presenting adequate levels of 25(OH)D. Participants aged ≥80 years, with an income <€500 and whose blood sample was collected during late autumn and winter were more likely to be at risk of 25(OH)D deficiency (table 1).

Using bivariable (unadjusted) analyses, presenting cognitive impairment and waist circumference >88 cm for women and >102 cm for men were associated with higher odds of being at risk of 25(OH)D inadequacy. Participants presenting 5–12 completed years of schooling, dark-haired or Latin people skin phenotype, having an income ≥€500 or having not declared the income, with moderate alcoholic beverage consumption, using medication or supplements with vitamin D, and whose blood sample collection occurred during spring and summer had lower odds of being at risk of 25(OH)D inadequacy (table 2).

After conducting multivariable multinomial logistic regression models, waist circumference >88 cm for women and >102 cm for men was associated with higher odds of being at risk of 25(OH)D inadequacy. Having 5–12 completed years of schooling, dark-haired or Latin people skin phenotype, having an income ≥€500 or having not declared the income, use of medication or supplements with vitamin D, and blood sample collection during spring and summer were the variables associated with lower odds of being at risk of 25(OH)D inadequacy (table 2).

Regarding the factors associated with the risk of 25(OH)D deficiency in the bivariable (unadjusted) analysis, being aged ≥80 years, being institutionalised, reporting low levels of physical activity, cognitive impairment, self-perception of health rated as moderate, as bad or very bad, unknown use or unknown composition of medication and supplementation, undernutrition risk or undernutrition according to MNA-SF, and waist circumference >88 cm for women and >102 cm for men were all associated with higher odds of being at risk of 25(OH) D deficiency. Otherwise, being a male, living in Lisbon Metropolitan Area and in Madeira, having any level of formal education (≥1 schooling years), being married or in a common-law marriage, having an income ≥€500 or having not declared the income, consumption of alcoholic beverages, use of medication or of supplements with vitamin D in their composition, and blood sample collection during spring and summer were associated with lower odds of being at risk of 25(OH)D deficiency (table 2).

In the multivariable procedure, having Arabic, Asian or Black skin phenotype and waist circumference >88 cm for women and >102 cm for men were associated with higher

Table 1 Baseline sociodemographic, clinical and nutritional characteristics of 1500 older Portuguese ≥65 years old participating in a cross-sectional observational study according to 25(OH)D status*

	At risk of deficiency <30.0 nmol/L 25(OH)D (n=594)	At risk of inadequacy 30.0–49.9 nmol/L 25(OH)D (n=441)	Adequacy ≥50.0 nmol/L 25(OH)D (n=465)	p
Sex, n (%)				
Women	400 (67.3)	235 (53.3)	237 (51.0)	<0.001†
Men	194 (32.7)	206 (46.7)	228 (49.0)	
Age, years, median (IQR)	77.0 (11.0)	72.0 (9.0)	72.0 (9.0)	<0.001‡
Age, years, n (%)				
65–79	368 (62.0)	353 (80.0)	384 (82.6)	<0.001†
≥80	226 (38.0)	88 (20.0)	81 (17.4)	
Regional area, n (%)				
North	200 (33.7)	131 (29.7)	139 (29.9)	<0.001†
Centre	185 (31.1)	103 (23.4)	103 (22.2)	
Lisbon Metropolitan Area	105 (17.7)	137 (31.1)	142 (30.5)	
Alentejo	65 (10.9)	34 (7.7)	37 (8.0)	
Algarve	26 (4.4)	21 (4.8)	18 (3.9)	
Madeira	4 (0.7)	10 (2.3)	16 (3.4)	
Azores	9 (1.5)	5 (1.1)	10 (2.2)	
Residence, n (%)				
Home	544 (91.6)	431 (97.7)	453 (97.4)	<0.001†
Institution	50 (8.4)	10 (2.3)	12 (2.6)	
Education, years, n (%)				
0	133 (22.4)	46 (10.4)	33 (7.1)	<0.001†
1–4	396 (66.7)	321 (72.8)	314 (67.5)	
5–12	50 (8.4)	52 (11.8)	87 (18.7)	
>12	15 (2.5)	22 (5.0)	31 (6.7)	
Marital status, n (%)				
Single, divorced or widowed	401 (67.5)	207 (47.0)	189 (40.6)	<0.001†
Married or common-law marriage	193 (32.5)	233 (53.0)	276 (59.4)	
Professional activity, n (%)				
No	584 (98.6)	432 (98.2)	449 (97.0)	0.149†
Yes	8 (1.4)	8 (1.8)	14 (3.0)	
Skin phenotype, n (%)				
Red-haired with freckles or fair-haired people	110 (18.6)	111 (25.2)	84 (18.1)	0.007†
Dark-haired or Latin people	441 (74.6)	306 (69.4)	364 (78.3)	
Arabic, Asian or Black people	40 (6.8)	24 (5.4)	17 (3.7)	
Household income, €, median (IQR)§	500 (384)	600 (543)	800 (690)	<0.001‡
Household income, €, n (%)				
<500	124 (20.9)	79 (17.9)	45 (9.7)	<0.001†
500–999	108 (18.2)	95 (21.5)	102 (21.9)	
≥1000	40 (6.7)	50 (11.3)	85 (18.3)	
Does not know or does not declare	322 (54.2)	217 (49.2)	233 (50.1)	

Continued

Table 1 Continued

	At risk of deficiency <30.0 nmol/L 25(OH)D (n=594)	At risk of inadequacy 30.0–49.9 nmol/L 25(OH)D (n=441)	Adequacy ≥50.0 nmol/L 25(OH)D (n=465)	p
Physical activity (IPAQ)¶, kcal/week, n (%)				
Normal	445 (75.0)	385 (87.5)	407 (87.5)	<0.001†
Low	148 (25.0)	55 (12.5)	58 (12.5)	
Cognitive performance (MMSE), n (%)				
Normal	543 (91.4)	409 (92.7)	449 (96.6)	0.003†
Impairment	51 (8.6)	32 (7.3)	16 (3.4)	
Smoking habits, n (%)				
No	569 (95.8)	418 (94.8)	445 (95.7)	0.713†
Yes	25 (4.2)	23 (5.2)	20 (4.3)	
Alcoholic beverages consumption, n (%)				
None	446 (75.3)	260 (59.0)	248 (53.3)	<0.001†
Moderate (women=1/day, men=1 or 2/day)	109 (18.4)	119 (27.0)	161 (34.6)	
Excessive (women≥2/day, men≥3/day)	37 (6.3)	62 (14.1)	56 (12.0)	
Self-perception of health, n (%)				
Very good/good	152 (25.6)	149 (33.8)	178 (38.5)	<0.001†
Moderate	286 (48.2)	219 (49.7)	227 (49.0)	
Bad/very bad	155 (26.2)	72 (16.4)	58 (12.5)	
Medication and supplements use, n (%)				
No use	489 (82.3)	391 (88.7)	382 (82.2)	<0.001†
With vitamin D	12 (2.0)	13 (2.9)	55 (11.8)	
Unknown composition or use	93 (15.7)	37 (8.4)	28 (6.0)	
Undernutrition status (MNA-SF), n (%)				
Not undernourished	468 (78.8)	383 (86.8)	408 (87.7)	<0.001†
Risk of undernutrition	119 (20.0)	50 (11.3)	53 (11.4)	
Undernutrition	7 (1.2)	8 (1.8)	4 (0.9)	
Waist circumference, cm, mean (SD)				
Men	100.8 (12.0)	96.1 (11.4)	92.5 (11.5)	<0.001**
Women	105.2 (11.3)	102.5 (10.0)	101.0 (10.1)	<0.001**
Waist circumference, cm, n (%)				
Women ≤80 cm, men ≤94 cm	47 (8.0)	60 (13.7)	82 (17.8)	<0.001†
Women 81–88 cm, men 95–102 cm	81 (13.8)	96 (21.8)	136 (29.4)	
Women >88 cm, men >102 cm	459 (78.2)	283 (64.5)	244 (52.8)	
Body mass index, kg/m^2				
Men	28.8 (5.7)	28.2 (5.2)	27.9 (5.3)	<0.001**
Women	30.3 (7.0)	29.7 (5.7)	28.2 (5.5)	0.035**
Body mass index, kg/m^2, n (%)				
Underweight/normal range (≤24.99 kg/m^2)	83 (14.0)	76 (17.3)	93 (20.0)	
Preobese (25.00–29.99 kg/m^2)	234 (39.6)	203 (46.1)	225 (48.4)	<0.001†
Obese (≥30.00 kg/m^2)	274 (46.4)	161 (36.6)	147 (31.6)	

Continued

Table 1 Continued

	At risk of deficiency <30.0 nmol/L 25(OH)D (n=594)	At risk of inadequacy 30.0–49.9 nmol/L 25(OH)D (n=441)	Adequacy ≥50.0 nmol/L 25(OH)D (n=465)	p
Season of blood sample collection, n (%)				
Late autumn or winter	406 (68.4)	197 (44.9)	164 (35.3)	<0.001†
Spring or summer	188 (31.6)	242 (55.1)	301 (64.7)	

*Values may not add up 100.0% due to rounding up. Data before multiple imputation. Number of missing data points (0.14% of all data points): marital status n=1 (0.1%), professional activity n=5 (0.3%), skin phenotype n=3 (0.2%), physical activity (IPAQ) n=2 (0.1%), alcoholic beverages consumption n=2 (0.1%), self-perception of health n=4 (0.3%), waist circumference n=12 (0.8%), body mass index n=4 (0.3%) and season of data collection n=2 (0.1%).

†χ^2 test.

‡Kruskal-Wallis test.

§Data presented for 728 subjects because 772 participants did not know or preferred not to declare their income.

¶Normal physical activity levels defined as ≥383 kcal/week (men) and ≥270 kcal/week (women); low physical activity levels defined as <383 kcal/week (men) and <270 kcal/week (women).

**One-way analysis of variance.

25(OH)D, 25-hydroxyvitamin D; IPAQ, International Physical Activity Questionnaire; MMSE, Mini Mental State Examination; MNA-SF: Mini Nutritional Assessment – Short-Form.

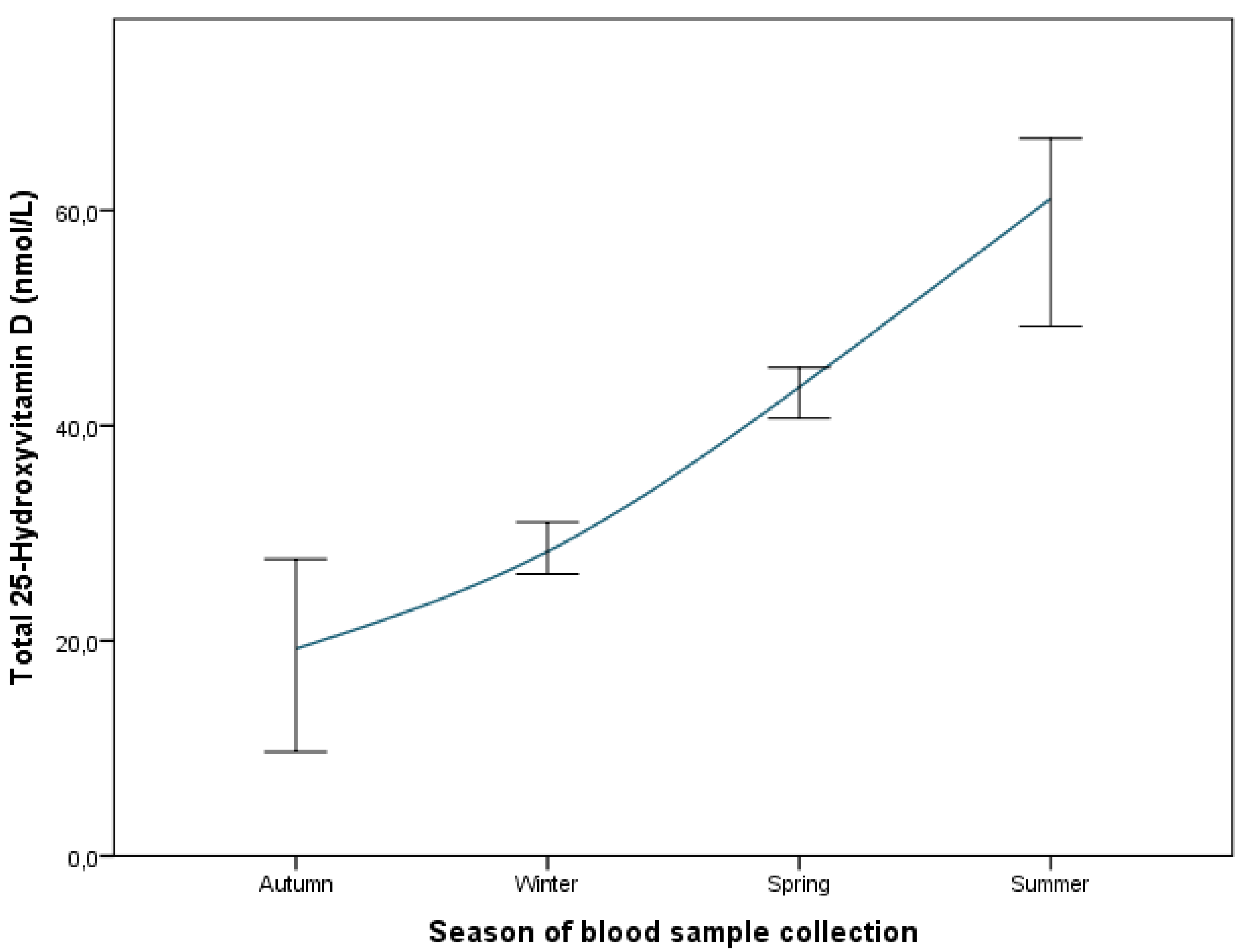

Figure 1 Total 25-hydroxyvitamin D (nmol/L) by season of blood sample collection.

Table 2 Factors associated with risk of vitamin D inadequacy (30.0–49.9 nmol/L 25(OH)D) and with risk of vitamin D deficiency (<30.0 nmol/L) by bivariable and multivariable multinomial logistic regression for 1500 older Portuguese ≥65 years old participating in a cross-sectional observational study

	At risk of inadequacy		At risk of deficiency	
	Crude OR (95% CI)	Adjusted OR (95% CI)	Crude OR (95% CI)	Adjusted OR (95% CI)
Sex				
Women	1	1	1	1
Men	0.91 (0.70 to 1.18)	1.23 (0.87 to 1.74)	0.50 (0.39 to 0.65)***	1.28 (0.88 to 1.87)
Age, years				
65–79	1	1	1	1
≥80	1.18 (0.85 to 1.65)	0.99 (0.67 to 1.46)	2.91 (2.18 to 3.90)***	1.49 (1.03 to 2.16)
Regional area				
North	1	1	1	1
Centre	1.06 (0.74 to 1.52)	1.24 (0.82 to 1.88)	1.25 (0.90 to 1.73)	1.23 (0.81 to 1.86)
Lisbon Metropolitan Area	1.02 (0.73 to 1.43)	1.10 (0.75 to 1.62)	0.51 (0.37 to 0.72)***	0.51 (0.33 to 0.79)*
Alentejo	0.98 (0.58 to 1.64)	0.88 (0.50 to 1.54)	1.22 (0.77 to 1.93)	1.14 (0.66 to 2.00)
Algarve	1.24 (0.63 to 2.43)	1.21 (0.57 to 2.57)	1.00 (0.53 to 1.90)	0.60 (0.27 to 1.32)
Madeira	0.66 (0.29 to 1.51)	0.72 (0.30 to 1.73)	0.17 (0.06 to 0.53)*	0.22 (0.07 to 0.74)*
Azores	0.53 (0.18 to 1.59)	0.56 (0.18 to 1.78)	0.63 (0.25 to 1.56)	0.80 (0.28 to 2.30)
Residence				
Home	1	1	1	1
Institution	0.88 (0.38 to 2.05)	0.84 (0.30 to 2.33)	3.47 (1.82 to 6.60)***	1.64 (0.73 to 3.72)
Education, years				
0	1	1	1	1
1–4	0.73 (0.46 to 1.18)	0.79 (0.46 to 1.33)	0.31 (0.21 to 0.47)***	0.59 (0.36 to 0.96)*
5–12	0.43 (0.24 to 0.75)*	0.51 (0.27 to 0.96)*	0.14 (0.09 to 0.24)***	0.40 (0.21 to 0.76)*
>12	0.51 (0.25 to 1.03)	0.82 (0.37 to 1.83)	0.12 (0.06 to 0.25)***	0.50 (0.22 to 1.36)
Marital status				
Single, divorced or widowed	1	1	1	1
Married or common-law marriage	0.77 (0.59 to 1.01)	1.03 (0.75 to 1.43)	0.33 (0.26 to 0.42)***	0.60 (0.43 to 0.84)*
Professional activity				
No	1	1	1	1
Yes	0.60 (0.25 to 1.43)	0.81 (0.51 to 1.31)	0.46 (0.19 to 1.11)	0.99 (0.35 to 2.78)
Skin phenotype				
Red-haired with freckles or fair-haired people	1	1	1	1
Dark-haired or Latin people	0.64 (0.46 to 0.88)*	0.67 (0.47 to 0.95)*	0.93 (0.68 to 1.27)	1.13 (0.77 to 1.66)
Arabic, Asian or Black people	1.07 (0.54 to 2.12)	0.88 (0.42 to 1.84)	1.80 (0.96 to 3.40)	2.24 (1.03 to 4.86)*
Household income, €				
<500	1	1	1	1
500–999	0.53 (0.33 to 0.84)*	0.58 (0.35 to 0.95)*	0.38 (0.25 to 0.59)***	0.70 (0.42 to 1.16)
≥1000	0.33 (0.20 to 0.56)***	0.41 (0.23 to 0.75)*	0.17 (0.10 to 0.28)***	0.52 (0.28 to 0.98)*

Continued

Table 2 Continued

	At risk of inadequacy		At risk of deficiency	
	Crude OR (95% CI)	**Adjusted OR (95% CI)**	**Crude OR (95% CI)**	**Adjusted OR (95% CI)**
Does not know or does not declare	0.53 (0.35 to 0.80)*	0.60 (0.38 to 0.95)*	0.50 (0.34 to 0.73)***	0.85 (0.53 to 1.34)
Physical activity (IPAQ)†				
Normal	1	1	1	1
Low	1.00 (0.67 to 1.48)	0.73 (0.47 to 1.14)	2.33 (1.67 to 3.25)***	1.26 (0.83 to 1.89)
Cognitive performance (MMSE)				
Normal	1	1	1	1
Impairment	2.20 (1.19 to 4.06)*	1.95 (0.99 to 3.81)	2.64 (1.48 to 4.69)**	1.38 (0.69 to 2.75)
Smoking habits				
No	1	1	1	1
Yes	1.22 (0.66 to 2.26)	1.31 (0.67 to 2.56)	0.98 (0.54 to 1.78)	1.84 (0.92 to 3.72)
Alcoholic beverages consumption, n/day				
None	1	1	1	1
Moderate (women=1, men=1 or 2)	0.70 (0.52 to 0.95)*	0.78 (0.54 to 1.13)	0.38 (0.28 to 0.50)***	0.49 (0.32 to 0.73)***
Excessive (women ≥2, men ≥3)	1.06 (0.71 to 1.58)	1.23 (0.77 to 1.96)	0.37 (0.24 to 0.57)***	0.48 (0.27 to 0.85)*
Self-perception of health				
Very good/good	1	1	1	1
Moderate	1.15 (0.87 to 1.53)	1.05 (0.77 to 1.44)	1.48 (1.12 to 1.95)*	1.13 (0.81 to 1.60)
Bad/very bad	1.50 (0.99 to 2.25)	1.30 (0.82 to 2.06)	3.14 (2.16 to 4.55)***	1.49 (0.93 to 2.40)
Medication and supplements use				
No use	1	1	1	1
With vitamin D	0.23 (0.12 to 0.43)***	0.20 (0.11 to 0.40)***	0.17 (0.09 to 0.32)***	0.11 (0.05 to 0.22)***
Unknown composition or use	1.29 (0.78 to 2.15)	1.15 (0.65 to 2.04)	2.60 (1.67 to 4.04)***	1.42 (0.85 to 2.39)
Undernutrition status (MNA-SF)				
Not undernourished	1	1	1	1
Risk of undernutrition or undernutrition	1.08 (0.73 to 1.60)	0.99 (0.64 to 1.53)	1.93 (1.37 to 2.71)***	1.39 (0.91 to 2.12)
Waist circumference, cm				
Women ≤80 cm, men ≤94 cm	1	1	1	1
Women: 81–88 cm, men: 95–102 cm	0.96 (0.63 to 1.47)	1.09 (0.69 to 1.71)	1.05 (0.67 to 1.65)	1.16 (0.68 to 2.00)
Women >88 cm, men >102 cm	1.59 (1.09 to 2.31)*	1.62 (1.07 to 2.50)*	3.28 (2.22 to 4.84)***	2.90 (1.79 to 4.69)***
Season of blood sample collection				
Late autumn or winter	1	1	1	1
Spring or summer	0.67 (0.51 to 0.88)*	0.60 (0.43 to 0.85)*	0.25 (2.07 to 2.97)***	0.34 (0.24 to 0.48)***

*, ** and ***: p values for multinomial logistic regression analyses. *p<0.05, **p=0.001, ***p<0.001.
†Normal physical activity levels defined as ≥383 kcal/week (men) and ≥270 kcal/week (women); low physical activity levels defined as <383 kcal/week (men) and <270 kcal/week (women).
25(OH)D, 25-hydroxyvitamin D; CI, Confidence Interval; IPAQ, International Physical Activity Questionnaire; MMSE, Mini Mental State Examination; MNA-SF: Mini-Nutritional Assessment – Short-Form.

odds of being at risk of 25(OH)D deficiency. Otherwise, living in Madeira, having 1–12 years of schooling, being married or in a common-law marriage, having an income ≥€1000, consumption of alcoholic beverages, use of medication or supplements with vitamin D in their composition, and blood sample collection during spring and summer were the factors associated with lower odds of risk of 25(OH)D deficiency (table 2).

DISCUSSION

In this cross-sectional study, the vitamin D status of Portuguese older adults was evaluated, and over two-thirds of the study participants presented inadequate or deficient 25(OH)D levels. The factors associated with 25(OH)D low levels were explored and some are potentially modifiable. There is no global agreement on the serum concentration of 25(OH)D that defines vitamin D deficiency. The Nutrition UP 65 project used the cut-off values specified by the IOM (ie, 30 nmol/L), which was established taking into consideration the risk of metabolic bone disease.[24] It has recently been argued that the IOM reference values for vitamin D have been misinterpreted and misapplied regarding the definition of deficiency and inadequacy. It is therefore essential to understand how the IOM nutrient reference values are defined. According to the IOM, a 25(OH)D serum level of 50 nmol/L would meet the requirements of 97.5% of the population; consequently, it cannot be used as a cut-off point for deficiency.[30] In Europe the serum 25(OH)D cut-off value typically used to define deficiency has been 25 nmol/L.[31] Interestingly, the Endocrine Society Clinical Guidelines define vitamin D deficiency when serum 25(OH)D is below 50 nmol/L. These guidelines also state that to take full advantage of the effect of vitamin D on calcium, bone and muscle metabolism, serum 25(OH)D concentration should surpass 75 nmol/L.[3] Within this sample, 15 participants had vitamin D values above 125 nmol/L. For 13 of those, blood samples were collected during the spring. Nine out the 15 took medication or supplementation with vitamin D, whereas five did not take the supplements and one could not provide the necessary information.

To our knowledge this work is the first to describe the 25(OH)D status and its associated factors in a nationally representative sample of Portuguese older adults. The fact that 39.6% of the participants in this study were at risk of 25(OH)D deficiency and 29.4% were at risk of 25(OH)D inadequacy, even when using relatively conservative cut-offs, is a matter of great concern. In fact, had we defined 25(OH)D deficiency when serum 25(OH)D was below 50 nmol/L, the percentage of participants with deficiency would have reached 69% of our sample.

Data of 595 Dutch adults, aged 65 years and above, revealed a high prevalence of vitamin D deficiency, with 36% of the participants having a 25(OH)D status <50 nmol/L.[32] Interestingly, this value is almost half the prevalence observed in our study when using the same cut-off point for deficiency.

There is a considerable variation in vitamin D status between other European countries. Almost three decades ago the Euronut-Seneca study, carried out on independent older persons, revealed mean serum 25(OH)D levels of 20–30 nmol/L in southern European centres versus 40–50 nmol/L in northern Europe. The Portuguese centre involved in this study included 59 older adults, aged 70–75 years, with a mean serum 25(OH)D level of 39 nmol/L, and 32% of this sample presented 25(OH)D levels below 30 nmol/L.[33] Very low 25(OH)D levels were also measured in free living and institutionalised Spanish older adults.[34] A study in Italy that included 700 women, aged 60–80 years, reported 25(OH)D levels lower than 12.5 nmol/L in 27% of them and lower than 30 nmol/L in 76% of the participants.[35] The Longitudinal Aging Study Amsterdam showed a serum 25(OH)D lower than 25 nmol/L in 8% of men and 14% of women, and lower than 50 nmol/L in 45% of men and 56% of the women.[36]

Apparently there is a higher prevalence of poor vitamin D status in the elderly from southern Europe. Studies of older adult populations revealed that the prevalence of vitamin D deficiency was much lower in higher latitude countries such as Finland, Norway and Iceland, while mid-latitude countries like the Netherlands, Germany, Ireland and the UK presented a higher prevalence, even when considering factors like ethnicity.[11] In higher latitude countries the breadth of the increase in prevalence in vitamin D deficiency between winter and summer values was considerably lower, which is probably due to the greater use of vitamin D supplements and/or fortified food items in these regions.[37]

It is possible that the high prevalence of 25(OH) D deficiency observed in our sample coexists with other micronutrient inadequacies. A recent review of micronutrient intakes and potential inadequacies of community-dwelling older adults revealed that of the 20 nutrients analysed, 6 were considered a possible public health concern: vitamin D, thiamine, riboflavin, calcium, magnesium and selenium.[7]

This raises the need to improve the knowledge on these nutrient levels status in older adults.

In our study waist circumference >88 cm for women and >102 cm for men was one of the factors more intensely associated with higher odds of 25(OH)D inadequacy or deficiency. Indeed, similar findings have been obtained in several studies. In the Rotterdam Study 25(OH)D serum levels were inversely and independently associated with waist circumference in the elderly.[38] This inverse association between waist circumference and 25(OH)D serum levels was also observed in the Framingham Heart Study. This study also showed that vitamin D status is strongly associated with changes in subcutaneous and particularly visceral adiposity.[39] The inverse association between waist circumference and 25(OH)D was also observed in elderly Korean women but not in men,[40] and over a 2-year period in adults with pre-diabetes in the Diabetes Prevention Program.[41]

Waist circumference relates closely to BMI and the proportion of body fat located intra-abdominally, as opposed to subcutaneous depots.[42]

The secretory component of visceral fat is rich in proinflammatory and low in anti-inflammatory adipokines. It is unclear if and how these factors affect blood 25(OH)D concentration; however, a few reports have suggested that inflammation may lower circulating 25(OH)D levels potentially by effects on vitamin D-binding proteins.[43]

Besides, low nutritional density diets were also found to be associated with lower levels of 25(OH)D.[7] Therefore, specific strategies to reduce abdominal obesity while increasing dietary nutrient density could be an important part of the modifiable factors associated with inadequacy or deficiency.

In the present study having 5–12 completed years of schooling, a higher household income and use of 25(OH)D medication or supplements were independently associated with lower odds of being at risk of 25(OH)D inadequacy or deficiency. A Canadian ecological study raised the possibility that the low levels of 25(OH)D in the elderly may not be related to age per se but to other correlated variables such as education because those with greater postsecondary education possibly achieved higher 25(OH)D levels through supplementation.[44] Indeed, a study in a Swedish nationwide population of older men and women showed that higher educational level was associated with use of calcium and vitamin D supplements, even after adjustment for the occurrence of osteoporotic fractures. The use of osteoporosis drug therapy (including calcium and vitamin D) seemed unequally distributed in that elderly population, even in a country with presumably equal access to healthcare.[45]

The inverse association between income and vitamin D status is a frequent finding in several studies. In the region of Wallonia (Belgium), it was reported that in the absence of vitamin D supplementation, subjects with very low household income were more likely to be vitamin D-deficient.[46] In Britain it has also been described that low-income/materially deprived population have lower vitamin D status than the general population.[47]

In our study the use of vitamin D medication or supplements was related to lower odds of 25(OH)D inadequacy or deficiency. Similarly, the total intake of vitamin D in the US population (diet and supplements) has been positively associated with higher income.[48]

We also found that those living in the Lisbon Metropolitan Area had lower odds of 25(OH)D deficiency. One possible explanation for this result may be related to the fact that this regional area had the higher mean income of all regions studied (data not shown), considering that a higher income may enable better dietary practices and the use of supplements.

We found that blood samples collected during spring or summer reduced the odds of 25(OH)D inadequacy or deficiency. This association may be explained by a rise in solar ultraviolet (UV) radiation during these months, thereby increasing the possibility of exposure to UV radiation of sufficient intensity to synthesise vitamin D in all skin phenotypes.[49] In our study blood samples were collected between December 2015 and June 2016; this means that the first samples were collected in late autumn with at least 2 months of low ultraviolet B radiation (UVB) exposure in our latitude and consequent low cutaneous synthesis of vitamin D. Otherwise in our latitude from the beginning of March, we have many days reaching a UV index >3, the threshold of UVB energy above which cutaneous vitamin D synthesis can occur.[50] Our interpretation is that some of those participants whose samples were collected in spring or summer were already benefiting from this increase in cutaneous synthesis of vitamin D, resulting in a reduced proportion of cases of vitamin D deficiency in the samples collected in this period. As illustrated by a Dutch study, sun exposure still appears to be an important determinant of serum 25(OH)D in older individuals, closely followed by genes and vitamin D intake.[51]

We observed that those individuals with higher skin pigmentation had markedly increased odds of 25(OH)D deficiency. In similar latitudes this inverse relation between skin pigmentation and 25(OH)D levels has been previously described.[49] However, to our surprise, dark-haired or Latin people skin phenotype subjects had lower odds of 25(OH)D inadequacy. Since our reference in this model were red-haired with freckles or fair-haired people phenotypes, we can speculate that as previously described[52] public health campaigns advocating sun avoidance in fair-skinned individuals may need to be revised in view of their risk of vitamin D deficiency.

Living in Madeira island reduced the odds of 25(OH)D deficiency. Although based on a small sample size, this result may be related to higher UV radiation exposure as this region has a much lower latitude (32° N) than the northern mainland Portugal (around 41° N), which was the reference region in our adjusted model.

In our study alcoholic beverages intake reduced the odds of 25(OH)D deficiency. The positive association between alcohol intake and vitamin D status has been described in many studies.[53–55] However, these results should be regarded with caution since excessive alcohol intake may have a negative effect on vitamin D status due to changes in vitamin D metabolism.[56] It has also been described in an animal model that alcohol treatment may decrease the serum levels of its active metabolite (1,25-dihydroxycholecalciferol) while 25(OH)D levels remained unchanged. This may limit the interest of using 25(OH)D when studying the effects of alcohol on vitamin D status.[57]

Being married or in common-law marriage was associated with lower odds of 25(OH)D deficiency. This finding could be explained by the fact that eating alone can severely influence nutritional practices. Studies of independently living elderly indicate that those living alone, or eating alone, eat less and are at higher risk of poor nutritional status.[58 59]

The main strengths of this study are the large number of studied subjects, the population-based recruitment of a nationally representative sample of Portuguese older adults and the centralisation of 25(OH)D assays in a central laboratory. Although also representing a strength of this study, the strict inclusion/exclusion criteria may also be a limitation, as it possibly resulted in the selection of a sample with better-than-average health and nutritional status. It could be argued that the prevalence of 25(OH)D deficiency might be higher in an otherwise selected population that for instance included a higher proportion of institutionalised older adults. This should be taken into account when generalising the results of the present study to other samples.

The participants' self-reported use of vitamin D medication or supplements did not allow an accurate quantification. Additionally, vitamin D dietary intake was not assessed in this study excluding the possibility to reflect its effect on 25(OH)D status. Although we employed the most widely used automated 25(OH) D immunoassays in Portugal, it is possible that another assay method would have given different results and thus different proportions of at risk of 25(OH)D inadequacy/deficiency individuals.[11] Data on parathyroid hormone (PTH) were not obtained within this study and could have been helpful since vitamin D controls the intestinal absorption of calcium together with PTH in a negative feedback loop, and together 25(OH)D, PTH and calcium are important for musculoskeletal health.[60]

In conclusion, the abdominal obesity observed in this sample is an important part of the modifiable factors associated with 25(OH)D inadequacy/deficiency. Therefore, specific strategies to reduce abdominal obesity could improve older adults' vitamin D status. Public health strategies may explore the implementation of food-based solutions for the prevention of vitamin D deficiency. The use of supplements and/or food fortification may be considered weighing the risks of these practices. Sun exposure is another modifiable factor greatly influencing vitamin D status, but at this point there is no consensus whether a minimal-risk approach to UVB exposure would allow vitamin D synthesis without increasing the risk of skin cancer.

The high prevalence in 25(OH)D inadequacy/deficiency observed in our study, even when using the IOM's conservative cut-off points, highlights the urgency to define strategies for the improvement of vitamin D status in Portuguese older adults.

Correction notice This paper has been amended since it was published Online First. Owing to a scripting error, some of the publisher names in the references were replaced with 'BMJ Publishing Group'. This only affected the full text version, not the PDF. We have since corrected these errors and the correct publishers have been inserted into the references. The Funding statement has been reinstated.

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