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Association between serum 25-hidroxyvitamin D concentrations and Ultraviolet Index in Portuguese older adults

Sofia Reis Alves Soares Cardoso

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Associação entre concentrações séricas de 25-hidroxivitamina D e Índice Ultravioleta em idosos Portugueses

Sofia Reis Alves Soares Cardoso

Faculdade de Ciências da Nutrição e Alimentação da Universidade do Porto, Porto

Orientador: Prof. Doutor Nuno Borges (Faculdade de Ciências da Nutrição e Alimentação da Universidade do Porto)

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Resumo

Introdução: A vitamina D possui funções endócrinas, parácrinas e autócrinas no organismo. As fontes de vitamina D incluem a síntese cutânea através da exposição solar, a alimentação e a suplementação. Em Portugal, verifica-se um envelhecimento populacional crescente. Os idosos são um grupo de risco para a deficiência de vitamina D, que pode ser considerada uma pandemia na Europa, associada a diversas complicações clínicas e a custos económicos. Esta problemática tem como principais causas uma ingestão e síntese cutânea inadequadas. A síntese cutânea ocorre por ação da radiação ultravioleta B, sendo influenciada por fatores como a idade e variáveis que afetam a radiação disponível. O Índice Ultravioleta (IUV) mede o nível de radiação que atinge a Terra, passível de causar eritema, variando com fatores como a estação do ano e a latitude. O IUV é um possível indicador do potencial de síntese de vitamina D. O conhecimento sobre a deficiência de vitamina D, no país, é limitado. É necessário aumentar os conhecimentos sobre este problema de saúde pública para garantir a prevenção e tratamento, através de uma adequada exposição solar, ingestão alimentar e suplementação.

Objetivo: Explorar a associação entre as concentrações séricas de 25-hidroxivitamina D [25(OH)D] de idosos portugueses e o IUV do distrito de residência, tendo em conta outros fatores potencialmente influentes nas concentrações de 25(OH)D.

Métodos: Este é um estudo observacional transversal realizado em Portugal, entre dezembro de 2015 e junho de 2016, com uma amostra de 1497 idosos (≥65 anos) para os quais se recolheram dados sociodemográficos, antropométricos, de estilo de vida, de saúde e de estado nutricional. As concentrações séricas de 25(OH)D foram determinadas através de um ensaio de eletroquimioluminescência. Todos os dados foram recolhidos no Projeto Nutrition UP 65, exceto os do IUV, providenciados pelo Instituto Português do Mar e da Atmosfera. Para cada indivíduo calculou-se a média do IUV, do distrito de residência, dos 30 dias prévios à colheita sanguínea. Através de análises de regressão linear múltipla explorou-se a associação entre as concentrações de 25(OH)D e o IUV, tendo em conta outros fatores potencialmente influentes das concentrações de 25(OH)D.

Resultados: A mediana das concentrações de 25(OH)D da amostra foi de 14.40 ng/ml. A frequência de deficiência de vitamina D na amostra foi de 69%, segundo o ponto de corte da *Endocrine Society* de 25(OH)D ≤20 ng/ml. Segundo o ponto de corte do *Institute of Medicine* de 25(OH)D <12 ng/ml**,** 39.5% da amostra apresentava risco de deficiência de vitamina D. O IUV esteve positivamente associado com as concentrações de 25(OH)D nos modelos de dezembro-junho (Coeficiente de regressão padronizado [sβ]=0.244, intervalo de confiança a 95% [95% IC] 0.198; 0.291, P<0.001) e abril-junho (sβ=0.295, 95% IC 0.229; 0.362, P<0.001) e negativamente

associado no modelo de dezembro-março (sβ=-0.149, 95% IC -0.211; -0.087, P<0.001). O modelo de regressão de dezembro-junho explicou 28% da variância nas concentrações 25(OH)D (R^2 ajustado=0.280), enquanto os modelos estratificados de dezembro-março e abril-junho explicaram cerca de 23% (R^2 ajustado=0.229 e 0.232, respetivamente).

Conclusão: A frequência de deficiência de vitamina D nesta amostra de idosos portugueses foi elevada. O IUV foi um preditor da concentração de 25(OH)D, mas a sua associação diferiu com o período de colheita sanguínea. Os resultados do estudo sugerem que pode ser importante que futuras investigações e recomendações sobre a exposição solar e o estado de vitamina D tenham em consideração os meses do ano. Outros fatores não avaliados como a alimentação, a exposição solar e a genética podem explicar a variância restante nas concentrações de 25(OH)D, influenciando o estado de vitamina D desta população.

Palavras-chave: Vitamina D, 25-hidroxitamina D, Deficiência de vitamina D, Idosos, Síntese Cutânea, Índice Ultravioleta.

Abstract

Introduction: Vitamin D has endocrine, paracrine and autocrine roles in the organism. Sources of vitamin D include cutaneous synthesis through sun exposure, diet and supplements. In Portugal, population ageing is accelerating. Older adults are a risk group for vitamin D deficiency, which could be considered as being pandemic in Europe, and is associated with several clinical complications and an economic burden. An inadequate cutaneous synthesis and intake are the principal causes for this problem. Cutaneous synthesis occurs by action of ultraviolet B radiation and is influenced by factors such as age and variables that affect radiation availability. The Ultraviolet Index (UVI) measures the ultraviolet radiation level reaching the Earth likely to cause erythema; which varies with factors such as seasons and latitude. The UVI can be an indicator of potential for vitamin D synthesis. Knowledge about vitamin D deficiency, in Portugal, is limited. There is a necessity to improve knowledge about this public health problem to assure prevention and treatment through adequate sun exposure and vitamin D intake from diet and supplements.

Objective: To explore the association between serum 25-hidroxyvitamin D [25(OH)D] concentrations of Portuguese older adults and Ultraviolet Index in the district of residence, accounting for other potential influential factors of 25(OH)D concentrations.

Methods: This is a cross-sectional observational study conducted in Portugal between December 2015 and June 2016, in a sample of 1497 older adults (≥65 years) for whom sociodemographic, anthropometric, lifestyle, health and nutritional status data were collected. Serum 25(OH)D concentrations were determined by a competitive electrochemiluminescence protein binding assay. All data were collected in the Nutrition UP 65 Study, except for the UVI, provided by the Portuguese Institute for Sea and Atmosphere. For each participant, the mean UVI in the residence district, within the 30 days prior to blood collection, was calculated. Multiple linear regression analyses were performed to assess the association between 25(OH)D concentrations and Ultraviolet Index, accounting for other potential influential factors of 25(OH)D concentrations.

Results: The median 25(OH)D concentration in our sample was 14.40 ng/ml. The frequency of deficiency was 69%, according to the Endocrine Society cut-off point of 25(OH)D ≤20 ng/ml**.** According to the Institute of Medicine cut-off point of 25(OH)D <12 ng/ml, 39.5% of the sample was at risk for deficiency. The UVI was positively associated with 25(OH)D in the models for December-June (Standardized regression coefficient [sβ]=0.244, 95% confidence interval [CI] 0.198; 0.291, P<0.001) and April-June (sβ= 0.295, 95% CI 0.299; 0.362, P<0.001) and negatively associated in the model for December-March (sβ=-0.149, 95% CI -0.211; -0.087,P<0.001). The regression model for December-June explained around 28% of variance in 25(OH)D (adjusted

R^2=0.280), whereas stratified models for December-March and April-June explained around 23% (adjusted R^2 =0.229 and 0.232, respectively).

Conclusion: In this sample of Portuguese older adults, the frequency of vitamin D deficiency was high. The UVI was a predictor of 25(OH)D concentrations but the association varied according to the blood collection period. Results of this study suggest that accounting for the time of the year in future research and recommendations about sun exposure and vitamin D status may be relevant. Other factors nor measured in this work, such as diet, sun exposure and genetics may explain the remaining variance in 25(OH)D, influencing vitamin D status of this population of older adults.

Keywords: Vitamin D, 25-hydroxyvitamin D, Vitamin D deficiency, Older adults, Cutaneous Synthesis, Ultraviolet Index.

Abbreviations

25(OH)D - 25-Hydroxyvitamin D

7DHC - 7- dehydrocholesterol

BMI - Body mass index

d - Day

DBP- Vitamin D Binding Protein

EURONUT-SENECA - Survey in Europe on Nutrition and the Elderly: A Concerted Action

InCHIANTI - Invecchiare in Chianti

IOM - Institute of Medicine

IPAC - International Physical Activity Questionnaire

IU - International Units

MED - Minimum erythemal dose

MeDi - Mediterranean diet

MET - Metabolic equivalent task

MMSE – Mini Mental State Examination

MNA-SF- Mini Nutritional Assessment Short Form

mUVI - Mean Ultraviolet Index

NUP65S - Nutrition UP 65 study

NUTS II - Nomenclature of Territorial Units for Statistics II

PREDIMED - Prevention with Mediterranean Diet tool

RDA - Recommended Dietary Allowance

UVB - Ultraviolet B

UVI - Ultraviolet Index

Requirements for vitamin D are listed in International Units (IUs). The biological activity of 40 IU is equal to 1 µg.

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I - Introduction

1. Vitamin D

1.1. Characterization, functions and metabolism

Vitamin D is a fat-soluble pro-hormone with endocrine, autocrine and paracrine roles in the human body [1, 2]. The classical function of this vitamin is to maintain bone health and phosphate and calcium homeostasis [3, 4]. In the 2000's, research began to focus on extra-skeletal functions and several studies have shown a role in diverse physiological functions. Additionally, vitamin D deficiency has been associated with mortality and several illnesses such as cardiovascular and autoimmune diseases, some types of cancers and type 1 and 2 diabetes [5,6].

The term "vitamin D" includes two forms which are secosteroids: vitamin D2 or ergocalciferol and vitamin D3 or cholecalciferol. While vitamin D2 is formed by ultraviolet B irradiation (UVB) of ergosterol existing in plants, fungi and invertebrates, vitamin D3 is formed by irradiation of 7-dehydrocolesterol (7DHC) found in vertebrates [7].

Throughout this dissertation, "vitamin D" refers to D2 or D3, unless stated otherwise.

Sources of vitamin D are cutaneous synthesis through sun exposure, diet and supplements [4]. Under normal conditions, photosynthesis in the skin is able to fulfil 80-100% of human vitamin D requirements [8, 9].

Ingested or synthesized vitamin D needs to undergo two hydroxylations to acquire biological activity [10].

When ingested vitamin D enters the circulation, it is bound to vitamin D binding protein (DBP) and lipoproteins or to DBP in case of synthesized vitamin D3 [10]. Afterwards, vitamin D can go to the liver or be taken up by peripheral tissues, particularly adipose tissue and muscle [11, 12]. Adipose tissue is the main site of storage of vitamin D, extending its total half-life in the body to two months [12].

The first hydroxylation of vitamin D occurs in the liver, by vitamin D-25-hydroxylase, to 25-hydroxyvitamin D [25(OH)D]. which is the predominant form in circulation [10] where it has a half-life of two to three weeks [13]. However, a serum half-life of one to two months has been reported [14, 15]. Serum 25(OH)D concentrations are typically measured to determine vitamin D status [10].

The rate and the extent of the increase in serum 25(OH)D concentrations following UV irradiation or vitamin D3 ingestion are a function of regulated activity of vitamin D-25-hydroxylase and are variable [12, 16].

In the kidney and other organs, 25(OH)D is hydroxylated to 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], which is the hormonal and biologically active form of vitamin D [10]. This activation is regulated by various factors including parathyroid hormone, calcium and phosphate levels [16].

Catabolism of 25(OH)D and 1,25$(OH)_2$D results in inactive products which are excreted mostly through the bile [10].

The molecule 1,25$(OH)_2$D is involved in genomic and extra genomic actions through interaction with the vitamin D receptor, which is present in more than 37 cell types, including intestine and bone cells [17].

1.2. Diet and supplements - recommendations and current intake

Very few foods naturally contain vitamin D2 and D3 [18]. The major sources are fatty fish such as salmon and mackerel, and cod liver oil [19, 20]. It is also present in small quantities in meat, egg yolk, milk and dairy as well as in some mushrooms [18, 20].

Fortification of food with vitamin D represents a way of meeting daily recommended intake [21]. Fortified foods usually include milk and dairy, breakfast cereals, margarine, bread and orange juice, depending on the country [19 ,21, 22].

Vitamin D supplements are commercially available and their use appears to be high in some countries [23].

The recommendations for vitamin D intake are different among health entities and countries [18, 24-26]. According to the Institute of Medicine (IOM), assuming minimal sun exposure, the Recommended Daily Allowance (RDA) for vitamin D, is 600 International Units (IU)/day(d) for ages 1 to 70 years and 800 IU/day for ages ≥71 years [11]. The RDA corresponds to serum 25(OH)D concentrations ≥20 ng/mL because the IOM focused only on bone health and concluded that higher concentrations were not consistently associated with greater benefits [11]. However, some authors consider that these recommendations are too low to reach/maintain optimal concentrations when UVB radiation does not allow synthesis [18, 23, 27] and when individuals are at risk for vitamin D deficiency (e.g. older people) [8, 18, 23, 28]. Without sun exposure, these authors recommend an intake of 1000-2000 IU/d of vitamin D [28-30].

In Portugal, the recommended daily dose (DDR) of vitamin D for adults is 200 IU/day [31]. In 2016, the European Food Safety Agency has set, for the European adult population, an adequate intake of 600 IU/d [32].

Current levels of food supply [19] and vitamin D intake from diet and supplements are inadequate to meet the recommendations, in several countries [33, 34], including in Europe [35, 36], and are insufficient during the seasons of deficit in sunlight [33, 35].

Data on vitamin D intake by the Portuguese population, particularly in older adults, is limited. In a sample of women living in Porto (2005), intake levels were inadequate in 96% of women aged 60-69 years [37].

1.3. Cutaneous synthesis

Exposure to sunlight constitutes the main source of vitamin D for most humans [4].

Cutaneous synthesis of vitamin D occurs during sun exposure, by the action of UVB radiation (290-315 nm) that causes the photolysis of 7DHC, in the skin, to previtamin D3 [4]. Production of previtamin D3 reaches a plateau at 10 to 15% of the original 7DHC content [38]. Previtamin D3 undergoes thermal isomerization in the skin to form vitamin D3, which binds to DBP, entering the bloodstream [3].

Endogenous synthesis of vitamin D is regulated by melanin pigmentation and mostly by photochemical degradation [3, 30]. Increased pigmentation decreases synthesis because melanin pigmentation absorbs UV radiation [3]. Therefore, to synthesize the same amount of vitamin D, people with darker skin need longer sun exposure than fair-skinned people [3].

Both previtamin D3 and vitamin D3 can absorb photons when exposed to sunlight and, respectively, isomerise and photolyse into inert photoproducts [3]. The concentration of these photoproducts increases with sun exposure time [3]. In this way, sunlight can regulate production of vitamin D3 in the skin [30] and vitamin D intoxication by excessive sun exposure is not possible[10].

The UV radiation allows vitamin D synthesis and increases serum 25(OH)D concentrations but the dose-response relationship is still unclear [39]. Besides allowing vitamin D synthesis, UV radiation has also deleterious effects such as erythema and some types of cancers [40, 41]. Thus, recommendations about how much and when individuals should be exposed to sunlight to ensure an adequate vitamin D status is a controversial topic [42, 43]. To discuss recommendations, it is helpful to understand the concept of action spectrum of vitamin D, which describes the relative effectiveness of energy at different wavelengths of UV radiation to synthesize previtamin D3 in the skin [44]. Several action spectra of vitamin D have been proposed, but there is still no standard and all have limitations [45, 46]. Some authors say that it is similar to the erythema action spectrum [42, 47], thus, it is possible to make recommendations of exposure in terms of minimum erythemal dose (MED), i.e. the amount (Joule per square metre [J/m^2]) of erythemally effective radiation that causes a just perceptible reddening of the skin [47]. Each person, according to their skin type, will have a different MED [47].

A UV exposure of one-quarter of a personal MED on one-quarter of skin area (hands, face and arms) yields a dietary equivalent vitamin D dose of about 1000 IU [45]. The exposure time to get this recommended UV dose depends on the skin type, time of day, season, location, ambient conditions and clothing [45].

According to various researchers, sensible exposure to sunlight of hands, face, and arms, 5 to 15 minutes per day, during the spring, summer and autumn in latitudes >37º N and throughout the year <37ºN, satisfies the requirements [28, 30]. Some authors indicate longer times for satisfying requirements whereas others say these requirements are insufficient [45]. Maximum vitamin D

synthesis occurs at sub-erythemogenic UV doses (less than one MED) and further sun exposure does not have beneficial effects [14, 40, 48]. Despite the recommendations, the effects of UV exposure on vitamin D3 production [48] and 25(OH)D concentrations are complex and remain under investigation [45].

1.3.1. Factors influencing cutaneous synthesis

Vitamin D synthesis in the skin is influenced by environmental and individual factors.

Environmental factors affect UVB available for skin synthesis and include atmospheric ozone and dispersion [49], cloudiness, air pollution, solar zenith angle (which is a function of latitude, season and time of day), altitude and surface reflection [1].

Atmospheric ozone and dispersion, cloudiness and air pollution diminish UVB available for synthesis [49, 50]. UV irradiance is higher for smaller solar zenith angle, i.e., in regions closer to the equator, in the summer and at solar noon (maximum solar elevation) [49]. Altitude and surface reflection also increase UVB available [49].

Latitude and season have substantial impact on UVB radiation [45]. Vitamin D synthesis usually increases from spring to summer and decreases after that [4]. This results in a seasonal variation of 25(OH)D serum concentrations, which reaches its nadir (minimum) in winter/early spring [4, 51]. Researchers have described a "vitamin D winter" referring to the months during which solar UV radiation is not intense enough to allow synthesis [4, 45, 52], which occurs at latitudes above approximately 35º-40ºN [3, 53-55]. Many European countries experience a four to six-month "vitamin D winter" [45, 52]. However, Webb *et al* (2006) estimated that at 45°N, UV exposure may result in vitamin D synthesis at any time of the year [56].

Kimlin *et al* (2007) concluded that latitude was the major predictor of the sun's ability to produce vitamin D in the winter but not in the summer, and that the seasonal difference is most marked at high latitudes such as 44.4ºN [57]. For latitudes >35ºN, the authors consider that monitoring UV is crucial to understand adequate sun exposure for vitamin D synthesis [57]. At the same time, a paradox exists in countries at higher latitudes which show lower prevalence of vitamin D deficiency than others closer to the equator, probably due to the population diet [58]. Thus, latitude by itself is not a good indicator for potential vitamin D production and is not very useful for estimating the vitamin D status of a population [41].

Personal variables determine if and how available UV radiation is used for vitamin D synthesis [47]. They include age, skin type and variables associated with exposure behaviours such as clothing, sun protection, skin area exposed and duration of exposure [1, 47].

As ageing occurs, the concentration of 7DHC decreases [59]. Above the age of 65 there is a fourfold reduction in the ability to synthesize vitamin D3 when compared to a younger adult [4, 47]. Older adults also tend to avoid the sun and be more covered up, decreasing the amount of UV that reaches the skin [47, 59, 60].

Clothing and sunscreen also represent barriers to production of vitamin D [47].

Given the environmental, social and physiological factors that may impair adequate exposure to UVB radiation [55, 61], sun exposure is often not sufficient for adequate vitamin D production [29]. For this reason, dietary intake and/or use of supplements are also of great importance [29] particularly during periods when vitamin D synthesis is not possible [22, 45, 62, 63].

1.4. The Ultraviolet Index

The Ultraviolet Index (UVI) is a parameter that describes the level of UV radiation that reaches the Earth's surface, susceptible of causing erythema [49]. It was developed to promote public awareness of the risks of UV radiation exposure and sun protection [49]. The UVI consists of an open-ended scale that ranges from 0 to 11+ [64] and values can be grouped into the following exposure categories: low (1-2), moderate (3-5), high (6-7), very high (8-10) and extreme (11+) [41]. As the UVI gets higher, the potential to cause erythema increases and the time it takes to occur decreases [41].

The UVI is defined as "the integral over the spectral UV irradiance on a horizontal plane (Watt per square metre per nanometre [$W.m^{-2}.nm^{-1}$]), weighted with the International Commission on Illumination (1987) erythemal action spectrum and multiplied by the factor 40" [65]. The UVI depends on several variables such as time of day, season and latitude, being higher in the summer, at solar noon and lower latitudes [47, 49, 50].

Typically, UVI forecast is estimated using a computer model that relates the strength of solar UV radiation to forecasted stratospheric ozone concentration, aerosol factors, cloud amounts and elevation of the ground [66]. The daily maximum UVI is usually the value forecasted by the media and represents the maximum UV level that occurs during a four-hour period around solar noon[50]. The UVI can be an indicator of potential for vitamin D synthesis [32, 47], meaning that the higher the UVI, the less exposure to sunlight will be needed to produce a certain amount of vitamin D[47]. Generally, little vitamin D is synthesized at UVI values between 0.5-3 [30]. Some authors consider that synthesis occurs at UV values ≥3 [67, 68] and inferior values do not supply sufficient vitamin D[56]. Despite this, other researchers argue that synthesis can occur at UVI <3, although usually longer sun exposure times are required [69, 70]. Regardless, synthesis is possible at sub-erythemogenic UV doses [41, 48, 71].

There are some studies about the relationship between solar exposure and vitamin D synthesis and/or status, but studies *in vivo* are complex [45]. The association between ambient UV radiation or UVI and 25(OH)D has also been investigated [15, 72-75]. Methods to estimate/evaluate sun exposure include questionnaires [76], devices to measure sunlight intensity [77] and modelled vitamin D-effective UVB availability [78]. Most studies that measured sun exposure and UV report an association with 25(OH)D concentrations [76, 79].

1.5 Vitamin D deficiency

The pleiotropic effects of vitamin D and its potential of reducing risk of disease indicates the importance of maintaining an adequate vitamin D status [4, 19]. In addition to vitamin D intake and cutaneous synthesis, other factors can influence vitamin D status and predispose individuals to deficiency, making them a risk group [80].

With ageing, there are several biological and behavioural alterations [80], frequently coupled with disabilities, that contribute to reduction of the rate of skin synthesis, hydroxylation and response of target tissues as well as reduced skin exposure and vitamin D intake [81]. Hence, older adults are a risk group for vitamin D deficiency [35].

Taking certain medications (e.g. cholestyramine, anticonvulsants, antiretroviral medications) also increases the risk of vitamin D deficiency [18].

There is an inverse association between body mass index (BMI) and 25(OH)D concentrations [82] possibly due to lower synthesis and the uptake of vitamin D by the adipose tissue [18, 83]. Obesity (BMI >30 kg/m^2) has been associated with vitamin D deficiency [18], thus, obese individuals can be considered at risk for this nutritional deficiency [18].

Some studies found that smokers have a lower vitamin D status, while physical activity is associated with higher 25(OH)D concentrations, although the causes remain to be determined[41].

There is still no consensus regarding the cut-off points for defining vitamin D deficiency or optimal concentrations. The IOM defines 25(OH)D concentrations of <12 ng/ml, 12-19 ng/ml, and ≥20 ng/m as risk of deficiency, risk of inadequacy and sufficiency of vitamin D, respectively [84]. The Endocrine Society as well as other experts [19], adopt higher 25(OH)D thresholds for deficiency, insufficiency and sufficiency: ≤20 ng/ml, 21–29 ng/ml and ≥30 ng/ml, respectively [18]. Cases of toxicity due to excess intake of vitamin D are rare [85]. Different toxicity levels, based on 25(OH)D concentrations, can be found in the literature. Holick (2010) states that toxicity levels are not met until 25(OH)D levels reach 150-200 ng/ml [4], while the IOM states that levels >50 ng/ml are possibly harmful [84].

The prevalence of deficiency depends on the cut-off points adopted by the researchers. The IOM states that higher serum 25(OH)D thresholds, such as 20 ng/ml, are too high and misclassify most people as deficient, creating the “pandemic” that other authors report [86]. Nevertheless, more experts are drawing attention to the dimension and possible consequences of this problem [7, 24].

Globally, vitamin D deficiency is a public health problem, estimated to affect one billion people across all age groups [7]. In Europe, the prevalence of deficiency (<20 ng/ml) is 40.4%, which can be considered as being pandemic and entails an economic burden [51].

In Portugal, data on vitamin D deficiency is scarce and not representative of the population [87].

Even though large variations in serum 25(OH)D concentrations exist between different European countries, inadequacy is most obvious during winter [52], occurring not only at higher latitudes but also in southern Europe, especially among older adults [19, 59, 88]. Prevalence of vitamin D

deficiency (25(OH)D <10 ng/ml) is even higher in the institutionalized elderly, reaching 80% in some studies [35, 59].

The major reasons for this prevalence of inadequacy are avoidance of sun exposure and the unawareness that vitamin D in dietary sources is scarce [4, 23].

In Europe, high latitudes, indoor living, low intake and ineffective vitamin D fortification in most countries contribute to low levels of 25(OH)D [89].

Population ageing has been an increasing trend in Europe, particularly in Portugal, where 19% of the population was ≥65 years in 2011 [90]. The present and projected scenarios pose challenges for the social, economic and health sectors [91, 92]**.** Consequences of vitamin D deficiency in this age group include osteomalacia and increased risk of muscle weakness [18, 56] falls and fractures [27, 93]. Additionally, hypovitaminosis D in geriatric population has been associated with an increased risk of neuropsychiatric, cardiovascular, endocrine and oncologic diseases [80].

Grant *et al* (2007) estimated that increasing 25(OH)D levels to 40 ng/ml could reduce the economic burden of disease, in Europe, by 187 million euros/year [89].

Vitamin D deficiency is easily treatable [35] by encouragement of safe and moderate UV exposure, increase in food fortification and the provision of higher doses of vitamin D supplements when necessary [94]. During the winter, fortification and supplementation may be strategies to achieve adequate levels [23, 61].

More research is needed to develop and prove effectiveness of new strategies to help ensure adequate vitamin D intakes critical to the overall health and prevention of chronic diseases in high-risk groups [23, 29, 61].

Additionally, it is important that nutritionists and other health care professionals educate the public and regulatory agencies about the relevance of implementing dietary strategies to achieve/preserve adequate vitamin D status in the population [29]. Public awareness coupled with health policies can improve public health at moderate costs [45].

All the aspects cited in this chapter highlight the relevance of studying the factors that can influence vitamin D status in the older population, namely UVI, in Portugal. This study is relevant because it is the first to explore the association of UVI and vitamin D status in this high-risk population for vitamin D deficiency, in Portugal, and may provide some suggestions to future research and to face this problem.

2. Nutrition UP 65 study

"Nutritional Strategies Facing an Older Demographic: The Nutrition UP 65 Study" (NUP65S) was a study developed by Faculty of Nutrition and Food Sciences of the University of Porto [87], conducted in Portugal in older adults, between December 2015 and June 2016. The main

objectives were to reduce the nutrition inequalities and provide knowledge about older Portuguese adults' nutritional status [87]. Vitamin D status was one of the investigated areas [87].

II – Objective

This study aimed to explore the association between serum 25-hidroxyvitamin D [25(OH)D] concentrations of Portuguese older adults and Ultraviolet Index in the district of residence, accounting for other potential influential factors of 25(OH)D concentrations.

III – Methods

Sampling and Recruiting

In order to achieve a representative sample of Portuguese older adults, a quota sampling approach, using data from Census 2011 regarding sex, age, educational level and regional area, was implemented.

Eligible individuals were Portuguese with 65 years of age or more. The sample was constituted by community-dwelling individuals and individuals institutionalized in retirement homes, representing the 5% proportionality prevailing in the population.

The regional areas used were defined in the Nomenclature of Territorial Units for Statistics: Alentejo, Algarve, Azores, Lisbon Metropolitan Area, Centre, Madeira and North (NUTS II).

A random and stratified cluster sampling was applied. In each regional area, three or more town councils with >250 inhabitants were randomly selected. Potential community-dwelling participants were contacted via home approach, telephone or via institutions (town councils and parish centres) whereas institutionalized participants were contacted via institutions. Potential participants were informed about the NUP65S and were invited to participate. In case of acceptance, participants signed the *Informed consent* form. Individuals presenting any condition that unbaled the collection of venous blood samples or urine (eg, dementia or urinary incontinence) were excluded from the NUP65S.

Study locations

In total, 15 districts were selected: 13 districts of mainland Portugal which were Aveiro, Braga, Coimbra, Évora, Faro, Leiria, Lisboa, Portalegre, Porto, Santarém, Setúbal, Viana do Castelo and Viseu; in addition to Ponta Delgada (São Miguel Island) from the Azores Archipelago and Funchal (Madeira Island) from the Madeira Archipelago.

Ultraviolet Index

Daily maximum UVI forecast was provided by the Portuguese Institute of Sea and Atmosphere (IPMA), for the studied districts and the period between November 2015 and July 2016. The provided UVI forecast corresponded to the daily maximum UVI around the solar noon and was obtained by the German Meteorological Service (DWD) [66, 95]. The DWD's UVI forecast has a modular structure and is adjusted for cloud modification factors [95].

Study Design and Setting

The NUP65S is a cross-sectional observational study conducted in Portugal, in a sample of 1500 subjects (≥65 years old), representative of the older Portuguese population in terms of age, sex, education and regional area. Data were collected between December 2015 and June 2016. The complete description of NUP65S can be read elsewhere [87].

The present study is a cross-sectional and observational study and was conducted with a sub-sample of 1497 older adults (≥65 years) from the NUP65S. Three individuals were excluded from the original NUP65S sample: one subject was excluded because the respective serum 25(OH)D concentration was 178.10 ng/ml, which was above the toxicity level (150 ng/ml), and two subjects were excluded due to missing data. All data of this study were obtained in the NUP65S except for the UVI and latitude data.

Data collected in Nutrition UP 65 Study

The following information was collected through a structured questionnaire: cognitive performance, social and demographic data (sex, age, educational level, professional occupation and activity, marital status, residence and monthly household income), lifestyle (current smoking habits, consumption of alcoholic beverages, physical activity and adherence to Mediterranean Diet), skin phenotype, health status and clinical history, as well as hydration, nutritional and vitamin D status. The interviews were conducted by eight registered nutritionists previously trained.

The methodology of NUP65S considered relevant to this dissertation is described in detail.

Skin phenotype

Skin phenotype was self-reported by the participants according to Fitzpatrick (1975) classification [96]. This scale comprises six skin phenotypes (I-VI) according to melanin pigmentation and the

response to UV exposure [97]. Across the scale, skin type becomes darker and less susceptible to erythema. The classification was the following: Type I - white, very fair, red or blond hair, blue eyes, freckles (always burns, never tans); Type II - white, fair, red or blond hair, blue, hazel, or green eyes (usually burns, tans with difficulty); Type III - cream white, fair with any eye or hair colour, (sometimes a mild burn, gradually tans); Type IV – brown, typical Mediterranean Caucasian skin, (rarely burns, tans with ease); Type V - dark brown, mid-eastern skin types (very rarely burns, tans very easily) and Type VI - black (never burns, tans very easily) [98].

Cognitive performance

Cognitive performance was assessed by the Portuguese version of the Mini Mental State Examination (MMSE) [99]. This examination consists of 30 questions (each scored one point if correct) and assesses the functions of registration, orientation, attention, calculation, recall, language and ability to follow simple commands. The cut-off scores for cognitive impairment were as follows: individuals with no education, ≤15 points; 1 to 11 years of school completed, ≤22 points; and >11 years of school completed, ≤27 points [99].

Physical activity

To estimate the physical activity level, the short form of the International Physical Activity Questionnaire (IPAQ) Short Form was applied [100]. This questionnaire collects information about the time spent on different activities (walking, moderate and vigorous activities) in the last seven days. Each activity corresponds to a specified energy expenditure in metabolic equivalent task (METs) [100]. Data collected with IPAQ was converted to MET-minutes. Median values were calculated for the different activities using established formulas [100, 101]. Kilocalories were computed from MET-minutes/week scores and participants were classified as either presenting a low physical activity, if <383 kcal/week (men) and <270 kcal/week (women), or normal physical activity if ≥383 kcal/week (men) and ≥270 kcal/week (women) [102].

Adherence to the Mediterranean diet and Fish/Shellfish consumption

Adherence to the Mediterranean diet (MeDi) was assessed by the Portuguese version of the Prevention with Mediterranean Diet tool (PREDIMED) [103]. This tool was created to test the association between Mediterranean diet and obesity indexes and comprises 14 questions, each scored with zero or one point. The criteria for assigning one point was previously established: a final score ≥10 designated a high adherence to MeDi and <10 designated a low adherence [104]. The final score of this tool was analysed because a greater adherence to a MeDi has been related to a better nutrient profile and a lower prevalence of inadequate micronutrient intake [105]. One of the 14 questions could include food with high content of vitamin D, which was: “How many servings of fish or shellfish do you consume per week (1 serving: 100-150g of fish or 4-5 units or

200g of shellfish)?" [104]. Criteria for one point was ≥3 servings per week [104]. The answer to this question was analysed because it could be related to vitamin D intake and status.

Health Status

Health status was assessed by subject's self-perceived health, which was categorized as follows: very good, good, moderate, bad and very bad [106].

Supplement intake

Supplement intake was self- reported by the participants. Supplement intake included vitamin D and/or multivitamins containing vitamin D.

Nutritional status

Detailed anthropometric measurements were performed by the registered nutritionists. Body weight was measured with a portable calibrated electronic scale (Seca 803) (with a 0.1-kilogram (kg) resolution) and standing height was measured with a stadiometer (with a 0.1-centimetres (cm) resolution), following standard procedures [107]. When it was not possible to obtain these measures, weight was estimated from mid-upper arm and calf circumferences, measured with metal tape measure (Lufkin) with 0.1centimetre resolution, and height was estimated from nondominant hand length, measured with a rated paquimeter (Fervi Equipment) with 0.1-centimetre resolution.

Body mass index (BMI) was calculated using the standard formula [weight(kg)/height^2(metres^2)]. According to BMI categories, participants were classified as underweight (<18.5 kg/m^2), normal range (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2) and obese (≥30 kg/m^2) [108].

The Mini Nutritional Assessment® – Short Form (MNA®-SF) was also administered to assess the nutritional status of the participants. The MNA®-SF is a validated nutrition screening and assessment tool that can identify older adults (≥65 years old) who are malnourished or at risk of malnutrition [109, 110]. This tool consists of six questions regarding food intake, weight loss, physical and mental status, and anthropometry through BMI assessment [109, 110]. According to the score, each participant was classified as: undernourished (≤7 out of 14 points), at risk of undernutrition (8-11 points) or normal nutritional status (12-14 points).

Vitamin D Status

Vitamin D status was assessed by dosing the serum levels of 25-hidroxyvitamin D3 [25(OH)D] (ng/ml). Blood samples were collected by qualified nurses after the questionnaire and after a 12-hour fasting period. Blood collection occurred between December 2015 and June 2016, although the time period was not the same for all the districts. Therefore, blood collection occurred only for five or less months in some districts.

All serum 25(OH)D concentrations were analysed in the same equipment (*Cobas Roche*) in one central laboratory (*General Lab*), in Portugal, by a competitive electrochemiluminescence protein binding assay using *Roche Diagnostic Vitamin D Total assay* (Roche Diagnostics GmbH, Mannheim, Germany) [111]. The detection limit of this test is 3.00 ng/ml [111].

Ethics

The NUP65 research was performed according to the guidelines established by the Declaration of Helsinki, and the study protocol was approved by the Ethics Committee of the department of Social Sciences and Health (Ciências Sociais e Saúde) from the Faculty of Medicine of University of Porto (PCEDCSS – FMUP 15/2015) and by the Portuguese National Commission of Data Protection (9427/2015) [87].

Statistical Analysis

The mean of daily maximum UVI for the 30 days prior to the respective blood collection date (mUVI) was calculated for each participant. This time period was chosen for several reasons. In the literature, half-life of serum 25(OH)D has been reported to be of 1 month [14], 4 weeks to 2 months [15] or from 2 to 3 weeks [12]. Also, the lag-time between the change in monthly UV dose and the corresponding change in 25(OH)D levels has been reported to range from 4 to 8 weeks [75] [112]. Additionally, associations between serum 25(OH)D and UVI over the previous 30 days[75] and over the previous 35 days [15] have been found in the literature.

The UVI was treated both as a categorical and a continuous variable.

The variable serum 25(OH)D concentrations (ng/ml) was treated as a continuous variable.

Mean latitudes of the studied districts were calculated based on the participants' postal code.

Data in descriptive statistics are presented as median and first and third quartile (Q1 and Q3) of 25(OH)D concentrations (due to a non-normal distribution) for each potential influential variable of serum 25(OH)D. For presentation of the results, variables were categorized as follows: age (65-69, 70-74, 75-79 and ≥80 years old), educational level (0, 1-4, 5-12 and ≥13 years of school completed), marital status (Single, divorced or widowed and Married or common-law marriage), household income (<500, 500-999, ≥1000 €/month and Does not know or does not declare), skin phenotype (I-II, III-IV and V-VI), alcoholic beverages consumption (None, Moderate if 1 drink/d for women and 1-2 for men, and Heavy if ≥2 drinks/d for women and ≥3 drinks/d for men) [113], nutritional status (Not undernourished and Undernourished/at risk of undernutrition) and period of blood collection (December-March which comprises late autumn, winter and early spring, and in April-June which comprises spring and early summer). Categorization of period of blood collection was based on the fact that there is a seasonal variation in 25(OH)D concentrations [30] and several authors found that 25(OH)D concentrations reached its minimum in March or late winter/early spring [51] [114-116]. Additionally, there have been reports that UV radiation may be too low to induce adequate vitamin D synthesis until March, in European countries [78] [117].

In descriptive statistics, mUVI was categorized as: Low (1-2), Moderate (3-5), High (6-7) and Very high (8-10) [41].

The assessment of normality of continuous variables was conducted by analysing the skewness and the kurtosis values. If both values were within the range of -2 to 2, the distribution was considered normal.

According to 25(OH)D concentrations, participants were compared for several sociodemographic, lifestyle, health, nutritional and environmental characteristics. For the dichotomous variables, statistical significance of differences in serum 25(OH)D concentrations was assessed with the Mann-Whitney test. For variables with >2 categories, differences were tested using Kruskal-Wallis test and Mann-Whitney test with Bonferroni correction.

To illustrate the variation of serum 25(OH)D concentrations and mUVI, during the blood collection months, a chart was plotted. The median, Q1 and Q3 of 25(OH)D concentrations, as well as the mean and standard deviation (SD) of mUVI were calculated for the participants evaluated in each month. Mann-Whitney test with Bonferroni correction was performed to test statistical significance of differences in serum 25(OH)D concentrations between consecutive months.

To explore the association between serum 25(OH)D concentrations and mUVI accounting for other potential influential factors, multiple linear regression analyses were conducted using the stepwise method. Due to a non-normal distribution of 25(OH)D, the dependent variable, a logarithm (log base 10) transformation was conducted [$\log_{10}$25(OH)D]. The following independent variables were included: mUVI (continuous), sex (dichotomous), age (continuous), education (categorical), professional activity (dichotomous), marital status (dichotomous), residence (dichotomous), household income (categorical), skin phenotype (categorical), cognitive performance (continuous), smoking habits (dichotomous), alcoholic beverages consumption (categorical), adherence to MeDi (continuous), fish or shellfish consumption ≥3 times/week (dichotomous), self-perceived health (categorical), supplement intake (dichotomous), nutritional status (continuous) and BMI (continuous). Cognitive performance, measured by MMSE score, and nutritional status, measured by MNA®-SF score, were exponentially transformed before computing the model in order to achieve a normal distribution. Independent variables were chosen based on previous studies. Three multiple linear regression analyses were conducted using the same method. A regression analysis was conducted for the entire blood collection period, between December and June (Dec-Jun). Additionally, two regression analyses stratified by period of blood collection were conducted: between December and March (Dec-Mar) and between April and June (Apr-Jun).

For the results of stepwise linear regression analyses, standardized regression coefficients (sβ) and the respective 95% Confidence Interval (95% CI) are presented. The adjusted R-square (R^2) of the models were used to estimate the proportion of the variance in 25(OH)D explained by the model. The regression coefficient was used to estimate the change in 25(OH)D concentrations (ng/ml) for each 1 unit increase in mUVI.

In the statistical tests, all P-values are two-tailed and statistical significance was assumed for $P<0.05$. Microsoft Excel and the IBM Statistical Package for Social Sciences (SPSS) software version 24 were used for data and statistical analysis.

IV – Results

Descriptive statistics

Distribution of the participants by the 15 districts included in the study and the respective mean latitudes are shown in **Table 1**. Mean latitudes of Mainland Portugal districts ranged from 37.1ºN to 41.7ºN. The Azores archipelago had a mean latitude of 38.3ºN and Madeira archipelago had a mean latitude of 32.7ºN, which was the closest to the equator.

Table 1. Distribution of the participants by the 15 districts and the respective mean latitude (in ascending order of latitude).

District	Latitude (ºN)	*n (%)*
Funchal (Madeira)	32.7	30 (2.0)
Faro	37.1	66 (4.4)
Ponta Delgada (São Miguel)	38.3	24 (1.6)
Setúbal	38.6	131 (8.8)
Évora	38.7	25 (1.7)
Lisboa	38.9	257(17.2)
Santarém	39.4	96 (6.4)
Portalegre	39.5	98 (6.5)
Leiria	39.8	114 (7.6)
Coimbra	40.2	145 (9.7)
Viseu	40.8	14 (0.9)
Aveiro	40.9	127(8.5)
Porto	41.2	179 (12.0)
Braga	41.5	173 (11.6)
Viana do Castelo	41.7	18 (1.2)

n- number of subjects

Descriptive data and median 25(OH)D concentrations of the sample, by potential influential factors of 25(OH)D, are shown in **Table 2**.

Table 2. Median 25(OH)D concentrations of the sample, by potential influential factors of 25(OH)D.

Variable	*n*	Median (Q1; Q3)	*P-value*
Sex			**<0.001**
Female	872	13.20 (7.75; 21.18)	
Male	625	16.90 (10.45; 24.75)	
Age (years)			**<0.001**
65-69	412	17.75 (12.00; 25.60)	
70-74	372	15.30 (10.20; 23.48)	
75-79	319	13.70 (8.10; 23.30)	
≥ 80	394	9.95 (5.80; 17.95)	

(Continued)

Table 2. *(Continued)*

Variable	*n*	Median (Q1; Q3)	*P-value*
NUTS II			**<0.001**
North	468	14.15 (8.13;23.35)	
Centre	391	12.60 (6.90; 20.30)	
Lisbon Metropolitan Area	383	16.90 (11.50; 24.90)	
Alentejo	136	12.25 (8.83; 20.60)	
Algarve	65	14.50 (8.30; 22.10)	
Madeira	30	21.75 (15.68; 29.93)	
Azores	24	17.10 (8.43; 23.98)	
Education (years)			**<0.001**
0	212	9.50 (5.40; 14.08)	
1-4	1029	14.80 (8.80; 22.80)	
5-12	188	18.00 (11.68; 26.25)	
≥13	68	18.55 (12.48; 29.98)	
Professionally active			**0.038**
No	1462	14.30 (8.70; 22.90	
Yes	30	18.60 (11.73; 26.30)	
Marital Status			**<0.001**
Single. divorced or widowed	796	11.90 (6.90; 19.30)	
Married or common-law marriage	700	17.40 (11.43; 25.68)	
Residence			**<0.001**
Home	1425	14.70 (8.95; 23.20)	
Institution	72	7.20 (4.12; 15.35)	
Household income (€/month)			**<0.001**
<500	248	12.00 (7.03;17.98)	
500-999	305	14.60 (9.65; 23.45)	
≥1000	174	19.45 (13.43; 27.95)	
Doesn't declare/Doesn't know	770	14.10 (8.00; 22.75)	
Skin Phenotype			0.064
I + II	305	14.90 (9.95; 20.75)	
III + IV	1109	14.50 (8.50;23.65)	
V + VI	80	12.00 (7.53; 18.53)	
Cognitive performance (MMSE)			**<0.001**
Maintenance	1398	14.60 (8.80; 23.30)	
Impairement	99	11.30 (5.60; 18.50)	
Smoking habits			0.852
No	1429	14.40 (8.70; 22.98)	
Yes	68	14.45 (9.23; 21.45)	

(Continued)

Table 2. (*Continued*)

Alcoholic Beverages Consumption			**<0.001**
Does not drink	952	13.20 (8.10; 20.73)	
Moderate	388	17.60 (9.70; 25.90)	
Heavy	155	16.70 (11.40; 24.70)	
Physical activity (IPAC)			**<0.001**
Normal	1234	15.30 (9.40; 23.60)	
Low	261	10.20 (5.95; 19.10)	
Adherence to Mediterranean Diet (PREDIMED)			**0.001**
Low	849	13.70 (9.48; 24.20)	
High	648	15.60 (8.20; 21.60)	
Fish/shellfish consumption ≥3 servings/week			0.149
No	372	14.80 (9.50; 23.93)	
Yes	1125	14.20 (8.40; 22.60)	
Self-perceived health			**<0.001**
Very good	69	17.70 (13.00; 25.70)	
Good	409	16.90 (9.80; 25.05)	
Moderate	730	14.10 (8.90; 22.80)	
Bad	232	11.30 (6.20; 17.70)	
Very bad	53	10.20 (6.90; 19.00)	
Supplement intake			**<0.001**
No	1369	13.90 (8.40; 22.00)	
Yes	128	21.85 (14.05; 32.95)	
Nutritional Status (MNA®-SF)			**<0.001**
Normal nutritional status	1256	15.00 (8.48; 21.45)	
Unndernourished/at risk of undernutrition	241	11.60 (5.90; 18.08)	
Body Mass Index			**<0.001**
Underweight	3	15.30 (8.80; [a])	
Normal range	248	17.10 (9.30; 26.05)	
Overweight	660	15.45 (9.40; 24.20)	
Obese	582	12.45 (7.60; 20.03)	
mUVI Categories			**<0.001**
Low	570	12.00 (6.88;19.40)	
Moderate	518	12.90 (7.98;20.43)	
High	222	18.20 (12.18; 26.23)	
Very High	187	20.80 (15.43; 27.98)	
Period of blood collection			**<0.001**
December-March	807	11.20 (6.70; 18.30)	
April-June	690	18.20 (12.20; 26.53)	

n number of subjects (does not always add up to 1497 because of missing data). Q1 First Quartile. Q3 Third Quartile. [a] Q3 was not possible to calculate. P-value for Mann-Whitney (dichotomous variables) or Kruskal-Wallis test (variables with >2 categories).

In this population of older adults, the median (Q1; Q3) of 25(OH)D concentration was 14.40 ng/ml (8.80; 22.95 ng/ml). Using cut-off points of the Endocrine Society, 69.0% of the participants had deficiency (≤20 ng/m); 19.2% had insufficiency (21-29 ng/ml) and 11.8% had sufficiency (≥30 ng/ml). If the IOM cut-off points were applied, 39.5% of the participants were at risk of deficiency (<12 ng/ml), 29.5% were at risk of inadequacy (12-19 ng/ml) and 31% were sufficient at serum 25(OH)D levels (≥20 ng/m). Seven individuals had 25(OH)D concentrations between 50-60 ng/ml and seven had 25(OH)D concentrations >60 ng/ml.

Analysing median 25(OH)D concentrations for each variable (**Table 2**), these were significantly lower in women than in men, as well as in participants: with no education (increased from 0 years to 5-12 years), institutionalized, not professionally active, not married, with a household income of <500 €/month, cognitively impaired, with no alcoholic beverages consumption, with low physical activity level, with low adherence to MeDi, with bad and a very bad self-perceived health, without supplement intake, undernourished or at risk of undernutrition and whose blood was collected in December-March. The 25(OH)D concentrations decreased significantly across the age categories, except for the 70-74 and 75-79 years. Obese individuals had lower 25(OH) concentrations than individuals with a BMI between 18.5 and 29.9 kg/m^2 (Mann-Whitney test P<0.001). Concentrations of 25(OH)D increased significantly across mUVI categories (Mann-Whitney test P<0.001) except for the low and moderate categories.

Variation of mUVI and 25(OH)D by month of blood collection

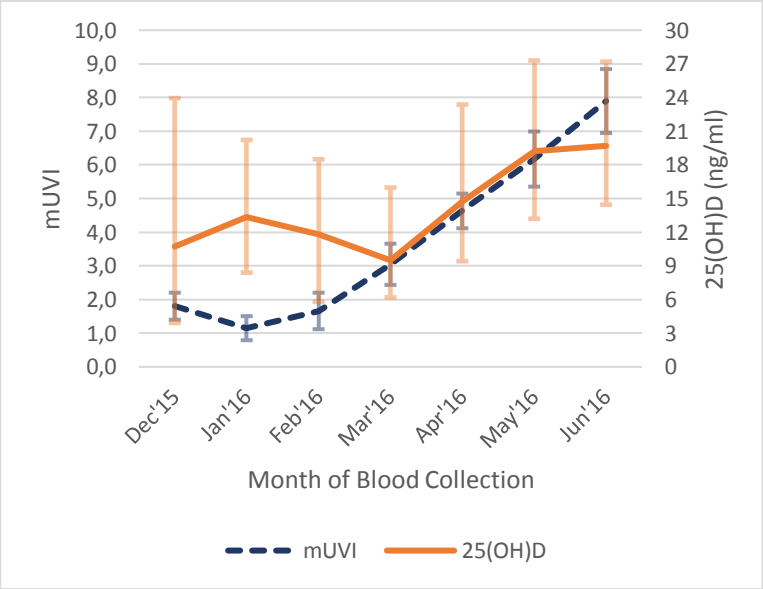

Figure 1. Variation of mUVI (mean and SD in error bars) and 25(OH)D (median, Q1 and Q3 in error bars) concentrations by month of blood collection.

The variation of mUVI and 25(OH)D concentrations during the blood collection period is shown in

Figure 1, where the mean of mUVI for all districts analysed in each month and the respective median 25(OH)D concentrations are presented. The mean of mUVI had its minimum in January (1.2) and increased until June, when it reached its peak (7.9). The mUVI was >3 between April and June. Although 25(OH)D concentrations increased from December to January and reached its nadir in March (median=9.50 ng/ml), differences were not statistically significant between consecutive months within this period. After March, 25(OH)D concentrations increased significantly between consecutive months until May (all P<0.001), when the median concentration (19.20 ng/ml) was not significantly different from June.

Multiple linear regression analysis for the entire blood collection period

Table 3. Factors associated with 25(OH)D by multiple stepwise linear regression analysis for 1486 older adults participating in a cross-sectional observational study. Model for the period of blood collection between December-June.

Independent variables	**Sβ (95%CI)**	***P-value***
mUVI	0.244 (0.198; 0.291)	<0.001
Sex [a]		
Women	Reference	
Men	--	--
Age (years)	-0.135 (-0.184; -0.086)	<0.001
Education (years)		
0	-0.060 (-0.106; -0.014)	0,010
1-4[a]	Reference	
5-12	0.052 (0.007; 0.096)	0.022
≥13 [a]	---	---
Professional activity [a]		
No	Reference	
Yes	--	--
Marital status		
Single, divorced or widowed	Reference	
Married or common-law marriage	0.089 (0.042; 0.137)	<0.001
Residence		
Home	Reference	
Institution	-0.064 (-0.110; -0.019)	0.006
Household income (€/month)		
<500 [a]	--	--
500-999	0.056 (0.011; 0.101)	0.015
≥1000	0.099 (0.052; 0.145)	<0.001
Does not know or does not declare	Reference	

(Continued)

Table 3. *(Continued)*

Independent variables	Sβ (95%CI)	*P-value*
Skin phenotype [a]		
I + II	--	--
III + IV	Reference	
V+ VI	--	--
Cognitive performance (MMSE score) [a]	--	--
Smoking habits [a]		
No	Reference	
Yes	--	--
Alcoholic beverages consumption		
None	Reference	
Moderate	0.054 (0.011; 0.098)	0.015
Heavy [a]	--	--
Physical activity (IPAQ)		
Normal	Reference	
Low	-0.078 (-0.124; -0.033)	0.001
Adherence to MeDI (PREDIMED score)	--	--
Fish/shellfish consumption ≥3 servings/week	--	--
No	Reference	--
Yes	--	--
Self-perceived health		
Very good [a]	--	--
Good [a]	--	--
Moderate	Reference	--
Bad	-0.051 (-0.096; -0.006)	0.025
Very bad [a]	--	--
Supplement intake		
No	Reference	
Yes	0.202 (0.158; 0.245)	<0.001
Nutritional status (MNA®-SF score)	0.070 (0.025; 0.115)	0.002
BMI (kg/m^2)	-0.123 (-0.168; -0.079)	<0.001

CI: confidence interval; IPAQ: International Physical Activity Questionnaire; MMSE: Mini Mental State Examination; MNA®-SF: Mini Nutritional Assessment® – Short Form. Sβ-Standardized regression coefficient

[a] Variables or categories that were not included in the final model.

The model included only 1486 subjects because of missing data.

The 25(OH)D concentrations were positively associated with: mUVI (P<0.001), 5-12 years of education (P=0.022), being married or in a common-law marriage (P<0.001), household income

of 500-999 €/month (P=0.015) and ≥1000 €/month (P<0.001), moderate alcoholic beverages consumption (P=0.015), supplement intake (P<0.001) and nutritional status (P=0.002). The 25(OH)D concentrations were negatively associated with age (P<0.001), BMI (P<0.001), 0 years of education (P=0.010), living in an institution (P=0.006), having a low physical activity (P=0.001) and a bad self-perceived health (P=0.025).

The variance in the set of independent variables included in the model explained 28% of the variance in 25(OH)D concentrations (adjusted R^2 =0.280).

Of all the continuous variables, UVI had the highest effect on 25(OH)D concentrations (sβ =0.244) while supplement intake was the variable with the highest effect of the categorical variables (sβ=0.202).

Multiple linear regression analysis stratified by period of blood collection

Table 4. Factors associated with 25(OH)D by multiple stepwise linear regression analysis for the period of blood collection between December-March (n=802).

December – March		
Independent variables	**Sβ (95%CI)**	***P-value***
mUVI	**-0.149 (-0.211; -0.087)**	**<0.001**
Age (years)	-0.211(-0.276; -0.146)	<0.001
Supplement intake	0.196 (0.134; 0.257)	<0.001
Nutritional status (MNA®-SF score)	0.097 (0.034; 0.159)	0.003
BMI (kg/m²)	-0.164 (-0.226; -0.102)	<0.001
Household income - ≥1000 €/month	0.147 (0.082; 0.211)	<0.001
Household income - 500-999 €/month	0.069 (0.006; 0.132)	0.033
Residence	-0.104 (-0.168; -0.041)	0.001
Alcoholic beverages consumption - Moderate	0.070 (0.009; 0.132)	0.025
Self-perceived health - Bad	-0.082 (-0.144; -0.020)	0.010

CI: confidence interval; IPAQ: International Physical Activity Questionnaire; MNA®-SF: Mini Nutritional Assessment® – Short Form. Sβ-Standardized regression coefficient

Only variables that were included in the final model are presented.

Table 5. Factors associated with 25(OH)D by multiple stepwise linear regression analysis for the period of blood collection between April-June (n=683).

April- June		
Independent variables	**Sβ (95%CI)**	***P-value***
mUVI	**0.295 (0.229; 0.362)**	**<0.001**
Age (years)	-0.092 (-0.163; -0.022)	0.010
Supplement intake	0.246 (0.181; 0.315)	<0.001
Nutritional status (MNA®-SF score)	0.076 (0.007; 0.145)	0.030
BMI (kg/m^2)	-0.117 (-0.185; -0.050)	0.001
Education - 0 years	-0.078 (-0.148; -0.010)	0.025
Marital Status	0.140 (0.072; 0.209)	<0.001
Skin phenotype - V +VI	-0.079 (-0.146; -0.013)	0.019
Cognitive performance (MMSE score)	0.085 (0.016; 0.156)	0.017
Physical Activity - Low	-0.102 (-0.172; -0.034)	0.004

CI: confidence interval; IPAQ: International Physical Activity Questionnaire; MMSE: Mini Mental State Examination; MNA®-SF: Mini Nutritional Assessment® – Short Form. Sβ-Standardized regression coefficient.
Only variables that were included in the final model are presented.

In the model for Dec-Mar, mUVI was negatively associated with 25(OH)D concentrations (Sβ=-0.149), contrarily to the models for Apr-Jun (Sβ=0.295) and Dec-June. Comparing models for Dec-Mar and Apr-Jun, mUVI had a lower effect on 25(OH)D in the first model (Sβ=-0.149 versus 0.295) and some independent variables included in the models were different. The following variables were included in all three models: mUVI, age, supplement intake, nutritional status and BMI. Both models explained approximately 23% of the variance in 25(OH)D concentrations (Dec-Mar: adjusted R^2=0.229; Apr-Jun: adjusted: R^2=0.232).

In the models for Dec-Jun and Apr-Jun, while keeping all the other independent variables constant, an increase of 1 unit in mUVI was associated with an increase in 25(OH)D of 1.07 ng/ml (Regression coefficient=0.030) and 1.12 ng/ml (Regression coefficient=0.049), respectively.

V - Discussion

In this cross-sectional study of Portuguese older adults, the median 25(OH)D concentration was 14.40 ng/ml. The majority of the subjects (69%) had 25(OH)D concentrations ≤20 ng/ml (Endocrine Society cut-off point for deficiency). If more conservative cut-off points, by the IOM, were applied, more than one third of the sample (39.5%) would be classified as being at risk of deficiency (<12 ng/ml).

The few studies conducted in Portugal focused on heterogenous population settings and did not include just the elderly, which hampers comparisons of results and highlights the relevance of this study. Prevalence of moderate (8/10-20 ng/ml) or severe deficiency (<8/10 ng/ml) in Portuguese studies ranged from 21 to 67.5% [118-121]. In the Survey in Europe on Nutrition and the Elderly: A Concerted Action (EURONUT-SENECA study), prevalence of 25(OH)D <12 ng/ml in Portuguese men and women was 31% and 33%, respectively [60]. The numbers found in the present study were higher than those found in most of previous works, but closer to the findings of Santiago *et al* (2012) in a sample of older adults [118].

In the EURONUT-SENECA study, the mean 25(OH)D levels ranged from 8 to 12 ng/ml in Southern European centers [122]**.** In a study with Spanish older adults, 86.3% had vitamin D insufficiency (≤30 ng/ml) [123]. In the Invecchiare in Chianti (InCHIANTI) study (2007), approximately 28.8% of women and 13.6% of men had 25(OH)D <10 ng/ml, and 74.9% of women and 51.0% of men had 25(OH)D <20 ng/ml [124]**.** Comparisons should be taken cautiously due to discrepancies in latitude of the sample, ethnicity and season of blood collection [51].

In agreement with previous reports, the observed differences in 25(OH)D levels in our sample, were as expected for the following variables: sex [56], age [56], physical activity [73, 125, 126] and BMI [114, 115, 127]. Institutionalized participants had less than half of the 25(OH)D concentrations of those living at home (7.20 versus 14.70 ng/ml), which was also expected [88, 128, 129]. Participants that reported supplement intake (8.6%) had higher 25(OH)D levels compared to those who did not, which is in line with other works [114, 130] (**Table 2**).

The serum 25(OH)D concentrations increased across ascending mUVI categories. Other works also showed a significant increase in 25(OH)D as UV radiation was more intense [73, 115] (**Table 2**).

As expected, 25(OH)D concentrations were lower in Dec-Mar (late autumn-early spring) than in Apr-Jun (spring-early summer) (P<0.001) which reflects the seasonal variation reported for 25(OH)D levels [114, 117] (**Table 2**).

Figure 1, which illustrates the variation of mean mUVi and median 25(OH)D concentrations during period of blood collection, showed that 25(OH)D concentrations varied during the blood collection period and it is noticeable that these were lower in Dec-Mar than in Apr-Jun. The variation of 25(OH)D from December to June was similar to other studies, which have also found a minimum in March (or late winter/early spring) [78, 88, 130] and an increase in spring and summer

[47, 51, 114-116]. In a Portuguese study, 25(OH)D levels were highest in the summer, followed by autumn, spring and winter [119]. However, similarly to our study, frequency of sufficiency of vitamin D (≥ 30 ng/ml) was below 50% in every month [119]. In the present study, despite the increase in 25(OH)D concentrations since April, of the 209 subjects sampled in June, only 17.2% had 25(OH)D concentrations ≥30 ng/ml and 48.3% had ≥20 ng/ml.

It is noticeable that although mUVI started to increase in February, the rise of 25(OH)D concentrations only started in April, when mUVI was >3. This is in line with statements that UVI <3 does not trigger adequate synthesis of vitamin D [67, 68]. At latitudes >37ºN, from November through February, the amount of UV radiation is usually not enough to initiate cutaneous synthesis [117]. O'Neill *et al* (2016) also found that the UV threshold for adequate synthesis was only reached in mid-March, in European countries [78]. At the same time, there might have been a lag-time between a change on monthly UVI and the corresponding change in 25(OH)D levels, which has been reported to range from 4 to 8 weeks [4, 72, 75, 112, 131]. This lag-time may be linked to synthesis of vitamin D and half-life of 25(OH)D [72]. One could speculate that the decrease in 25(OH)D between January and March might have been influenced by the fact that UVI was not intense enough to trigger vitamin D synthesis [67, 68], and/or exposure was not likely to occur due to low temperatures, limited hours of sunshine and/or individual factors such as clothing [60, 76, 127]. Therefore, although UVI was rising, individuals may have been relying on their vitamin D stores [116], which could have been insufficient, and 25(OH)D concentrations still declined until March (**Figure 1**).

The set of independent variables in the model for Dec-Jun (**Table 3**) explained 28% of the variance in 25(OH)D concentrations. This is in line with other studies that explored the relationship between 25(OH)D and vitamin D intake, UV exposure, environmental and sociodemographic factors, which obtained an adjusted R^2 between 21% and 33% [73, 114]. The model obtained in a study on Dutch older people was able to explain 27% of the variance in 25(OH)D concentrations, with sun exposure being the major determinant [114].

Associations between the independent variables and 25(OH)D were expected, according to previous research in middle aged and older populations, for: age [73, 115, 132], household income [133, 134], education [115], residence [133, 135], alcoholic beverages consumption [56, 132] self-perceived health [73] and BMI [56, 73, 132, 136].

Most works that included sun exposure measurements or UV radiation availability found these were predictors of 25(OH)D concentrations [73, 76, 115, 130, 132]. The present study did not measure sun exposure, but used UVI as an indicator of potential for synthesis of vitamin D. It was possible to find four studies that have explored the association between UVI and 25(OH)D, including by multiple regression analysis. Three have shown that UVI was positively associated with 25(OH)D and was a predictor of 25(OH)D levels [74, 75] or 25(OH)D <30 ng/ml [137]. Greer *et al* (2013) found no correlation between the two variables, presumably due to no sun exposure of their sample [15].

The fact that mUVI was a predictor of 25(OH)D levels and that there was a positive association between the two is in line with other works. This was also expected since UVB radiation induces photolysis of 7DHC and initiates synthesis of vitamin D, if adequate sun exposure occurs [3].

Supplement intake was an important predictor of 25(OH)D levels, as seen in the literature [132, 125, 138, 139]. Physical activity has also been associated with higher 25(OH)D concentration, similarly to this study. Higher sun exposure amongst other factors may be responsible for higher vitamin D levels in more active individuals [125, 140].

Nutritional status, measured by the MNA®-SF score, was positively associated with 25(OH)D. A lower score can be related to a decline in food intake and impaired mobility [110]. Impaired mobility or institutionalization discourages sun exposure in older people and have been associated with vitamin D deficiency [128, 141]. Thus, participants with lower scores might have been more prone to have an inadequate sun exposure and vitamin D intake, which could have contributed to lower 25(OH)D concentrations.

Skin phenotype was not included in the model, which occurred in some [142] but not all of the studies [115, 133]. This could have been influenced by the relative narrow range of skin types in our sample.

The majority of researchers concluded that dietary vitamin D intake [114, 115, 132, 136] and fatty fish consumption [138], which were not possible to estimate in the present study, were predictors of 25(OH)D concentrations. However, fish and shellfish consumption ≥3 servings/week and adherence to MeDI were not included in the final model. The lack of discrimination between lean and fatty fish, inadequacy of servings and the absence of questions linked to food with a high vitamin D content might have contributed to the exclusion of these variables.

Other unassessed factors, such as sun-related behaviors, diet and genetics, or other factors not yet known may account for the remaining variance in 25(OH)D status (72%).

Stratification by period of blood collection resulted in models with different predictors of 25(OH)D and opposite associations between UVI and 25(OH)D (**Tables 4** and **5**). The coincident predictors of 25(OH)D concentrations among the three models were: mUVI, age, supplement intake, nutritional status and BMI.

In the model for Dec-Mar, mUVI was inversely associated with 25(OH)D. This association was expected as **Figure 1** showed that between Dec-March, 25(OH)D declined despite the fact that mUVI was rising. Factors that have been previously discussed for Figure 1 and that were observed in other studies could have contributed to this negative association [76, 127, 143].

Given the Portuguese latitudes, UV may not be high enough to trigger vitamin D synthesis between late autumn and early spring in all the districts [3, 53-55]. During this period, individuals may have to rely on diet and supplements as well as on their vitamin D reserves, which may not last all “vitamin D winter” [70]. Since vitamin D intake from diet may not be adequate, supplement intake may be advisable in older adults [41, 60, 67, 132, 139, 142].

In the model for Apr-Jun**,** mUVI was positively associated with25(OH)D, which is in line with previous studies [74, 75, 137]. Between spring and early summer, sun exposure is more likely to occur [130] and it was expected that the higher UVI (mUVI>3) would promote synthesis and contribute (among other factors) to the higher 25(OH)D concentrations, comparatively to Dec-Mar [67, 68].

The low 25(OH)D concentrations despite UVI >3 is in line with reports of high levels of deficiency even in regions with high UVI, particularly in risk groups as the elderly [127, 144]. In similar latitudes, normal levels are barely reached after summer-autumn [145]. Additionally, older people synthesize vitamin D less efficiently [67] and tend to avoid the sun, staying at home or in the shade [145], and also wear long sleeves and cover the face even when temperatures are high [60, 146, 147]. The widespread public health advice on skin protection and harmful effects of UV radiation can also contribute to limited sun exposure [35, 148].

The fact that different predictors have been selected in both stratified models and UVI associations were opposite may also be related with the characteristics of the different groups of participants.

Nevertheless, UV exposure can increase 25(OH)D in older adults, depending on the season [149, 150]. Moreover, sun exposure during summer is a major determinant for stores of vitamin D [151]. Therefore, even though deficiency was frequent in this and other works, optimizing vitamin D stores is still important to maintain (or to minimize the decline of) vitamin D status during winter, for as long as possible [47, 56].

Limitations

The UVI does not account for human body orientation [68] and is based on the UV spectrum for erythema, which some authors argue that diverges from the vitamin D action spectrum [44, 47, 152].

It was not possible to estimate sun exposure from the collected data and UVI may indicate only the potential to synthesize vitamin D. Various factors that were not possible to assess may have interfered in this potential, including sun exposure behaviors (such as sunscreen use, clothing, duration and location of exposure) and ambient factors such as pollution [45]. Genetic factors and inter-individual variability might also have affected synthesis and 25(OH)D concentrations [59, 153].

On the other hand, 25(OH)D concentrations reflect not only sun exposure but also vitamin D intake, from diet and supplements [59] and might have been influenced by several factors.

Although analysis did not account for medication use, some individuals reported the use of medication that can influence vitamin D status, which may have interfered with their 25(OH)D levels.

Some participants (9% of the sample) reported to have chronic kidney problems, including renal failure. Chronic kidney disease, nephrotic syndrome and other unreported health conditions, which can influence vitamin D metabolism, might have affected vitamin D status of our sample.

One should also note that the dispersion of 25(OH)D concentrations in this sample was high.

There are some limitations inherent to the laboratorial methods of dosing 25(OH)D concentrations [154]. Comparison of 25(OH)D concentrations found in this study with previous works has limitations given the considerable variability between different methods and laboratories [154].

This was a cross-sectional observational study and the measured 25(OH)D concentrations may not reflect the long-term 25(OH)D levels [144]. Blood collection only encompassed months between December and June, which does not represent the variation during the entire year.

The fact that this is a cross-sectional observational study does not allow the establishment of causal relationships, as exposures and findings are seen simultaneously at one particular moment in time. Further, the effect of reverse causality may exist in the present work.

The sample was not randomly selected and a participation bias might have existed. Hence, our sample may not be representative of the Portuguese older adults' population (with exception for the characteristics of sex, age, educational level and regional area), which does not allow inference of results to the population and constitutes a limitation of this study.

Strengths

This is the first study to explore the relationship between UVI and 25(OH)D in older people in Portugal. The main strengths of this study include the large sample size and the fact that it is representative of the population in terms of in terms of age, sex, education, and regional area. Also, all the blood samples were analysed at the same laboratory using the same equipment and assay, which decreased the variability of 25(OH)D analysis that exists in several works [154].

The use of UVI instead of latitude and season, as an indicator of potential for vitamin D synthesis, constitutes an advantage since UVI accounts for latitude, season and intensity of UV that reaches the Earth adjusted for nebulosity and ozone absorption [70].

VI - Conclusions

Vitamin D deficiency was highly frequent in this sample of Portuguese older adults (approximately 7 in 10 individuals).

Despite all the factors that can decrease vitamin D synthesis and sun exposure in older adults, UVI was a predictor of 25(OH)D concentrations in all the multiple linear regression models.

The UVI was positively associated with 25(OH)D in December-June and April-June but inversely associated in December-March. The studied factors in the overall model explained 28% of the variance in 25(OH)D concentrations.

This study suggests that the association between UVI and vitamin D may be different depending on the time of the year and thus, future research and recommendations about sensible sun exposure to maintain/achieve vitamin D status should take this into account. Moreover, diet and supplements may be more important sources of vitamin D during months when UVI and sun exposure are expected to be low/insufficient and should be reinforced accordingly.

Other predictors of 25(OH)D concentrations included in the models might suggest some modifiable factors that could be important to assess in future studies, which can elucidate how these can affect vitamin D status in older adults. Nevertheless, other parameters not measured in this study, such as sun exposure behaviors and diet may have an impact on vitamin D levels in the older population.

In future works, it may be useful to investigate the long-term variation of 25(OH)D concentrations of a population. Additionally, it is important to develop and include in future studies, adequate and validated tools that estimate sun exposure, accounting for personal behaviors, as well as vitamin D intake from diet and supplements.

References

1. Mostafa WZ, Hegazy RA. Vitamin D and the skin: Focus on a complex relationship: A review. J Adv Res. 2015; 6(6):793-804.
2. Lai YH, Fang TC. The pleiotropic effect of vitamin D. ISRN Nephrol. 2013; 2013:6.
3. Chen TC, Lu Z, Holick MF. Photobiology of Vitamin D. 2010:35-60.
4. Holick MF. Nutrition and Health-Vitamin D Physiology, Molecular Biology, and Clinical Applications. Chapter 1. Vitamin D and Health: Evolution, Biologic Functions, and Recommended Dietary Intakes for Vitamin D. 2nd ed. Boston; 2010.
5. Grober U, Spitz J, Reichrath J, Kisters K, Holick MF. Vitamin D: Update 2013: From rickets prophylaxis to general preventive healthcare. Dermatoendocrinol. 2013; 5(3):331-47.
6. Theodoratou E, Tzoulaki I, Zgaga L, Ioannidis JP. Vitamin D and multiple health outcomes: umbrella review of systematic reviews and meta-analyses of observational studies and randomised trials. BMJ. 2014; 348(g2035):1-19.
7. Holick MF. Vitamin D Deficiency. New England Lournal of Medicine. 2007; 357:266-81.
8. Glerup H, Mikkelsen K, Poulsen L, Hass E, Overbeck S, Thomsen J, et al. Commonly recommended daily intake of vitamin d is not sufficient if sunlight exposure is limited. J Intern Med. 2000; 247(2)
9. Holick MF. Vitamin D: A millenium perspective. J Cell Biochem. 2003; 88(2):296-307.
10. Holick MF. Resurrection of vitamin D deficiency and rickets. J Clin Invest. 2006; 116(8):2062-72.
11. Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium. Dietary reference intakes for Calcium and Vitamin D 2011
12. Jones G. Pharmacokinetics of vitamin D toxicity. Am J Clin Nutr. 2008; 88((suppl.)):582S–6S.
13. Jones KS, Assar S, Vanderschueren D, Bouillon R, Prentice A, Schoenmakers I. Predictors of 25(OH)D half-life and plasma 25(OH)D concentration in The Gambia and the UK. Osteoporos Int. 2015; 26(3):1137-46.
14. Vieth R. Vitamin D supplementation, 25-hydroxyvitamin D concentrations and safety. Am J Clin Nutr. 1999; 69:842-56.
15. Greer RM, Portelli SL, Hung BS, Cleghorn GJ, McMahon SK, Batch JA, et al. Serum vitamin D levels are lower in Australian children and adolescents with type 1 diabetes than in children without diabetes. Pediatr Diabetes. 2013; 14(1):31-41.
16. Tsiaras WG, Weinstock MA. Factors influencing vitamin D status. Acta Derm Venereol. 2011; 91(2):115-24.
17. Aikaterini IT, Vaia K, Konstantinos S, Georgina X. Vitamin-D in the Immune System: Genomic and Non-Genomic Actions. Mini-Reviews in Medicinal Chemistry. 2015; 15(11):953-63.
18. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011; 96(7):1911-30.
19. O'Mahony L, Stepien M, Gibney MJ, Nugent AP, Brennan L. The potential role of vitamin D enhanced foods in improving vitamin D status. Nutrients. 2011; 3(12):1023-41.
20. Schmid A, Walther B. Natural vitamin D content in animal products. Adv Nutr. 2013; 4(4):453-62.
21. Parreira D, Serra MC, Dias MG. Doseamento de vitamina D em produtos lácteos consumidos em Portugal. Boletim Epidemiológico Observações Artigos breves. 2015; 7:27-28.
22. Calvo M, Whiting S, Barton C. Vitamin D fortification in the United States and Canada: current status and data needs. Am J Clin Nutr. 2004; 80:1710-16.
23. Lamberg-Allardt C. Vitamin D in foods and as supplements. Prog Biophys Mol Biol. 2006; 92(1):33-8.
24. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Guidelines for preventing and treating vitamin D deficiency and insufficiency revisited. J Clin Endocrinol Metab. 2012; 97(4):1153-8.
25. Płudowski P, Karczmarewicz E, Bayer M, Carter G, Chlebna-Sokół D, Czech-Kowalska J, et al. Practical guidelines for the supplementation of vitamin D and the treatment of deficits in

Central Europe — recommended vitamin D intakes in the general population and groups at risk of vitamin D deficiency. Endokrynologia Polska. 2013; 64(4):319-27.
26. Members of Recommending Committee. Prophylaxis of vitamin D deficiency— Polish Recommendations 2009. Endokrynologia Polska/Polish Journal of Endocrinology. 2010; 61(2)
27. Hollis BW. Symposium. Circulating 25-Hydroxyvitamin D Levels Indicative of Vitamin D Sufficiency:Implications for Establishing a New Effective Dietary Intake Recommendation for Vitamin D. Journal of Nutrition. 2005:317:22.
28. Holick MF. Vitamin D: important for prevention of osteoporosis, cardiovascular heart disease, type 1 diabetes, autoimmune diseases, and some cancers. Southern medical journal. 2005; 98(10):1024-7.
29. Calvo MS, Whiting SJ, Barton CN. Vitamin D Intake A Global Perspective of Current Status. J Nutr 2005; 135:310-16.
30. Feldman D, Wesley Pike J, Adams JS. Vitamin D. Academic Press; 2011.
31. Ministério da Agricultura do Desenvolvimento Rural e das Pescas. Decreto-Lei 54/2010, de 28 de maio.nexo I - Vitaminas e minerais que podem ser declarados e respectiva dose diária recomendada (DDR). Diário da República, 1ª série, nº 104. 2010:p.1847.
32. European Food Safety Authority. Dietary reference values for vitamin D. EFSA Journal. 2016; 14(10):e04547.
33. Hall LM, Kimlin MG, Aronov PA, Hammock BD, Slusser JR, Woodhouse LR, et al. Vitamin D intake needed to maintain target serum 25-hydroxyvitamin D concentrations in participants with low sun exposure and dark skin pigmentation is substantially higher than current recommendations. J Nutr. 2010; 140(3):542-50.
34. Moore C, Murphy MM, Keast DR, Holick MF. Vitamin D intake in the United States. J Am Diet Assoc. 2004; 104(6):980-3.
35. Spiro A, Buttriss JL. Vitamin D: An overview of vitamin D status and intake in Europe. Nutr Bull. 2014; 39(4):322-50.
36. Quraishi SA, Camargo CA, Jr., Manson JE. Low vitamin D status in Europe: moving from evidence to sound public health policies. Am J Clin Nutr. 2016; 103(4):957-8.
37. Lucas R, Costa L, Barros H. Ingestão de cálcio e vitamina D numa amostra urbana de mulheres portuguesas. Arquivos de Medicina. 2005; 19(1-2):7-14.
38. Holick MF, MacLaughlin JA, Doppelt SH. Regulation of cutaneous previtamin D3 photosynthesis in man: skin pigment is not an essential regulator. Science (New York, NY). 1981; 211(4482):590-3.
39. Bogh MK, Schmedes AV, Philipsen PA, Thieden E, Wulf HC. Vitamin D production depends on ultraviolet-B dose but not on dose rate: a randomized controlled trial. Experimental dermatology. 2011; 20(1):14-8.
40. Wolpowitz D, Gilchrest BA. The vitamin D questions: how much do you need and how should you get it? J Am Acad Dermatol. 2006; 54(2):301-17.
41. World Health Organization, International Agency For Research On Cancer. Vitamin D and cancer / a report of the IARC Working Group on Vitamin D. 2008.
42. Moan J, Porojnicu AC, Dahlback A, Setlow RB. Addressing the health benefits and risks, involving vitamin D or skin cancer, of increased sun exposure. Proc Natl Acad Sci U S A. 2008; 105(2):668-73.
43. Panou N, Georgopoulos S, Panou M, Sergentanis T, Maropoulos G, Papalambros E. Sun exposure and vitamin D. Perspectives in Public Health. 2012; 132(1):7.
44. Fioletov VE, McArthur LJ, Mathews TW, Marrett L. On the relationship between erythemal and vitamin D action spectrum weighted ultraviolet radiation. J Photochem Photobiol B. 2009; 95(1):9-16.
45. Engelsen O. The relationship between ultraviolet radiation exposure and vitamin D status. Nutrients. 2010; 2(5):482-95.
46. van Dijk A, den Outer P, van Kranen H, Slaper H. The action spectrum for vitamin D3: initial skin reaction and prolonged exposure. Photochem Photobiol Sci. 2016; 15(7):896-909.
47. Webb AR. Who, what, where and when-influences on cutaneous vitamin D synthesis. Prog Biophys Mol Biol. 2006; 92(1):17-25.
48. Allinson S, Asmuss M, Baldermann C, Bentzen J, Buller D, Gerber N, et al. Validity and use of the UV index: report from the UVI working group, Schloss Hohenkammer, Germany, 5-7 December 2011. Health Phys. 2012; 103(3):301-6.

49. Vanicek K, Frei T, Litynska Z, Schmalwieser A. A guide for publication and interpretation of solar UV Index forecasts for the public. COST-713 Action. 1999
50. World Health Organization, World Meteorological Organization, United Nations Environment Programme, International Commission on Non-Ionizing Radiation Protection. Global Solar UV Index - A Practical Guide. 2002.
51. Cashman KD, Dowling KG, Skrabakova Z, Gonzalez-Gross M, Valtuena J, De Henauw S, et al. Vitamin D deficiency in Europe: pandemic? Am J Clin Nutr. 2016; 103(4):1033-44.
52. European Commission. New data on Vitamin D from the ODIN Project.Food-based solutions for optimal vitamin D nutrition andhealth through the life cycle. Short summary of the Symposium of 3rd of March 2016 in Brussels. 2016
53. Webb AR, Holick MF. Influence of Season and Latitude on the Cutaneous synthesis of vitamin D exposure to winter sunlight in boston and edmonton will not promote vit d synthesis in human skin. Journal of Clinical Endocrinology and Metabolism. 1988; 67(2)
54. Santos A. Efeitos pleiotrópicos da vitamina D. Efeitos na saúde óssea e cardiovascular. Associação Portuguesa de Nutrição Entérica e Parentérica. 2011; 1(1):25.
55. Bandeira F, Griz L, Dreyer P, Eufrazino C, Bandeira C, Freese E. Vitamin D Deficiency A Global Perspective. Arq Bras Endocrinol Metab 2006; 50(4)
56. Brouwer-Brolsma EM, Berendsen AAM, Vaes MMA, Dullemeijer C, de Groot LCPGM, Feskens EJM. Collection and analysis of published scientific information as preparatory work for the setting of Dietary Reference Values for Vitamin D.EFSA supporting publication 2016:EN-766. External Scientific Report. 2016:171.
57. Kimlin MG, Olds WJ, Moore MR. Location and vitamin D synthesis: is the hypothesis validated by geophysical data? J Photochem Photobiol B. 2007; 86(3):234-9.
58. Correia A, Azevedo MdS, Gondim F, Bandeira F. Ethnic aspects of vitamin D deficiency. Arquivos Brasileiros de Endocrinologia & Metabologia. 2014; 58(5):540-44.
59. Mosekilde L. Vitamin D and the elderly. Clin Endocrinol (Oxf). 2005; 62(3):265-81.
60. Van der Wielen RP, Löwik MR, van den Berg H, de Groot LC, Haller J, Moreiras O, et al. Serum vitamin D concentrations among elderly people in Europe. The Lancet. 1995; 346:207-10.
61. Calvo MS, Whiting SJ. Prevalence of Vitamin D Insufficiency in Canada and the United States:Importance to Health Status and Efficacy of Current Food Fortcation and Dietary Supplement Use. Nutrition Reviews 2003; 61(3):107-13.
62. Gilchrest BA. Sun protection and Vitamin D: three dimensions of obfuscation. J Steroid Biochem Mol Biol. 2007; 103(3-5):655-63.
63. Grant W, Holick MF. Benefits and Requirements of Vitamin D for Optimal Health: A Review. Alternative Medicine Review. 2005; 10(2):94-111.
64. Instituto Português do Mar e da Atmosfera. A Radiação Ultravioleta - A radiação solar.; sem data. Disponível em: http://www.ipma.pt/pt/enciclopedia/amb.atmosfera/uv/index.html.
65. Wetterdienst SHD. Global UV Index Forecasting by the Deutscher Wetterdienst (DWD). no date
66. German Meteorological Service (DWD). UV Index Forecasting. 2015
67. Nair R, Maseeh A. Vitamin D: The “sunshine” vitamin. Journal of Pharmacology & Pharmacotherapeutics. 2012; 3(2):118-26.
68. Seckmeyer G, Schrempf M, Wieczorek A, Riechelmann S, Graw K, Seckmeyer S, et al. A novel method to calculate solar UV exposure relevant to vitamin D production in humans. Photochem Photobiol. 2013; 89(4):974-83.
69. Gill P, Kalia S. Assessment of the feasibility of using sunlight exposure to obtain the recommended level of vitamin D in Canada. CMAJ Open. 2015; 3(3):E258-63.
70. Webb AR, Engelsen O. Calculated Ultraviolet Exposure Levels for a Healthy Vitamin D Status. Photochem Photobiol. 2006 82(6):1697-703.
71. Gilchrest BA. Sun exposure and vitamin D sufficiency. Am J Clin Nutr. 2008; 88:570-77.
72. Maeda SS, Saraiva GL, Hayashi LF, Cendoroglo MS, Ramos LR, Correa Mde P, et al. Seasonal variation in the serum 25-hydroxyvitamin D levels of young and elderly active and inactive adults in Sao Paulo, Brazil: The Sao PAulo Vitamin D Evaluation Study (SPADES). Dermatoendocrinol. 2013; 5(1):211-7.
73. Tran B, Armstrong BK, McGeechan K, Ebeling PR, English DR, Kimlin MG, et al. Predicting vitamin D deficiency in older Australian adults. Clin Endocrinol (Oxf). 2013; 79(5):631-40.

74. Mesquita KC, Igreja AC, Costa IMC. Ultraviolet index: a light in atopic dermatitis and vitamin D research? Anais Brasileiros de Dermatologia. 2016; 91(1):34-39.
75. Prodam F, Zanetta S, Ricotti R, Marolda A, Giglione E, Monzani A, et al. Influence of Ultraviolet Radiation on the Association between 25-Hydroxy Vitamin D Levels and Cardiovascular Risk Factors in Obesity. J Pediatr. 2016; 171:83-9 e1.
76. Brot C, Vestergaard P, Kolthoff N, Gram J, Hermann AP, Sørensen OH. Vitamin D status and its adequacy in healthy Danish perimenopausal women: relationships to dietary intake, sun exposure and serum parathyroid hormone. British Journal of Nutrition. 2001; 86(S1):S97-S103.
77. Lee SH, Park SJ, Kim KM, Lee DJ, Kim WJ, Park RW, et al. Effect of sunlight exposure on serum 25-hydroxyvitamin d concentration in women with vitamin d deficiency: using ambulatory lux meter and sunlight exposure questionnaire. Korean J Fam Med. 2012; 33(6):381-9.
78. O'Neill CM, Kazantzidis A, Ryan MJ, Barber N, Sempos CT, Durazo-Arvizu RA, et al. Seasonal Changes in Vitamin D-Effective UVB Availability in Europe and Associations with Population Serum 25-Hydroxyvitamin D. Nutrients. 2016; 8(9)
79. Brustad M, Alsaker E, Engelsen O, Aksnes L, Lund E. Vitamin D status of middle-aged women at 65-71 degrees N in relation to dietary intake and exposure to ultraviolet radiation. Public Health Nutr. 2004; 7(2):327-35.
80. Kositsawat J, Geraci SA. Screening for vitamin D deficiency in the elderly. Journal of the Mississippi State Medical Association. 2010; 51(1):7-10.
81. Liu BA GM, Labranche JM, Murray TM, Vieth R, Shear NH. Seasonal Prevalence of Vitamin D Deficiency in institutionalized older adults. J Am Geriatr Soc 1997; 45(5):598-603.
82. Saneei P, Salehi-Abargouei A, Esmaillzadeh A. Serum 25-hydroxy vitamin D levels in relation to body mass index: a systematic review and meta-analysis. Obesity Reviews. 2013; 14(5):393-404.
83. Vanlint S. Vitamin D and obesity. Nutrients. 2013; 5(3):949-56.
84. Looker AC, Johnson CL, Lacher DA, Pfeiffer C, Schleicher RL, Sempos CT. Vitamin D Status: United States, 2001–2006. NCHS Data Brief. 2011(59)
85. Hathcock JN, Shao A, Vieth R, Heaney R. RisK Assessment fot vitamin D. Am J Clin Nutr. 2007; 85:6-18.
86. Manson JE, Brannon PM, Rosen CJ, Taylor CL. Vitamin D Deficiency — Is There Really a Pandemic? New England Journal of Medicine. 2016; 375(19):1817-20.
87. Amaral TF, Santos A, Guerra RS, Sousa AS, Alvares L, Valdiviesso R, et al. Nutritional Strategies Facing an Older Demographic: The Nutrition UP 65 Study Protocol. JMIR Res Protoc. 2016; 5(3):e184.
88. Papapetrou PD, Triantaphyllopoulou M, Karga H, Zagarelos P, Aloumanis K, Kostakioti E, et al. Vitamin D deficiency in the elderly in Athens, Greece. J Bone Miner Metab. 2007; 25(3):198-203.
89. Grant WB, Cross HS, Garland CF, Gorham ED, Moan J, Peterlik M, et al. Estimated benefit of increased vitamin D status in reducing the economic burden of disease in western Europe. Prog Biophys Mol Biol. 2009; 99(2-3):104-13.
90. Instituto Nacional de Estatística. Censos 2011 Resultados Definitivos -Portugal. 2012
91. Organização Mundial da Saúde. Relatório Mundial de envelhecimento e saúde. 2015
92. Comission E. The 2015 Ageing Report - Economic and budgetary projections for the 28 EU Member States (2013-2060). 2015
93. Janssen H, Samson M, Verhaar H. Vitamin D deficiency, muscle function, and falls in elderly people. Am J Clin Nutr 2002; 75:611-5.
94. Vieth R B-FH, Boucher BJ, Dawson-Hughes B, Garland CF, Heaney RP, Holick MF, Hollis BW, Lamberg-Allardt C, McGrath JJ, Norman AW, Scragg R, Whiting SJ, Willett WC, Zittermann A. The urgent need to recommend an intake of vitamin D that is effective. Am J Clin Nutr. 2007; 85(3):649-50.
95. Deutscher Wetterdiens. Large-Scale UV Index Forecasts. 2016. Disponível em: http://www.dwd.de/EN/climate_environment/consultancy/health/uvi/wmo_rsmc_index/wmo_rsmc_node.html. .
96. Fitzpatrick TB. Soleil et peau [Sun and skin]. . Journal de Médecine Esthétique (in French). 1975(2):33-34.

97. Fitzpatrick TB. THe validity and practicality of sun-reactive skin types i through vi. Archives of Dermatology. 1988; 124(6):869-71.
98. Holm EA. Skin Colour and Pigmentation. In: Fluhr JW, editor. Practical Aspects of Cosmetic Testing: How to Set up a Scientific Study in Skin Physiology. Berlin: Springer; 2011. p. 181.
99. Guerreiro M. Testes de rastreio de defeito cognitivo e demência: uma perspectiva prática. Rev Port Clin Geral. 2010; 26:46-53.
100. Craig CL MA, Sjöström M, Bauman AE, Booth ML, Ainsworth BE,. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc 2003; 35(8):1381-95.
101. International Physical Activity Questionnaire. Guidelines for Data Processing and Analysis of the International Physical Activity Questionnaire (IPAQ) - Short Form version 2.0. 2004
102. Fried L, Tangen M, Walston J, Newman A, Hirsch C, Gottdiener J, et al. Frailty in Older Adults: Evidence for a Phenotype. Journal of Gerontology. 2001; 56
103. Afonso L, Moreira T, Oliveira A. Índices de adesão ao padrão alimentar mediterrânico - a base metodológica para estudar a sua relação com a saúde. . Revista Factores de Risco. 2014; 31:48-55.
104. Martinez-Gonzalez MA, Garcia-Arellano A, Toledo E, Salas-Salvado J, Buil-Cosiales P, Corella D, et al. A 14-item Mediterranean diet assessment tool and obesity indexes among high-risk subjects: the PREDIMED trial. PLoS One. 2012; 7(8):e43134.
105. Castro-Quezada I, Román-Viñas B, Serra-Majem L. The Mediterranean Diet and Nutritional Adequacy: A Review. Nutrients. 2014; 6(1):231.
106. Vintém J. Inquéritos Nacionais de Saúde: auto-percepção do estado de saúde: uma análise em torno da questão de género e da escolaridade. Psicologia da Saúde. 2008
107. Stewart A, Marfell-Jones M, Olds T, H. R. International Standards for Anthropometric Assessment. Potchefstroom,South Africa; 2011.
108. World Health Organization. BMI classification. 2017. Disponível em: http://apps.who.int/bmi/index.jsp?introPage=intro_3.html.
109. Institute NN. Mini Nutritional Assessment. MNA. Português.; sem ano.
110. Kaiser MJ, Bauer JM, Ramsch C, Uter W, Guigoz Y, Cederholm T, et al. Validation of the Mini Nutritional Assessment short-form (MNA®-SF): A practical tool for identification of nutritional status [journal article]. JNHA - The Journal of Nutrition, Health and Aging. 2009; 13(9):782.
111. Roche Diagnostics Cobas. Vitamin D total. 25-Hidroxivitamina Dl.ms_05894913190.V7.0. 2015
112. Amy K. Kasahara RJS, Andrew Noymer. Vitamin D (25OHD) Serum Seasonality in the United States. PLoS One. 2013; 8(6)
113. US Department of Health and Human Services and US Department of Agriculture. 2015–2020 Dietary Guidelines for Americans. 8th Edition. 2015
114. Brouwer-Brolsma EM, Vaes AMM, Zwaluw NLvd, Wijngaarden JPv, Swart KMA, Ham AC, et al. Relative Importance of Summer Sun Exposure, Vitamin D Intake, and Genes to Vitamin D Status in Dutch Older Adults: The B-Proof Study. J Steroid Biochem Mol Biol. 2015; 164(168-176)
115. Millen AE, Wactawski-Wende J, Pettinger M, Melamed ML, Tylavsky FA, Liu S, et al. Predictors of serum 25-hydroxyvitamin D concentrations among postmenopausal women: the Women's Health Initiative Calcium plus Vitamin D clinical trial. Am J Clin Nutr. 2010; 91(5):1324-35.
116. Papadakis G, Keramidas I, Kakava K, Pappa T, Villiotou V, Triantafillou E, et al. Seasonal Variation of Serum Vitamin D Among Greek Female Patients with Osteoporosis. In vivo 2015; 29:409-14.
117. Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. Am J Clin Nutr 2004; 80((suppl.)):1678S–88S.
118. Santiago T, Rebelo M, Porto J, Silva N, Vieira J, Costa JMN. Hipovitaminose D em Doentes Internados num Serviço de Medicina Interna. Acta Med Port. 2012; 25(2):68-76.
119. Santos AM, Fernandes V, Garcia FM. Carência de Vitamina D numa População Hospitalar: Uma Fotografia pela Perspetiva Laboratorial. Acta Med Port. 2015; 28(6):726-34.
120. Garrido S, Oliveira JC, Carvalho A. Vitamin D Status in the Outpatient Setting of Northern Portugal. Endocrine Review. 2015; 36(2)

121. Silva L, Freitas JM, Sampaio L, Terroso G, Pinto JA, Veludo V, et al. Níveis séricos de vitamina D em portugueses com fracturas de fragilidade. 2010; 3(Acta Reumatológica Portuguesa):352-57.
122. Mithal A, Wahl DA, Bonjour JP, Burckhardt P, Dawson-Hughes B, Eisman JA, et al. Global vitamin D status and determinants of hypovitaminosis D. Osteoporos Int. 2009; 20(11):1807-20.
123. Mateo-Pascual C, Julian-Vinals R, Alarcon-Alarcon T, Castell-Alcala MV, Iturzaeta-Sanchez JM, Otero-Piume A. [Vitamin D deficiency in a cohort over 65 years: prevalence and association with sociodemographic and health factors]. Revista espanola de geriatria y gerontologia. 2014; 49(5):210-6.
124. Houston KD, Cesari M, Ferrucci L, Cherubini A, Maggio. D, Bartali B, et al. Association Between Vitamin D Status and Physical Performance: The InCHIANTI Study. Journal of Gerontology:. 2007; 62(4):440-46.
125. Wanner M, Richard A, Martin B, Linseisen J, Rohrmann S. Associations between objective and self-reported physical activity and vitamin D serum levels in the US population. Cancer Causes Control. 2015; 26(6):881-91.
126. Hibler EA, Sardo Molmenti CL, Dai Q, Kohler LN, Warren Anderson S, Jurutka PW, et al. Physical activity, sedentary behavior, and vitamin D metabolites. Bone. 2016; 83:248-55.
127. Kimlin M, Harrison S, Nowak M, Moore M, Brodie A, Lang C. Does a high UV environment ensure adequate vitamin D status? J Photochem Photobiol B. 2007; 89(2-3):139-47.
128. Malik R. Vitamin D and Secondary Hyperparathyroidism in the Institutionalized Elderly. Journal of Nutrition For the Elderly. 2008; 26(3-4):119-38.
129. Papapetrou PD, Triantafyllopoulou M, Korakovouni A. Severe vitamin D deficiency in the institutionalized elderly. Journal of endocrinological investigation. 2008; 31(9):784-7.
130. Rodríguez Sangrador M, Beltrán de Miguel B, Quintanilla Murillas L, Cuadrado Vives C, Moreiras Tuny O. Contribución de la dieta y la exposición solar al estatus nutricional de vitamina D en españolas de edad avanzada; Estudio de los Cinco Países (Proyecto OPTIFORD). Nutr Hosp. 2008; 23(6):567-76.
131. Pasco JA, Henry MJ, Kotowicz MA, Sanders KM, Seeman E, Pasco JR, et al. Seasonal periodicity of serum vitamin D and parathyroid hormone, bone resorption, and fractures: the Geelong Osteoporosis Study. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2004; 19(5):752-8.
132. Bertrand KA, Giovannucci E, Liu Y, Malspeis S, Eliassen AH, Wu K, et al. Determinants of plasma 25-hydroxyvitamin D and development of prediction models in three U.S. cohorts. The British journal of nutrition. 2012; 108(10):1889-96.
133. Naugler C, Zhang J, Henne D, Woods P, Hemmelgarn BR. Association of vitamin D status with socio-demographic factors in Calgary, Alberta: an ecological study using Census Canada data. BMC Public Health. 2013; 13:316.
134. Lips P. Worldwide status of vitamin D nutrition. J Steroid Biochem Mol Biol. 2010; 121(1-2):297-300.
135. Shinkov A, Borissova A-M, Dakovska L, Vlahov J, Kassabova L, Svinarov D, et al. Differences in the prevalence of vitamin D deficiency and hip fractures in nursing home residents and independently living elderly. Archives of Endocrinology and Metabolism. 2016; 60:217-22.
136. Jacques PF, Felson DT, Tucker KL, Mahnken B, Wilson PW, Rosenberg IH, et al. Plasma 25-hydroxyvitamin D and its determinants in an elderly population sample. Am J Clin Nutr. 1997; 66(4):929-36.
137. Klassen KM, Fairley CK, Kimlin MG, Kelly M, Read TR, Broom J, et al. Ultraviolet index and location are important determinants of vitamin D status in people with human immunodeficiency virus. Photochem Photobiol. 2015; 91(2):431-7.
138. van der Meer IM, Boeke AJ, Lips P, Grootjans-Geerts I, Wuister JD, Deville WL, et al. Fatty fish and supplements are the greatest modifiable contributors to the serum 25-hydroxyvitamin D concentration in a multiethnic population. Clin Endocrinol (Oxf). 2008; 68(3):466-72.
139. Jolliffe DA, Hanifa Y, Witt KD, Venton TR, Rowe M, Timms PM, et al. Environmental and genetic determinants of vitamin D status among older adults in London, UK. J Steroid Biochem Mol Biol. 2016; 164:30-35.

140. van den Heuvel EG, van Schoor N, de Jongh RT, Visser M, Lips P. Cross-sectional study on different characteristics of physical activity as determinants of vitamin D status; inadequate in half of the population. Eur J Clin Nutr. 2013; 67(4):360-5.
141. Zhang R, Naughton DP. Vitamin D in health and disease: current perspectives. Nutr J. 2010; 9:65.
142. Burgaz A, Åkesson A, Oster A, Michaelsson K, Wolk A. Associations of diet, supplement use, and ultraviolet B radiationexposure with vitamin D status in Swedish women during winter. Am J Clin Nutr. 2007; 86:1399-404.
143. van der Wielen RP, Löwik MR, van den Berg H, de Groot LC, Haller J, Moreiras O, et al. Serum vitamin D concentrations among elderly people in Europe. Lancet. 1995; 346
144. Millen AE, Bodnar LM. Vitamin D assessment in population-based studies: a review of the issues. Am J Clin Nutr. 2008; 87:1102-5.
145. Navarro Valverde C, Quesada- Gomez J. Vitamin D deficiency in Spain. Reality or myth? Rev Osteoporos Metab Miner. 2014; 6(1)
146. Boucher B. The Problems of Vitamin D Insufficiency in Older People. Age and Ageing. 2012; 3(4):313-29.
147. Matsuoka LY, Wortsman J, Dannenberg MJ, B.W. H, Lu Z, Holick MF. Clothing Prevents Ultraviolet-B radiation Dependent photosynthesis of vitamin D3. J Clin Endocrinol Metab. 1992; 75(4):1099-103.
148. Reichrath J. The challenge resulting from positive and negative effects of sunlight: how much solar UV exposure is appropriate to balance between risks of vitamin D deficiency and skin cancer? Prog Biophys Mol Biol. 2006; 92(1):9-16.
149. Durvasula S, Gies P, Mason RS, Chen JS, Henderson S, Seibel MJ, et al. Vitamin D response of older people in residential aged care to sunlight-derived ultraviolet radiation. Arch Osteoporos. 2014; 9:197.
150. Reid IR, Gallagher DJA, Bosworth J. Prophylaxis against vitamin D deficiency in the elderly by regular runlight exposure Age and Ageing. 1986; 15(1):35-40.
151. Maxwell JD. Seasonal variation in vitamin D. Proceedings of the Nutrition Society. 2007; 53(03):533-43.
152. Olds WG, Kimlin MG. Availability of Vitamin D UV and a “Vitamin D index” for public UV exposure advice.
153. Binkley N, Novotny R, Krueger D, Kawahara T, Daida YG, Lensmeyer G, et al. Low vitamin D status despite abundant sun exposure. J Clin Endocrinol Metab. 2007; 92(6):2130-5.
154. Alves M, Bastos M, Leitão F, Marques G, Ribeiro G, Carrilho F. Vitamina D–importância da avaliação laboratorial. Revista Portuguesa de Endocrinologia, Diabetes e Metabolismo. 2013; 8(1):32-39.