Associations of vitamin D status with dietary intakes and physical activity levels among adults from seven European countries: the Food4Me study

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56

ABSTRACT

Purpose: To report the vitamin D status in adults from seven European countries and to identify behavioural correlates.

Methods: In total, 1,075 eligible adult men and women from Ireland, Netherlands, Spain, Greece, UK, Poland and Germany, were included in the study.

Results: Vitamin D deficiency and insufficiency, defined as 25-hydroxy vitamin D_3 (25-OHD_3) concentration of <30 and 30-49.9 nmol/L, respectively, were observed in 3.3% and 30.6% of the participants. The highest prevalence of vitamin D deficiency was found in the UK and the lowest in the Netherlands (8.2% vs. 1.1%, $P<0.05$). In addition, the prevalence of vitamin D insufficiency was higher in females compared with males (36.6% vs. 22.6%, $P<0.001$), in winter compared with summer months (39.3% vs. 25.0%, $P<0.05$) and in younger compared with older participants (36.0% vs. 24.4%, $P<0.05$). Positive dose-response associations were also observed between 25-OHD_3 concentrations and dietary vitamin D intake from foods and supplements, as well as with physical activity (PA) levels. Vitamin D intakes of ≥5 μg/day from foods and ≥5 μg/day from supplements, as well as engagement in ≥30 min/day of moderate- and vigorous-intensity PA were associated with higher odds ($P<0.05$) for maintaining sufficient (≥50 nmol/L) 25-OHD_3 concentrations.

Conclusions: The prevalence of vitamin D deficiency varied considerably among European adults. Dietary intakes of ≥10 μg/day of vitamin D from foods and/or supplements and at least 30 min/day of moderate- and vigorous-intensity PA were the minimum thresholds associated with vitamin D sufficiency.

Keywords: vitamin D, 25-hydroxyvitamin D, diet, supplements, physical activity, adults Europe

INTRODUCTION

During the last 15 years, vitamin D has attracted increased attention from the scientific community, the food industry, policy makers and the public [1]. This is mainly due to new discoveries about the impact of vitamin D on several health outcomes beyond its known metabolic actions on bone and mineral metabolism [2]. Specifically, the presence of the vitamin D receptor in many body tissues supported evidence linking vitamin D deficiency to increased risk of certain auto-immune diseases, cancers, cardiovascular disease, diabetes and psychiatric disorders [3-5]. In addition to its effects on health and metabolism, vitamin D has raised interest because of the large variation in the prevalence of vitamin D deficiency across countries worldwide, with estimates ranging from 2% to 90% [6-9].

The major source of vitamin D for humans is endogenous synthesis via skin exposure to solar ultraviolet B radiation (wavelength, 290 to 315 nm). Skin exposure to sunlight stimulates the conversion of 7-dehydrocholesterol to pre-vitamin D_3 and its subsequent conversion to vitamin D_3 (one of the two major forms of vitamin D that is synthesized endogenously and is also found to animal food sources) [10]. However, several environmental factors, including seasonality, latitude and prevailing weather conditions, determine whether sufficient sunlight may induce cutaneous vitamin D_3 synthesis [11]. In addition, sex, age, adiposity status and skin pigmentation, as well as clothing habits, sunscreen use and physical activity (PA) levels, as a proxy of outdoor activities and consequently sunlight exposure, have also been associated with vitamin D status [12,13]. Regarding PA, positive associations irrespective of sun exposure have also been reported with vitamin D status [56-58], suggesting an independent association.

When environmental conditions, personal traits or lifestyle prevent adequate exposure to sunlight, dietary intake of vitamin D from fortified foods and/or supplements is considered as a good alternative to reach and to maintain blood 25-hydroxy vitamin D (25-OHD) concentrations (the main index of vitamin D status) in the normal range. In countries where availability of vitamin D fortified foods is low, it is very difficult to meet the recommended

dietary intakes of vitamin D from its limited natural food sources (e.g. oily fish) [14]. This is the case for several European countries where dietary intakes of vitamin D from fortified foods is particularly low [15].

Although the evidence on vitamin D status worldwide is increasing, there is large heterogeneity between studies, mainly due to differences in the methods used to estimate vitamin D concentration in blood [9]. The scarcity, as well as the heterogeneity of data regarding vitamin D intake and status in Europe, highlights the need for multi-centre studies that can provide relevant data for free-living populations in a consistent and standardized manner. The primary aim of the present study was to assess the prevalence of vitamin D deficiency and insufficiency in adults from seven European countries who participated in the baseline measurements of the Food4Me 'Proof of Principle' study [16]. The secondary aim was also to identify behavioural correlates of vitamin D status in these populations.

METHODS

Study design

The Food4Me 'Proof of Principle' study was a 6-month, four-arm, randomized controlled trial (RCT) conducted across 7 European countries to compare the effects of three levels of Personalized Nutrition (PN) with standard population advice on health-related outcomes. The current study presents data obtained at baseline from the study participants.

Recruitment

Participants were recruited in 7 European countries (Ireland, the Netherlands, Spain, Greece, the UK, Poland and Germany) using identical standardised protocols in all recruitment centres, as described in detail elsewhere [17]. In brief, local and national advertising of the study via the Internet, radio, newspapers, posters, e-flyers, social media and word of mouth, were used to recruit adult men and women. Prior to participation, an information sheet was provided to potential volunteers who completed an online informed consent form before

submitting personal data. This signed online consent form was automatically directed to the study coordinator to be counter-signed and archived. In total, 5,562 volunteers were screened online between August 2012 and August 2013 [18]. A second online informed consent form was completed before randomisation to the intervention study only for participants who met the inclusion criteria. A total of 1,607 study participants aged ≥18 years were recruited [17]. The current study presents results on 1,075 participants with full data on dietary intake, PA, anthropometrics, genetics and 25-hydroxy vitamin D_3 (25-OHD_3) concentrations.

Ethics approval

The Research Ethics Committees at each University or Research Centre granted ethics approval for the study.

Eligibility

Regarding eligibility criteria, volunteers aged >18 years were included in the study. In all these volunteers, the following exclusion criteria were applied in identifying eligible study participants: pregnant or lactating women; no or limited access to the Internet; following a prescribed diet for any reason, including weight loss, in the last 3 months; diabetes, coeliac disease, Crohn's disease, or any metabolic disease or condition altering nutritional requirements such as thyroid disorders (if condition was not controlled); allergies or food intolerances. Exclusion based on prescribed diet or specific diseases was to avoid that participating in the intervention study could be disadvantageous.

Measurements

To ensure that procedures were similar, standardized operating procedures were adopted in the recruiting centres and for all study procedures and researchers were trained in their use [17]. Two screening questionnaires, including a Food Frequency Questionnaire (FFQ) that was specifically developed and validated for the purposes of this study [19,20], were used to

identify participants for the Food4Me study. Participants self-reported online their ethnicity and occupations. Occupations were grouped according to the European classifications of occupations and the respective salaries of these occupations, as described in details elsewhere [21]. Based on this classification, the following groups and group names were generated: "Professional and managerial"; "Intermediate"; "Routine and manual". Categories for "Students" and "Retired and unemployed" were also added. Participants also provided health and anthropometric data at screening and detailed information on dietary intake and food preferences [17]. Anthropometric and PA data, as well as blood and buccal cell samples were collected from all study participants and the latter were used for metabolic marker analysis and genotyping, respectively. Detailed information on the inclusion/exclusion criteria used and the information collected are provided elsewhere [17].

Anthropometry

Participants self-measured their height and body weight and uploaded their anthropometric measurements to their personal Food4Me online account [17]. Standardised instructions on how to perform these measurements were provided to participants in printed and digital format. Validation of the self-reported anthropometry is described elsewhere [22]. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2), whereas participants with a BMI $\geq$30 kg/m^2 were categorized as obese.

Food Frequency Questionnaire (FFQ)

Habitual dietary intake was quantified using an online FFQ, developed for this study, which included food items consumed frequently in each of the 7 recruitment countries. The average daily intakes of foods and nutrients consumed over the last month were computed in real time using a food composition database based on McCance & Widdowson's ''The composition of foods'' [23]. For each one of the seven countries participating in the Food4Me study, the McCance & Widdowson food composition database was updated with the nutritional

composition of local foods and recipes included in the FFQ. Following this procedure, dietary intakes of vitamin D from individual food items and supplements were computed and used in the current analysis. Nevertheless, it should be noted that the agreement between the FFQ and the 4-day weighted food record used to assess the validity of the FFQ in estimating dietary intakes was lower for vitamin D compared to other nutrients [19]. More information on the design, reproducibility, validity and computations of food and nutrient intakes of the online FFQ has been previously described [19,20].

Metabolic markers

Blood samples were collected from all eligible study participants at their baseline evaluation that took place some time within the period from August 2012 to August 2013. Finger-prick blood samples were collected by the participants using a collection pack provided by Vitas Ltd, Oslo, Norway. Before spotting blood, cards for vitamin D analysis (Whatman Protein Saver 903 Card; GE Healthcare) were pre-treated with 1% of 2,6-di-tert-butyl-4-methylphenol (BHT) dissolved in methanol (MeOH); 30 µL of 1% BHT in MeOH were pipetted to each circle on the card and allowed to dry for at least 30 min at room temperature. These pre-treated cards were packed in an airtight aluminium bag (Whatman Foil Bags, item no. 10534321; Whatman Inc.) with a drying agent (Sorb-it, item no. 10548234; Süd-Chemie) and stored at room temperature until analysis. To help with blood collection, participants had access to an online video demonstration, written instructions and frequently asked questions in the local language. For the finger pricks, 2.0-mm contact-activated lancets (BD Microtainer; Becton, Dickinson and Company) were used. Each participant was asked to fill two Dry Blood Spot cards (equivalent to five drops of blood or up to 250 µL of blood per card) at each collection time point. When the 10 blood spots were filled, participants were instructed leave the cards to dry at room temperature for at least 2 h, but not longer than 4 h, before samples were put in the airtight aluminium bag with drying sachet and returned by post to the corresponding recruiting centre. The centres shipped the samples to DSM (DSM

Nutritional Products Ltd, Switzerland) for measurements of vitamin D (25-OHD_2 and 25-OHD_3). Although the shipments were done at ambient temperature, the closed bags were stored at the centres and at DSM at nominal −20°C. Calibration was carried out using whole-blood samples received from blood donors of the 'Blutspendezentrum SRK beider Basel' (Blood Donation Centre at Basel Hospital), including haematocrit values for each sample. More information on the procedures followed for the calibration is provided in detail elsewhere [17]. Before analysis, the samples were assessed to check whether they met the quality criteria. Samples meeting quality criteria, which are described elsewhere [17], were prepared for analysis. Chromatography was performed using an Ascentis Express C18 column (Supelco), and detection was carried out by an AB Sciex 5500 Qtrap instrument with APPI positive mode and MRM scan type at unit resolution. The resulting 25-OHD_3 concentration was corrected for sex-specific mean haematocrit values. The corrected 25-OHD_3 values are used in the present study.

Vitamin D status in study participants was assessed using the threshold values recently proposed by the Institute Of Medicine (IOM) Dietary Reference Intake (DRI) Committee [24]. More specifically, vitamin D deficiency, insufficiency and sufficiency were defined as 25-OHD_3 concentrations <30 nmol/L, 30-49.9 nmol/L and ≥50 nmol/L, respectively.

Gene analyses

Buccal cell samples were collected by participants at baseline using Isohelix SK-1 DNA buccal swabs and Isohelix Dri-capsules (LGC Genomics, Hertfordshire, UK). Samples were returned to the recruiting centres and shipped to LGC Genomics, who extracted the DNA and used competitive allele-specific polymerase chain reaction (KASP) genotyping assays to provide biallelic scoring of single nucleotide polymorphisms (SNPs) rs1544410 and the rs2228570 in the Vitamin D Receptor (VDR) gene [17] among other SNPs. These two VDR SNPs were used as covariates in the associations of vitamin D status with dietary intake and physical activity levels examined in the present study.

Physical activity

Physical activity (PA) was measured objectively using the DirectLife triaxial accelerometer for movement registration (TracmorD) (Philips Consumer Lifestyle, the Netherlands) [25-27]. The PA monitor was sent by post to each participant. Online video demonstrations as well as digital and printed instructions were provided at baseline. Participants were instructed to wear the monitor throughout the 6 months intervention and to upload their PA data fortnightly via an online interface. Data were recorded with a time-sampling interval of 1 minute. A day was considered valid if the participant had worn the PA monitor for at least 10 hours, but not longer than 18 hours. Wear time was defined as 24 hours minus non-wear time. To define non-wear time, the recommendations of Choi et al. [28] were adapted to the TracmorD. The R software version 3.1.2 was used for PA data processing.

Activity energy expenditure (AEE) and time spent in PAs of different intensity were derived from accelerometers [29]. Classification into sedentary activities and light-, moderate- and vigorous- intensity PA was based on the application of thresholds for AEE [29]. Time spent in sedentary activities, as well as in light-, moderate- and vigorous-intensity PA was calculated. Lastly, to account for the fact that 1 minute of vigorous-intensity PA is equivalent to 2 minutes of moderate-intensity PA [30], the time spent in moderate-equivalent PA was also calculated as follows: Moderate-equivalent PA (min/day) = Moderate-intensity PA (min/day) + 2 x Vigorous-intensity PA (min/ day).

Statistical analysis

Normality of the distribution of continuous variables was evaluated using the Kolmogorov-Smirnov test. Continuous variables were expressed as mean values ± standard deviations (sd), whereas categorical variables were presented as frequencies (%). Differences in mean values of continuous variables were examined using the one-way Analysis Of Variance (ANOVA) or the non-parametric Kruskal-Wallis test in the case of normally and non-normally

distributed variables, respectively. Differences in frequencies were tested using the chi square (χ^2) test and the two-sample z-test for proportions for multiple post-hoc comparisons. Analyses of co-variance and multivariate logistic regression analysis were also performed to examine the dose-response effect on 25-OHD_3 concentrations and the likelihood of vitamin D sufficiency derived from different doses of vitamin D intake from foods or supplements, as well as different moderate-equivalent PA levels. These analyses were adjusted for age, sex, dietary energy intake (kcal per day), country, VDR rs1544410, VDR rs2228570, BMI, study centre and seasonality. To model the seasonal variation at the study sites, the study centre and the interaction of study centre with the functions sin (sample year×2×π) and cos (sample year×2×π) were included as confounders in the analyses. The 20th January was the consensus date across all study centres when the 25-OHD_3 concentrations reached their nadir. To simplify the subsequent modelling and interpretation, a single normalised sine function was derived, which oscillated between −1.0 when the 25-OHD_3 concentration was at its lowest on the 20th January and +1.0 when the 25-OHD_3 concentration was at its highest on the 21st July. Including a single season function and its interaction with each centre in the model assumes synchronised timing of seasons across all study centres and enables the model to differentiate mean concentrations and seasonal amplitudes by study centre. More details are provided elsewhere [17]. All reported P-values were based on two-sided tests. The level of statistical significance in all analyses was set at $P<0.05$. SPSS version 22.0 (SPSS Inc., Texas, USA) was used for all statistical analyses.

RESULTS

Vitamin D status by country, sex, age, season, weight status, ethnicity and occupation

The prevalence of vitamin D deficiency and insufficiency in each of the 7 participating countries is presented in *Figure 1*. The highest prevalence of vitamin D deficiency, 25-OHD_3 concentration of <30 nmol/L, was observed in the UK, while the lowest prevalence was observed in the Netherlands (8.2% vs. 1.1%, $P<0.05$). *Supplementary Table 1* presents more

information on the sociodemographic characteristics of the Food4Me study participants as well as the seasonal distribution of the measurements. In addition, *Table 1* presents the prevalence of vitamin D deficiency and insufficiency in the total sample, by sex, seasonality, age and weight status groups. Overall, the prevalence of vitamin D deficiency and insufficiency was 3.3% and 30.6%, respectively. Furthermore, the prevalence of vitamin D deficiency and insufficiency was significantly higher in females compared with males (5.4% vs. 0.7%, $P<0.001$ for vitamin D deficiency; 36.6% vs. 22.6%, $P<0.001$ for vitamin D insufficiency). Regarding seasonal differences, the prevalence of vitamin D insufficiency was higher from January to March (i.e. typical winter months) as compared to the periods from April to June (i.e. typical spring months) and July to September (i.e. typical summer months) (39.3% vs. 25.6% and 25.0%, $P<0.001$). In addition, younger study participants (18-35 y) and students had higher prevalence of vitamin D insufficiency compared with older ones (≥51 y) and participants with "routine and manual" occupations (36.0% vs. 24.4%, $P<0.001$ and 40.7% vs. 24.1%, $P=0.030$ respectively). There were no statistically significant differences in the prevalence of vitamin D deficiency and insufficiency between obese and non-obese participants as well as between Caucasians, which represents ~97% of the total study sample, and other ethnic groups.

Dietary intake of vitamin D and PA levels by vitamin D status groups

Table 2 summarizes the differences in mean dietary intake of vitamin D from food and/or dietary supplements and in the mean time spent in PAs of different intensity among study participants with deficient (<30 nmol/L), insufficient (30-49.9 nmol/L) and sufficient (≥50 nmol/L) 25-OHD_3 concentrations. Participants with sufficient 25-OHD_3 concentrations had significantly higher dietary intakes of vitamin D from foods and/or dietary supplements compared with participants with vitamin D insufficiency and deficiency ($P<0.01$). Regarding food-derived vitamin D, participants with sufficient concentrations of 25-OHD_3 had higher dietary vitamin D intake from meat and fish ($P=0.001$), as well as from fats and spreads

(*P*=0.047) compared with their vitamin D-insufficient and -deficient counterparts and a significant difference was reached for eggs (P=0.030) compared to vitamin deficient participants. Furthermore, study participants with vitamin D sufficiency spent less time on sedentary activities (*P*=0.026) and more time on light- (*P*=0.004) and moderate-intensity PA (*P*=0.003), as well as in moderate equivalent PA (*P*=0.013) in comparison with vitamin D deficient and/or insufficient study participants.

Associations of different intakes of vitamin D and PA levels with vitamin D status

Table 3 displays the dose-response effect of different intakes of vitamin D, derived from foods or supplements, and different durations/ amounts of time spent in moderate-equivalent PA with 25-OHD_3 concentrations. Mean 25-OHD_3 concentrations were higher with higher intakes of vitamin D from foods (*P*=0.035) and supplements (*P*<0.001), as well as with more time spent in moderate-equivalent PA (*P*=0.007). Furthermore, *Table 3* presents the likelihood (adjusted OR, 95% CIs and *P*-values) of having sufficient 25-OHD_3 concentrations for different intakes of vitamin D, derived from foods or supplements, and different amounts of time spent in moderate-equivalent PA. The odds of having sufficient 25-OHD_3 concentration was 1.58 (95% CI 1.01-2.52) times more likely for study participants with 5-9.9 µg/day of vitamin D intake derived from foods compared to their counterparts with dietary vitamin D intake <2.5 µg/day. The odds for having sufficient 25-OHD_3 concentrations were 1.87 (95% CI 1.05-3.35), 5.49 (95% CI 1.87-16.1) and 14.2 (95% CI 1.86-36.2) times higher for study participants with 5-9.9, 10-19.9 and 20-80 µg/day, respectively, of vitamin D intake from dietary supplements compared with participants with <2.5 µg/day of vitamin D intake from supplements. Lastly, study participants engaged in 30-59.9 and ≥60 min/day of moderate-equivalent PA had 1.79 (95% CI 1.24-2.58) and 1.78 (95% CI 1.23-2.57) higher odds to be vitamin D-sufficient compared to study participants spending <30 min/day on moderate-equivalent PA.

DISCUSSION

The available literature on vitamin D status among populations in Europe is characterised by a high degree of variability among countries [6,9]. Differences among European countries in the prevalence of vitamin D deficiency and insufficiency may be explained in part by the confounding effect of different methods used to measure 25-OHD_3 concentrations in serum or plasma, as well as by different thresholds used to define deficiency and insufficiency [31-33]. Nevertheless, despite the use of the same methods to measure 25-OHD_3 and application of the same thresholds for vitamin D insufficiency for all centres, the current study confirmed similar variability in the prevalence of vitamin D insufficiency and deficiency in 7 European countries, ranging from 23.5% and 1.1% in the Netherlands to 34% and 8.2% in the UK, respectively (*Figure 1*).

The current study also reported sex, seasonal, and other socio-demographic differences in 25-OHD_3 concentrations (*Table 1*). In this context, the prevalence of vitamin D insufficiency and deficiency was higher in females than males, but these sex differences were smaller than reported by other studies [9]. Regarding seasonal differences, as expected the highest and lowest prevalence rates of vitamin D insufficiency were observed during typical winter (January to March) and summer (July to September) months, respectively. The prevalence of vitamin D insufficiency was lower in the older (≥ 51 y) compared with the younger (18-35 y) participants. The relevant evidence available in the literature concerning age-specific trends in vitamin D status across the lifespan is inconsistent since higher and lower prevalence rates of poor vitamin D status have been reported for both younger and older adults [9,33,34]. In the very old age (usually ≥ 85 y) several factors such institutionalization, especially when combined with concurrent health and mobility problems, declining efficiency of the skin to endogenously produce vitamin D [35], as well as poor dietary vitamin D intake, and general nutritional status [36], usually lead to a high prevalence of vitamin D deficiency [37]. However, in the present study older study participants were in the age range of 50 to 79 years, were healthy, which was due to the disease and clinical conditions exclusion criterion, and

379 quite physically active, which may explain their better vitamin D status as compared to
380 younger study participants.
381 Humans obtain vitamin D from the diet, dietary supplements and from endogenous synthesis
382 in the skin due to sunlight exposure, often in an ascending order [3]. The present study
383 confirmed the relatively low contribution of foods to meeting the Estimated Average
384 Requirement (EAR) value of 10μg/day for vitamin D proposed by the IOM [24]. Specifically,
385 vitamin D intakes from meat, fish, fats, spreads and eggs were significantly higher in
386 participants with sufficient vitamin D concentrations compared with their counterparts with
387 vitamin D insufficiency and/or deficiency (*Table 2*). Among the limited food sources of
388 vitamin D, either natural or fortified ones, the consumption of foods mentioned above has
389 been reported also by other studies to be linked to better vitamin D status [38]. In addition,
390 even after adjusting for several potential confounders, a dose-response association was
391 observed between dietary vitamin D intakes from foods with 25-OHD_3concentrations (**Table**
392 **3**). Nevertheless, the contribution of foods in the total dietary intake of vitamin D seems to be
393 particularly low [39] and this is also supported by our observations showing that the average
394 dietary intake of vitamin D derived from foods was less than the recommended EAR threshold
395 of 10 μg/day.
396 Taking into account the low contribution from foods to total dietary vitamin D intake, the
397 findings of the present and those of other European studies [40,41], highlight the significant
398 role of other sources, notably dietary supplements. The present study showed that the average
399 vitamin D intake from dietary supplements in participants with sufficient vitamin D levels was
400 3.14 μg/day compared with only 0.63 and 0.53 μg/day by vitamin D insufficient and deficient
401 participants, respectively (*Table 2*). Data from the National Adult Nutrition Survey in Ireland
402 showed that the average dietary vitamin D intake coming from supplements was 9 μg/day and
403 was much higher compared with that coming from foods, which in the Irish survey was found
404 to be 4 μg/day [42]. This relatively low average vitamin D intake from foods (i.e. exactly 4
405 μg/day) was also observed in the present study of adults in different parts of Europe and

406 together with the observed high prevalence of vitamin D insufficiency, may indicate the need
407 for more effective dietary strategies to enhance vitamin D intake. With the exception of fatty
408 fish, increasing the intake of natural (non-fortified) food sources of vitamin D is the least
409 likely strategy to counteract low dietary vitamin D intake [43]. As a consequence, the use of
410 dietary supplements has been proposed by many as the second most effective strategy (with
411 food fortification proposed as the primary one) [44]. However, the degree to which the use of
412 vitamin D dietary supplements can increase 25-OHD_3 concentrations depends on the dose of
413 vitamin D in the supplements [45]. In this regard, the present study showed that vitamin D
414 intakes of ≥10 μg/day from foods and/or supplements can ensure sufficient 25-OHD_3
415 concentrations.
416 Because dietary vitamin D intake from its natural food sources and from supplements cannot
417 account for the total variability of serum or plasma 25-OHD_3 concentrations, another major
418 determinant of vitamin D status is sun exposure [46-48]. Depending on the time of the day,
419 season, latitude and skin pigmentation, exposure of the skin, e.g. of arms or legs for 5 to 30
420 minutes can promote adequate endogenous synthesis of vitamin D_3 [49]. PA, when executed
421 outdoors, can be a proxy measure of sunlight exposure and probably explains the significant
422 positive association observed in the present study between time spent on moderate-equivalent
423 PA and 25-OHD_3 concentrations (*Table 3*). Similar positive linear associations between PA
424 and circulating 25-OHD_3 concentrations have been reported also by other recent studies [50-
425 52]. Interestingly, some studies have reported significant positive associations between
426 vitamin D status and PA even after adjusting for sun exposure [50], and others have found
427 similar significant positive associations with both outdoor and indoor activities [53]. In
428 addition to the wider health benefits from increased PA, the present study showed that at least
429 30 min per day spent on MVPA is related to sufficient 25-OHD_3 concentrations. This
430 observation is very important from a public health perspective, because it is in line with the
431 daily target of moderate- and/or vigorous-intensity PA proposed by the American College of
432 Sports Medicine and the American Heart Association for adults [54].

The findings of the current study should be interpreted in light of its strengths and limitations. Regarding strengths, the web-based design of the Food4Me study facilitated participation by volunteers regardless of distance from the research centre. In addition, the Food4Me research team developed and implemented a novel remote system for data and biological sample collection enabling study participants to provide dietary, anthropometric, PA and other health-related information via the Internet, as well as biological samples (dry blood spots and buccal cells) for nutritional, metabolic and genotypic measurements. In addition, the dried blood spot methodology used to measure 25-OHD_3 concentrations was applied for the first time in a fairly large study population and demonstrated to be highly applicable, cost effective and reliable [17]. Regarding limitations, because of the cross-sectional design of the current study, we cannot attribute causality to our observations. Furthermore, most data were self-reported or derived from biological samples collected remotely with the potential for introduction of measurement errors and change of samples. However, studies examining the reliability of data collected in web-based interventions [55,56], including the present one [22], have shown good agreement between self-reported and objectively measured indices. Moreover, in order to minimize measurement errors, all measurement protocols in the present study were standardized across all centres and were provided in the native languages of each recruitment country. Participants were assisted in recording of information and in sample collection by the provision of detailed instructions, video clips and a frequently asked questions leaflet. Lastly, participants were a convenience sample of those who volunteered for the Food4Me intervention study and are not necessarily nationally representative of the countries involved, which limits generalizability of findings from the present study. However, in several respects, participants were broadly similar to those of the adult population in Europe [17].

Conclusions

In conclusion, the present study reported a considerable variability in vitamin D status among adults examined in 7 European countries. The highest prevalence of vitamin D insufficiency

and/or deficiency was observed in the UK compared with the Netherlands, in females compared with males, in winter (January to March) compared with summer (July to September) months and in younger (18-35 y) compared with older (≥51 y) study participants. Regarding behavioural correlates of vitamin D status, there were positive dose-response associations between 25-OHD_3 concentrations and dietary vitamin D intake from foods and supplements, as well as with physical activity levels, most likely as a proxy of sun exposure. Dietary intakes of ≥10 μg/day of vitamin D from foods and/or supplements, as well as >30 min/day of moderate-equivalent PA were the minimum thresholds for ensuring sufficient circulating 25-OHD_3 concentrations.

Conflict of interest

The authors declare that they have no conflict of interest.

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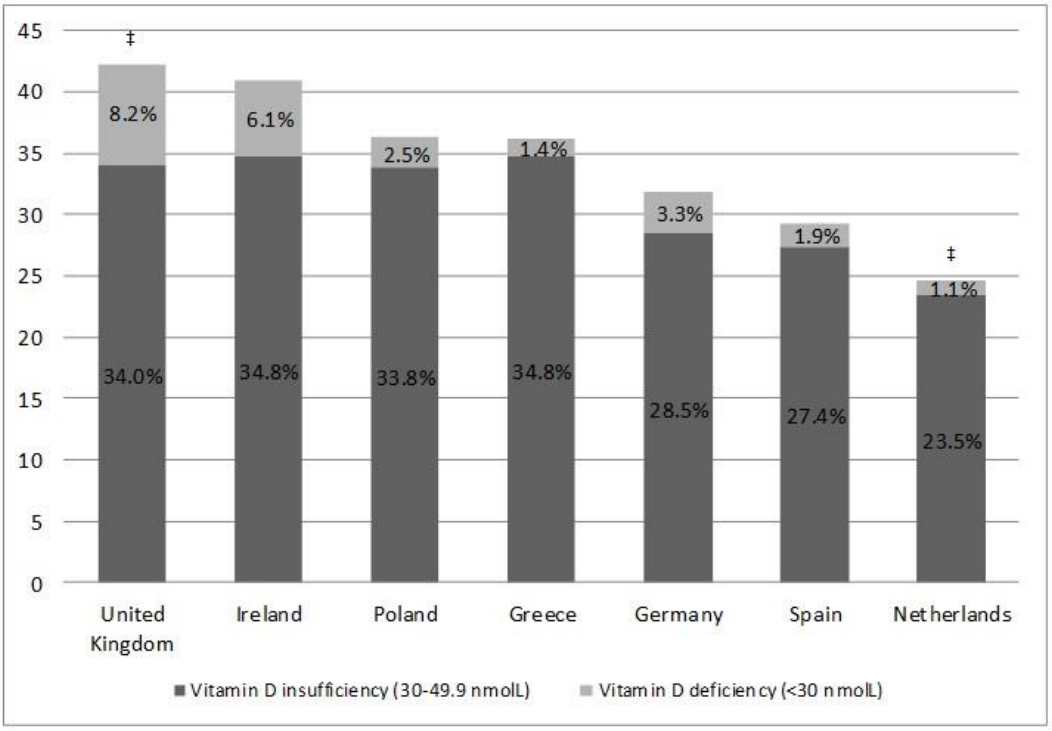
685

Figure 1. Prevalence of vitamin D insufficiency and deficiency by country.



‡: $P<0.05$ for the differences in the prevalence of vitamin D deficiency (25-OHD_3 <30 nmol/L) between countries sharing the same symbol.

Table

Table 1. Prevalence of vitamin D deficiency and insufficiency in the total sample, by sex, seasonality, age group and adiposity status.

	Vitamin D Deficiency: 25-OHD$_3$ <30 nmol/L		**Vitamin D Insufficiency: 25-OHD$_3$: 30-49.9 nmol/L**	
	n (%)	***P*-value**[†]	**n (%)**	***P*-value**[†]
Total sample (n=1075)	36 (3.3)		329 (30.6)	
Sex		<0.001		<0.001
Males (n=461)	3 (0.7)		104 (22.6)	
Females (n=614)	33 (5.4)		225 (36.6)	
Season		0.062		<0.001
October-December (n=54)	3 (5.6)		15 (27.8)	
January-March (n=384)	19 (4.9)		151 (39.3) [a, b]	
April-June (n=545)	13 (2.2)		140 (25.6)[a]	
July-September (n=92)	1 (1.1)		23 (25.0)[b]	
Age		0.208		0.003
18-35 y (n=445)	20 (4.5)		160 (36.0)[a]	
36-50 y (n=372)	10 (2.7)		106 (28.5)	
≥51 y (n=258)	6 (2.3)		63 (24.4)[a]	
Ethnicity		0.846		0.468
Caucasian (n=1039)	35 (3.4)		316 (30.4)	
Other (n=36)	1 (2.8)		13 (36.1)	
Occupations[*]		0.481		0.030
Intermediate (n=277)	9 (3.3)		84 (30.4)	
Routine and manual (n=116)	4 (3.4)		28 (24.1)[a]	
Professional and managerial (n=453)	13 (2.9)		131 (28.9)	
Retired or unemployed (n=67)	1 (1.5)		20 (29.9)	
Student (n=162)	9 (5.6)		66 (40.7)[a]	
Adiposity Status[‡]		0.682		0.459
Non-obese (n= 899)	31 (3.4)		271 (30.1)	
Obese (n=176)	5 (2.8)		58 (33.0)	

[†]*P*-values were derived from Pearson's Chi-square tests. [a, b] $P<0.05$ for pairwise *post-hoc* comparisons between groups/prevalence rates sharing the same superscript letter, derived from the 2-sample z-test for proportions. [‡] Obese study participants were those with a BMI ≥30 Kg/m^2.

[*]Occupations were grouped according to the European classifications of occupations and the respective salaries of these occupations. Based on this classification, the following groups and group names were generated: "Professional and managerial"; "Intermediate"; "Routine and manual". Categories for "Students" and "Retired and unemployed" were also added.

Table 2. Differences in Dietary intake of vitamin D from foods and supplements, and physical activity levels in study participants with insufficient or sufficient 25-OHD3 concentrations.

	Vitamin D Deficiency: 25-OHD$_3$ <30 nmol/L (n=36)	**Vitamin D Insufficiency: 25-OHD$_3$: 30-49.9 nmol/L (n=329)**	**Vitamin D Sufficiency: 25-OHD$_3$: ≥50 nmol/L (n=710)**	
	Mean (SD)	Mean (SD)	Mean (SD)	***P*-value**[†]
Vitamin D intake (µg/ day) from foods only	3.26 (2.08)[a]	3.79 (2.32)[c]	4.20 (2.61)[a, c]	0.006
Vitamin D intake (µg/ day) from:				
Cereals	0.17 (0.31)	0.19 (0.36)	0.27 (0.39)	0.097
Bread and savoury snacks	0.02 (0.03)	0.03 (0.08)	0.03 (0.07)	0.813
Starchy foods (Pasta, rice and potatoes)	0.08 (0.08)	0.08 (0.08)	0.07 (0.06)	0.702
Meat and Fish	1.59 (1.69)[a]	1.95 (1.96)[c]	2.29 (2.09)[a, c]	0.001
Dairy	0.16 (0.14)	0.16 (0.15)	0.16 (0.14)	0.761
Fats and Spreads	0.26 (0.56)[a]	0.26 (0.39)[c]	0.34 (0.63)[a, c]	0.047
Sweets and Snacks	0.19 (0.18)	0.20 (0.25)	0.21 (0.24)	0.836
Soups and Sauces	0.03 (0.02)	0.03 (0.03)	0.03 (0.02)	0.128
Drinks	0.24 (0.42)	0.37 (0.71)	0.32 (0.54)	0.368
Fruit	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	-
Vegetables	0.0020 (0.004)	0.0014 (0.004)	0.0013 (0.004)	0.503
Eggs	0.41 (0.44)[a]	0.51 (0.71)	0.58 (0.76)[a]	0.030
Vitamin D intake (µg/ day)only from supplements	0.53 (1.68)[a]	0.63 (2.40)[c]	3.14 (11.0)[a, c]	<0.001
Vitamin D intake (µg/ day) both from foods and supplements	3.68 (2.94)[a]	4.41 (3.41)[c]	7.44 (11.2)[a, c]	<0.001
Time (min/day) in physical activities of different intensity				
Sedentary PA	765.0 (63.1)[a]	752.6 (75.7)[c]	741.1 (78.5)[a, c]	0.026
Light PA	68.5 (40.3)[a]	70.8 (30.3)[c]	77.1 (30.7)[a, c]	0.004
Moderate PA	31.9 (20.7)	30.2 (19.8)[c]	34.8 (20.6)[c]	0.003
Vigorous PA	6.9 (8.3)	11.1 (16.1)	12.5 (16.1)	0.064
Moderate equivalent PA[*]	45.7 (33.0)[a]	52.3 (44.1)[c]	59.8 (46.1)[a, c]	0.013

[*]*P*-values were derived from one way Analysis of Variance (or the non-parametric Kruskal-Wallis whenever appropriate). The Bonferroni rule was used to correct for the inflation of type I error in the post hoc multiple comparisons. Mean values sharing the same superscript letter differentiate significantly between them ($P<0.05$).

[*]Moderate equivalent PA = Moderate PA + 2* Vigorous PA

Table 3. Associations of 25-OHD_3 concentrations and of vitamin D sufficiency with different vitamin D intakes from foods and supplements and with different levels of physical activity.

	Dependent variable: 25-OHD_3 concentrations (nmol/L)	**Dependent variable: Sufficient 25-OHD_3 concentrations (25-OHD ≥50nmol/L)**		
Independent variables	Mean (SD)	OR	(95% CI)	*P*-value‡
Vitamin D intake from foods				
<2.5 μg/day	60.9 (24.5)[a, b]	1.00		
2.5-4.9 μg/day	62.0 (23.9)	0.95	(0.68 – 1.34)	0.770
5-9.9 μg/day	66.3 (25.7)[b]	1.58	(1.01 – 2.52)	0.049
10-40 μg/day	70.5 (27.8)[a]	2.29	(0.80 – 6.56)	0.123
P-value†	0.035			
Vitamin D intake from supplements				
<2.5 μg/day	60.5 (22.9)[a, b, c]	1.00		
2.5 -4.9 μg/day	68.7 (23.6)[d]	1.80	(0.81– 4.01)	0.150
5-9.9 μg/day	69.1 (28.1) [c, e]	1.87	(1.05 – 3.35)	0.035
10-19.9 μg/day	74.6 (21.7)[b, f]	5.49	(1.87 – 16.1)	0.002
20-80 μg/day	99.9 (35.5) [a, d, e, f]	14.2	(1.86 – 36.2)	0.010
P-value†	<0.001			
Time (min/day) on moderate equivalent PA*				
<30 min/day	59.6 (24.4)[a]	1.00		
30-59.9 min/day	62.2 (23.2)	1.79	(1.24 – 2.59)	0.002
≥60 min/day	66.0 (25.5)[a]	1.78	(1.23 – 2.57)	0.002
P-value†	0.007			

†*P*-values were derived from the Analysis of Covariance after adjusting for age (y), sex, dietary energy intake (kcal per day), VDR rs1544410, VDR rs2228570, BMI, ethnicity, occupation, study site and interaction of study site with season. The Bonferroni rule was used to correct for the inflation of type I error in post hoc multiple comparisons. Mean values sharing the same superscript letter differentiate significantly between them (P<0.05).

OR: Odds Ratios; 95% C.I: 95% Confidence Interval.

‡*P*-values were derived from a multivariate logistic regression analysis. Adjustments were made for age (y), sex, dietary energy intake (kcal per day), VDR rs1544410, VDR rs2228570, BMI, ethnicity, occupation, study site and interaction of study site with season.

Moderate equivalent PA = Moderate PA + 2 Vigorous PA