Clinical Nutrition ESPEN 34 (2019) 23–31

Contents lists available at ScienceDirect

Clinical Nutrition ESPEN

journal homepage: http://www.clinicalnutritionespen.com

Original article

Association of serum vitamin D status with dietary intake and sun exposure in adults

Ioannis Dimakopoulos [a], Emmanuella Magriplis [a], Anastasia-Vasiliki Mitsopoulou [a], Dimitra Karageorgou [a, 1], Ioanna Bakogianni [a], Renata Micha [a, 1], George Michas [a, 2], Michail Chourdakis [b], Triantafyllia Ntouroupi [a], Sophia-Maria Tsaniklidou [a], Konstantina Argyri [a], Demosthenes B. Panagiotakos [c], Antonis Zampelas [a, *], HNNHS Contributors, HNNHS Advisory Committee[3]

[a] Department of Food Science and Human Nutrition, Agricultural University of Athens, Iera odos 75, 118 55, Athens, Greece
[b] Laboratory of Hygiene, Social & Preventive Medicine and Medical Statistics, Department of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, University Campus, 54 124, Thessaloniki, Greece
[c] Department of Nutrition and Dietetics, School of Health Science and Education Harokopio University, Eleftheriou Venizelou 70, 176 76, Athens, Greece

ARTICLE INFO

Article history:
Received 16 February 2019
Accepted 13 September 2019

Keywords:
Vitamin D
25-hydrovitamin D
Diet
Public health
Nutrition survey
Greece

SUMMARY

Background & aims: Serum 25(OH)D deficiency is becoming an epidemic. The aim was to assess vitamin D status of the adult Greek population in relation to intake, sun exposure and other factors, using data from the Hellenic National Nutrition and Health Survey (HNNHS).

Methods: Data from 1084 adult participants (37.8% males) were analyzed. Vitamin D intake was assessed using 24-h recalls. Serum 25(OH)D concentration was evaluated and related to anthropometric measurements and other covariates including supplements used, by sex. Variables significantly associated with 25(OH)D < 20 ng/ml were assessed using simple and multiple logistic regression.

Results: Median vitamin D intake from food was 1.23 mcg/day (0.60, 2.44), with 9.1% consuming supplements. Median serum 25(OH)D was 16.72 ng/ml, with no sex differences (P = 0.923). The odds of having 25(OH)D < 20 ng/ml significantly decreased with being very active (OR 0.55, 95% CI 0.35, 0.98), increasing length of sun exposure [1–3 h/day (OR 0.59, 95% CI 0.44, 0.80), >3 h/day (OR 0.36, 95% CI 0.24, 0.55)], and skin colour [light to medium skin (OR 0.47, 95% CI 0.24, 0.91), fairly dark skin colour (OR 0.34, 95% CI 0.17, 0.67) and dark or very dark skin colour (OR 0.34, 95% CI 0.15, 0.75)], compared to respective baseline levels. The odds significantly increased with obesity (OR 1.95, 95% CI 1.24, 3.08), and spring season of blood sample collection (OR 1.75, 95% CI 1.22, 2.50).

Conclusions: Vitamin D deficiency is highly prevalent in Greek adults. Relevant public health policies are highly recommended, which could include vitamin D fortification. and suggestion for increased but safe sun exposure.

* Corresponding author.
E-mail address: azampelas@aua.gr (A. Zampelas).
[1] Present address: Friedman School of Nutrition Science and Policy, Tufts University, Boston, USA.
[2] Present address: Department of Cardiology, "Elpis" General Hospital of Athens.
[3] Members of HNNHS Contributors and HNNHS Advisory Committee are listed in Appendix A section.

https://doi.org/10.1016/j.clnesp.2019.09.008
2405-4577/

Abbreviations

25(OH)D	25-hydroxyvitamin-D
AMPM	Automated Multiple Pass Method
ARCHES	Arkansas Cardiovascular Health Examination Survey
BMI	Body Mass Index
BRFSS	Behavioural Risk Factor Surveillance System
CAPI	Computer Assisted Personal Interview
EFSA	European Food Safety Authority
HDPA	Hellenic Data Protection Authority
HNNHS	Hellenic National Nutrition & Health Study
IoM	Institute of Medicine
iPAQ	International Physical Activity Questionnaire
ML	milliliter
NDNS	National Diet & Nutrition Survey
NG	nanograms
NHANES	National Health & Nutrition Examination Survey
NIAAA	National Institute on Alcohol Abuse and Alcoholism
OR	Odds ratio
UVBR	ultraviolet-B radiation

1. Introduction

In recent years vitamin D deficiency has become the most common nutritional deficiency in the world with more than 1 billion people having inadequate levels of serum vitamin D concentration [1,2]. In populations living in low latitude ultra violet B radiation (UVBR) is assumed adequate for vitamin D skin synthesis and in industrialized nations specific foods are fortified with vitamin D, however, deficiency still persists [3] across all age groups and subgroups [4–6]. These levels are observed in the national [4,7–10], European [4–6,11] and International level [3,5]. Apart from low serum concentrations, published data have also reported high prevalence of low vitamin D intake even at levels that are far below the recommended reference intake, as per the Institute of Medicine (IoM) [6,12].

Vitamin D has many physiological roles, including being a facilitator for calcium absorption, maintaining bone health and regulation of cell growth. Recently, its role to other health conditions, such as cardiovascular disease and diabetes have also been investigated [13], emphasizing on the importance of a high prevalence of vitamin D deficiency at the population level.

Vitamin D is primarily produced endogenously following skin exposure to UVBR, and its synthesis is influenced by a plethora of factors, such as geographical location, use of sunscreen and type of clothing, time spent outdoors and time of the day, age, skin pigmentation as well as environmental pollution [14].

In parallel, dietary intake is also important and significantly contributes to maintaining serum 25-hydroxyvitamin D (25(OH)D) concentration above 20 ng/ml. Vitamin D is found in a few foods, mainly in the form of cholecalciferol (vitamin D_3) which is highly bioavailable. It can also be found as ergocalciferol (vitamin D_2) from plant sources. Vitamin D supplementation from various forms can also be added to the intake and should therefore be considered when evaluating vitamin D status.

Given the significant prevalence of vitamin D deficiency worldwide, in all age groups, irrespectively of sunlight prevalence, as well as the number of factors that influence vitamin D status, studies addressing vitamin D population status are warranted. In Greece, to our best knowledge, a study on the population prevalence of vitamin D deficiency in adults, using a national representative sample, has not been performed.

The aim of this study is therefore to assess vitamin D status of the adult Greek population, using serum 25(OH)D concentration, in relation to total vitamin D intake (from diet and supplements) and sun exposure.

2. Materials & methods

2.1. Study design & sample

Data from the Hellenic National Nutrition and Health Survey (HNNHS), was used in this study, whereas collection took place from September 2013 to May 2015. Details of HNNHS's methods have been reported elsewhere [15]. Eligible for participation were males and females ≥6 months old that reside in Greece who were (i) not pregnant/breastfeeding (ii) not institutionalized (e.g. military service, hospital, other institution), and selection was performed using a random stratified design based on the 2011 census data.

A total of 3836 adults (≥18 years, 40.8% males) were initially enrolled in the HNNHS, for whom anthropometric and medical history was assessed by trained interviewers and according to the International Classification of Diseases (ICD-10) codes. All participants were also invited to provide blood samples for biochemical – hematological evaluation and anthropometric measurements. Of them, 1197 (26.2% of total population; 28.7% of adult population) agreed; no age distribution differences were found between the total population and those who provided blood sample ($p = 0.677$). A total sample of 1084 adults (23.7% participation; 37.8% males) with available data on serum 25(OH)D concentration were included.

2.2. Data collection

In HNNHS data collection included (i) an initial household Computer Assisted Personal Interview (CAPI) consisting of multiple questionnaires and a 24hr dietary recall, (ii) additional validated questionnaires, (iii) a second 24hr recall via telephone 8–20 days after the first interview, selecting a different non-consecutive day, as specified by HNNHS study-protocol and (iv) a visit to a mobile unit in order to perform the medical, biochemical and anthropometric examinations/measurements. HNNHS fieldwork protocol. Interviews were performed throughout all seasons of the year, to account for season vitamin D status and decrease error in results. Seasons of blood sample collection were defined based on the date that blood was drawn from each volunteer as follows: Summer (June, July and August), Fall (September, October and November), Winter (December January and February) and Spring (March, April and May).

The list of questionnaires used, relevant to this study, included information on demographic, psychosomatic health, vitamin and prescribed drug intake. These can be found in detail in the methodology HNNHS paper [15]. All questionnaires included in this study were adapted from previously validated and used by other large National Health Surveys [16].

Blood samples were collected in the morning, between 8:00 and 10:00 am, upon having fasted for at least 10 h. These were collected using BD vacutainer® safety blood collection set 21G and holder 21G and Greiner vacuette K_3EDTA. After centrifugation with the Nuve® NF400 centrifuge, samples were stored in cryovials in −80 °C. Total 25-OH vitamin D was measured in human plasma using the Roche Diagnostics Vitamin D total assay, a electrochemiluminescence binding assay (Elecsys Vitamin D total assay)

used on a Cobas e 411 immunoassay analyzer [17]. The intra-assay coefficients of variation (CVs) were 1.7–7.8% and interassay CVs were 2.2–10.7%. For quality control, PreciControl Varia (Roche Diagnostics, Mannheim, Germany) was used, in two concentration levels. After comparison of the assay with samples measured with LC-MS/MS method, a Pearson correlation coefficient r = 0.894 was reported.

Dietary intake was assessed using a detailed interviewer-administered 24-h dietary recall using the Automated Multiple-Pass Method (84.5% completed both 24-h recalls) [18]. The FoodEx2 food classification and description system developed by the European Food Safety Authority (EFSA) was used and recommendations for the harmonization of data across European Union countries were followed [19]. Food quantification was performed with the use of validated food atlases as the primary option along with standardized household measures. The Nutrition Data System for Research (NDSR) developed by the University of Minnesota was used for nutrient analysis. From the initial n = 1964 24-h recalls there were 62 recalls with extreme intakes (<600 or >6000 kcal per day) which were excluded from the analysis (67.7% females, 32.3% males).

A drug and supplement questionnaire was developed using validated questionnaires from other studies, mainly from the United States [15]. The questionnaire was applied twice, during the initial interview as well as during the mobile unit visit. From this data, vitamin D supplement intake was categorized as follows: “no supplement use” or “supplement use”. Although initially thought appropriate to have three categories (no supplement use, <10 μg/day and ≥10 μg/day), as those receiving ≥10 μg/day are supposed to intentionally seek vitamin D supplementation [20] rather than obtaining it as part of a multivitamin supplement, the number of observations in the ≥10 μg/day category was too small (males = 2, females = 45). Although the ideal method to assess sun exposure is dosimetry [21] in HNNHS, due to limited funding, sun exposure was assessed using a questionnaire; statistically significant correlations have been reported between the two methods [22]. The questionnaire's aim is to evaluate the amount of sun exposure as well as the parameters that could influence vitamin D skin synthesis to rank individuals accordingly. It included questions relating to sun exposure for each of the four seasons (weekends and weekdays), exposure for the last 30 days, sunscreen use and skin colour. Over 99% of respondents were white Caucasians and hence were classified by pallidity and ability to tan (using non-classic skin type definitions, More specifically, skin colour was categorized according to skin pallor and easiness in tanning as: 1) very light-fair skin, 2) light, 3) medium light, 4) fairly dark, 5) dark-tans easily and 6) very dark-tans very easy. For statistical power reasons categories 2 and 3 as well as 5 and 6 were combined in the analysis. Participants with constitutive pigment (representing <1% of the study population) were added to the darkest skin type category (category 6).

Physical activity was measured using the International Physical Activity Questionnaire (IPAQ) short form for those ≥18 years - <65 years and a modified version for those ≥65 years old. Alcohol was assessed using questionnaire from NHANES, BRFSS, Arkansas Cardiovascular Health Examination Survey (ARCHES) and Recommended Alcohol Questions by the National Institute on Alcohol Abuse and Alcoholism (NIAAA) were used [15]. Volunteers were classified as “alcohol” or “non-alcohol” consumers, based on their intake over the past 30 days.

Anthropometric measurements were performed according to the NHANES study protocol [23] with the use of Seca 213 portable stadiometer, and InBody 270 Biospace analyser was used to measure body weight following the required preparation. Height was measured by asking the volunteer to (i) remove any ornaments, jewelry, etc. from the top of the head, (ii) stand up straight against the backboard with the body weight evenly distributed from feet flat on the platform, (iii) stand with the heels together and toes apart at approximately a 60° angle and (iv) field researcher made sure that the back of the head, shoulder blades, buttocks and heels made contact with the backboard. Weight was measured by asking the volunteer to (i) remove heavy clothing and objects as well as shoes and (ii) to stand in the center of the scale platform facing the recorder with hands at the sides and looking straight ahead. Each of these individuals visited one of the 5 mobile units where medical and anthropometric measurements were completed.

2.3. Statistical analyses

Normality of continuous data was checked using P–P and kernel density plots. Categorical variables were expressed as frequencies and percentages (N %), whereas continuous variables were expressed as mean with standard deviation (SD) if normal and median if skewed and interquartile range (25%, 75%). Differences in categorical variables were derived using chi square test and Kruskal-Wallis test for sex differences between continuous variables, since continuous variables used in this analysis were not normally distributed.

The 25(OH)D cut-off for logistic regression chosen was 20 ng/ml as values < 20 ng/ml reflect inadequate (12 to <20 ng/ml) or deficient (<12 ng/ml) levels for bone and overall health in healthy individuals [24].

Univariate logistic regression analysis for each potential factor associated with 25(OH)D, based on a-priori knowledge was assessed, in total and by sex. Variables that were found significantly associated with the odds of serum 25(OH)D levels <20 ng/ml were included in the multivariate regression, as well as a priori known factors. The Likelihood Ratio test (LR test) was used to check the significance of the models with significance level at 0.05.

All analyses were performed in STATA statistical software (STATA 13.1, Stata Corp LP, Texas, USA) with a significance level set at P < 0.05.

2.4. Ethical statement

The study was approved by the Ethics Committee of the Department of Food Science and Human Nutrition of the Agricultural University of Athens. It was also approved by Hellenic Data Protection Authority (HDPA). All members of the staff signed confidentiality agreements. All volunteers were asked to sign a detailed consent form.

3. Results

3.1. Demographic and general characteristics of the population

The baseline characteristics of this subgroup of the HNNHS population are presented in Table 1 in total as well as by sex. According to the Shapiro–Wilk test for normality continuous variables did not follow a normal distribution. Statistically significant sex differences were found for dietary vitamin D intake, Body Mass Index (BMI) category, activity level, vitamin D supplement use, sun exposure and skin colour (P for all <0.05).

The median (interquartile range) age of the total sample was 36 years (27, 52), 35 (28, 48) for males and 36.5 (26, 54) for females. With regards to BMI category of the total population, 57.8% normal weight or were underweight (4.9% underweight), 28.6% overweight and 13.6% obese. Furthermore, males' prevalence of overweight (38% vs. 22.8%) and obesity (16.1% vs. 12.0%) were significantly higher compared to that of female participants (P < 0.001) (Table 1).

3.2. Serum 25(OH)D concentration, dietary intake and skin synthesis

Median (interquartile range) serum 25(OH)D concentration for the total population was 16.7 ng/ml (9.8, 23.6), 16.67 ng/ml (11.1, 23.46) for males and 16.74 ng/ml (9.72, 23.64) for females. According to the IoM cut-off levels for vitamin D deficiency, 28.8% of the total sample was found deficient (<12 ng/ml), 36.0% insufficient (12–19.9 ng/ml), 35.1% sufficient (20–49.9 ng/ml) and only 0.1% being in the high (≥50 ng/ml) category. There were no significant differences in serum 25(OH)D concentration between males and females. In addition, there were no significant differences in serum 25(OH)D concentration in different age groups (≥18 years, data not shown). Significant differences were found in serum 25(OH)D concentration <20 ng/ml per month of blood sample collection for the total sample ($P < 0.01$), for males ($P = 0.003$) and females ($P = 0.007$) (Fig. 1) and per season (for the total sample: $P = 0.03$; for males: $P = 0.002$; and for females: $P = 0.03$). In the total sample, higher prevalence of 25(OH)D levels <20 ng/ml was found during March, (end of Winter, beginning of Spring season), and overall during Spring season, when seasons were considered as a whole.

Median (interquartile range) vitamin D intake from food for the total population was 1.23 μg/day (0.60, 2.44), 1.45 μg/day (0.66, 3.07) for males and 1.16 μg/day (0.56, 2.05) for females. Regarding vitamin D supplement use, 9.1% reported taking a supplement containing vitamin D (Table 1). Among those, 4.8% reported taking

Table 1
Descriptive characteristics of study participants (Median values and interquartile ranges (25%, 75%), numbers and percentages).

	Total		Males		Females		P value[a]
	n = 1084		n = 410		n = 674		
	median	Range	median	Range	median	Range	
Age **(years)**	36	27, 52	35	28, 48	36.5	26, 54	0.972
Dietary vitamin D intake **(mcg)**[b]	1.23	0.60, 2.44	1.45	0.66, 3.07	1.16	0.56, 2.05	<0.001***
Serum 25(OH)D levels **(ng/ml)**	16.72	9.8, 23.6	16.67	11.1, 23.46	16.74	9.72, 23.6	0.923
Serum PTH levels **(pg/ml)**	38.04	28.7, 49.8	38.06	28.9, 51.3	38.04	28.0, 49.7	0.987
	n	%	n	%	n	%	
BMI category							<0.001***
Normal weight[c]	627	57.8	188	45.7	439	65.1	
Overweight	310	28.6	156	38.0	154	22.8	
Obese	147	13.6	66	16.1	81	12.0	
Education level							0.068
Primary school	50	4.6	12	2.9	38	5.6	
Secondary school	374	34.5	157	38.3	217	32.2	
Professional/Private higher education	98	9.0	31	7.6	67	9.9	
University degree	402	37.1	153	37.3	249	36.9	
MSc or PhD	160	14.8	57	13.9	103	15.3	
Activity level							0.048*
Sedentary	120	11.1	36	8.8	84	12.6	
Little active	197	18.3	80	19.5	117	17.5	
Moderately active	436	40.4	155	37.8	281	42.0	
Very active	326	30.2	139	33.9	187	27.9	
Smoking status (**last 30 days**)							0.471
Not smoking	700	64.7	260	63.4	440	65.6	
Smoking	381	35.2	150	36.6	231	34.4	
25(OH)D status[d]							0.886
<12 ng/ml (Deficient)	312	28.8	118	28.8	194	28.8	
12–19.9 ng/ml (Insufficient)	390	36.0	149	36.3	241	35.8	
20–49.9 ng/ml (Sufficient)	381	35.1	143	34.9	238	35.3	
≥50 ng/ml (High)	1	0.1	0	0.0	1	0.1	
Vitamin D supplement use							<0.001***
No supplement use	985	90.9	386	94.1	599	88.9	
Supplement use	99	9.1	24	5.9	75	11.1	
Sun exposure (**last 30 days**)							0.020*
0–1 h/d	520	49.2	179	45.2	341	51.7	
1–3 h/d	388	36.7	146	36.9	242	36.7	
>3 h/d	148	13.0	71	17.9	77	11.7	
Skin colour							<0.001***
Very light	72	6.8	11	2.8	61	9.2	
Light colour/Medium light	638	60.2	224	56.4	414	62.5	
Fairly dark	271	25.6	124	31.2	147	22.2	
Dark or very dark	78	7.4	38	9.6	40	6.0	
Season of blood sample collection							0.442
Summer	309	28.6	116	28.4	193	28.7	
Fall	125	11.6	51	12.5	74	11.0	
Winter	318	29.4	128	31.3	190	28.3	
Spring	329	30.4	114	27.9	215	32.0	

mcg/d, micrograms/day. 25(OH)D, 25-hydroxyvitamin D. ng/ml, nanograms per milliliter. PTH, Parathyroid hormone. pg/ml, picogram per milliliter. BMI, Body Mass Index. kg/m^2, kilograms per meters squared, h/d, hours/day.
*P < 0.5, **P < 0.01, ***P < 0.001.

[a] Categorical variables depicted as frequencies and percentages, continuous variables depicted as medians and 95% confidence intervals (because they are not normally distributed). Normality was tested using the Shapiro–Wilk test. P values indicate sex differences. Kruskal-Wallis test was used in the case of continuous variables and Peason's chi square in the case of categorical variables.

[b] Excluding 24-h recalls with extreme energy intake (<600 or >6000 kcal/day.

[c] Due to the small sample size of underweight individuals (n = 53) they were merged with normal weight individuals.

[d] Based on Institute's of Medicine cut-off points. Values are rounded to the first decimal (except for p values).

<10 μg/day vitamin D, mostly from multivitamins, and 4.3% reported taking ≥10 μg/day. There was statistically significant difference in vitamin D supplement intake between sexes (P < 0.001) as 94.1% of males and 88.9% of females reported not taking a supplement containing vitamin D. Additionally, a low 0.5% of males reported taking a supplement containing ≥10 μg/day compared to the 6.7% of females.

Most of the total sample reported sun exposure for the last 30 days (before the blood sample collection) of 0–1 h/day (49.2%) and 1–3 h/d (36.7%) compared to 13.0% that reported >3 h/d. On the other hand, 45.2% and 36.9% of males reported 0–1 h/d and 1–3 h/d of sun exposure respectively compared to 51.7% and 36.7% of females. In addition, 17.9% of males and 11.7% of females reported sun exposure >3 h/d. There was statistically significant difference in sun exposure between the two sexes (P = 0.020).

There were no statistically significant differences in season of blood sample collection between the two sexes (P = 0.442). For 28.6% of the total sample blood collection was performed during summer, 11.6% during fall, 29.4% during winter and 30.4% during spring.

In terms of skin colour 60.2% of the total sample had light colour/medium light as (56.4% of total males and 62.5% of total females), 25.6% had dark skin (31.2% of males and 22.2% of females), 7.4% had dark or very dark skin colour, and 6.8% very light skin colour (Table 1). There was statistically significant difference in skin colour between males and females (P < 0.001).

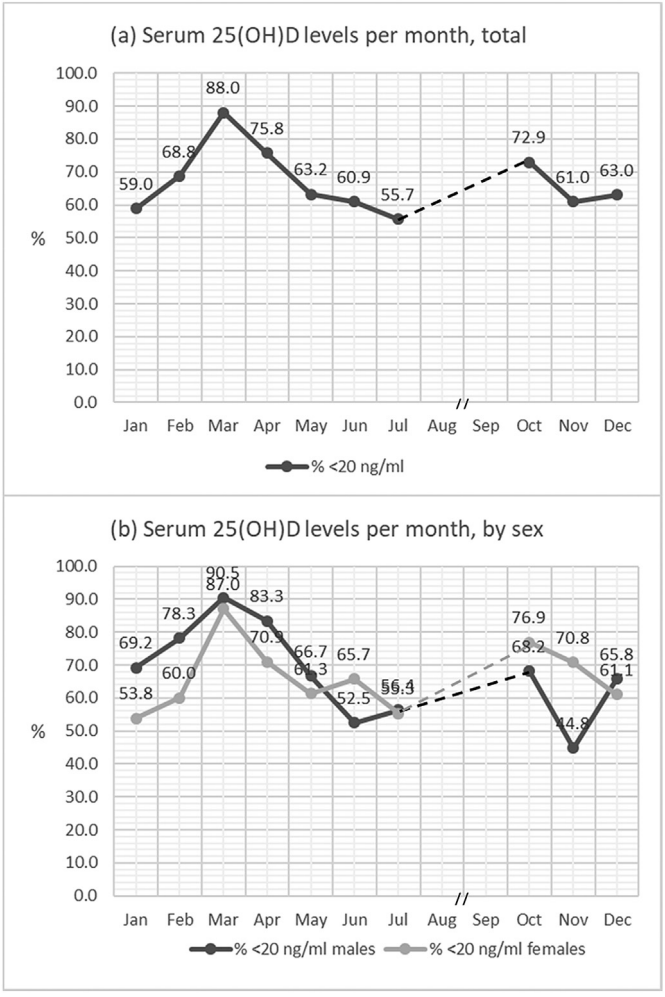

Fig. 1. Prevalence of serum 25(OH)D levels <20 ng/ml and per month[a] of blood sample collection (a) total (P < 0.01[b]), (b) for males (P = 0.003[b]) and females (P = 0.007 [b]). [a,b]There were no blood samples collected during August and September, depicted by dashed lines (–). [b]P values indicate differences by month.

3.3. Odds of having 25(OH)D concentration <20 ng/ml

As can be seen in Table 2 the odds of having 25(OH)D levels <20 ng/ml were lower for the total sample with high activity, with supplement use, increased hours of sun exposure and darker skin colour. On the other hand, spring season of blood sample collection was associated with increased odds of having 25(OH)D concentration <20 ng/ml as was being obese. In more detail, very active individuals had 41% reduced odds (OR 0.59, 95% CI 0.35, 0.98, P = 0.044). Individuals using vitamin D supplements were 46% less likely of having insufficient serum vitamin D concentration (OR 0.54, 95% CI 0.34, 0.86, P = 0.010). One to 3 h of sun exposure per day reduces the odds of 25(OH)D < 20 ng/ml by 41% (OR 0.59, 95% CI 0.44, 0.80, P = 0.001), >3 h/day by 64% (OR 0.37, 95% CI 0.24, 0.55, P < 0.001) as compared with 0–1 h/day of sun exposure dy. Compared to very light skin coloured individuals, those with light to medium light colour had 53% lower odds of 25(OH)D concentration <20 ng/ml (OR 0.47, 95% CI 0.24, 0.91, P = 0.025), those with fairly dark 66% reduced odds (OR 0.34, 95% CI 0.17, 0.67, P = 0.002) as those with dark or very dark skin (OR 0.34, 95% CI 0.15, 0.75, P = 0.007). Furthermore, blood samples collected during spring compared to those collected during summer were related with 87.5% increased odds of 25(OH)D concentration <20 ng/ml (OR 1.73, 95% CI 1.22, 2.48, P = 0.002). No significant association was found between dietary vitamin D intake and serum levels. This model was adjusted for age. The likelihood ratio test for the whole model was 88.85 with a p value < 0.001.

For males (Table 2) smoking status was associated with increased odds of 25(OH)D concentration <20 ng/ml (OR 1.74, 95% CI 1.04, 2.90, P = 0.034) as was spring season of blood sample collection (OR 2.91, 95% CI 1.52, 5.53, P = 0.001). On the other hand, compared to 0–1 h per day of sun exposure individuals with 1–3 h per day had 44% reduced odds (OR 0.56, 95% CI 0.32, 0.95, P = 0.033), >3 h per day 80% reduced odds (OR 0.21, 95% CI 0.11, 0.42, P < 0.001). The model was adjusted for age, BMI category, activity level, vitamin D supplement use and skin colour. The likelihood ratio test for the model was 68.09 with P < 0.001.

For females (Table 2) being obese was associated with increased odds of 25(OH)D concentration <20 ng/ml (OR 2.22, 95% CI 1.19, 4.16, P = 0.012). In contrary, supplement use compared to no supplement use was associated with 51% reduced odds (OR 0.49, 95% CI 0.29, 0.85, P = 0.012) as was sun exposure 1–3 h/day (OR 0.61, 95% CI 0.42, 0.89, P = 0.012), >3 h/day (OR 0.50, 95% CI 0.29, 0.89, P = 0.019) compared to 0–1 h/day of sun exposure. Furthermore, light to medium skin colour (OR 0.44, 95% CI 0.21, 0.89, P = 0.023), fairly dark skin colour (OR 0.33, 95% CI 0.15, 0.70, P = 0.004) and dark to very dark skin colour (OR 0.28, 95% CI 0.11, 0.75, P = 0.011) were associated with reduced odds of 25(OH)D concentration <20 ng/ml compared to individuals with very light skin colour. The model was adjusted for age, season of blood sample collection and PTH levels. The likelihood ratio test for the whole model was 55.23 with P < 0.001.

Several demographic variables (marital status, health insurance and income) as well as other variables, mentioned in the literature, such as alcohol intake, serum cholesterol, serum magnesium, serum creatinine, serum lead (Pb) levels, dietary factors (e.g. Ca, Vitamin K), skeletal muscle mass and sunscreen use were not significantly associated with 25(OH)D levels (data not shown). Fiber (OR 1.00, 95% CI 1.00, 1.01, P = 0.046) and magnesium intake (OR 1.00, 95% CI 1.00, 1.00, P = 0.035) were significantly associated

Table 2
Regression analyses examining the associations of 25(OH)D < 20 ng/ml (deficiency & insufficiency based on IoM cut-off points) in the total sample and by sex.

Variables	Total 25(OH)D levels <20 ng/ml				Males 25(OH)D levels <20 ng/ml				Females 25(OH)D levels <20 ng/ml			
	Crude		Model[a]		Crude		Model[b]				Model[c]	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Age (**years**)	0.99	0.98, 1.00	0.98	0.97, 0.99*	0.97	0.96, 0.99**	0.96	0.95, 0.98***	1.00	0.99, 1.01	0.99	0.98, 1.00
BMI category												
Normal weight	Ref.	–	Ref.	–	Ref.	–	Ref.	–	Ref.	–	Ref.	–
Overweight	1.07	0.80, 1.42	1.21	0.88, 1.66	0.96	0.61, 1.49	1.21	0.71, 2.04	1.14	0.78, 1.68	1.22	0.78, 1.89
Obese	1.84	1.22, 2.78**	1.95	1.24, 3.08*	1.36	0.74, 2.52	1.66	0.79, 3.46	2.33	1.32, 4.12**	2.22	1.19, 4.16*
Activity level												
Sedentary	Ref.	–	Ref.	–	Ref.	–	Ref.	–	Ref.	–	Ref.	–
Little active	0.81	0.48, 1.35	0.85	0.49, 1.47	1.01	0.42, 2.44	1.03	0.38, 2.76	0.72	0.38, 1.35	0.74	0.38, 1.47
Moderately active	0.60	0.38, 0.95*	0.66	0.40, 1.08	0.74	0.33, 1.64	0.74	0.30, 1.85	0.55	0.31, 0.95*	0.59	0.32, 1.09
Very active	0.51	0.32, 0.82**	0.59	0.35, 0.98*	0.53	0.24, 5.39	0.55	0.21, 1.39	0.52	0.29, 0.92*	0.57	0.30, 1.08
Education level												
Primary school	Ref.	–	–	–	Ref.	–	–	–	Ref.	–	–	–
Secondary school	0.95	0.51, 1.78	–	–	2.99	0.90, 9.90	–	–	0.59	0.27, 1.29	–	–
Professional/Private higher education	0.84	0.41, 1.73	–	–	1.93	0.50, 7.48	–	–	0.63	0.26, 1.53	–	–
University degree	0.98	0.52, 1.83	–	–	2.88	0.87, 9.54	–	–	0.65	0.30, 1.40	–	–
MSc or PhD	0.88	0.45, 1.71	–	–	2.06	0.58, 7.32	–	–	0.66	0.29, 1.52	–	–
Vitamin D intake from food (**μg/day**)	0.97	0.93, 1.00	–	–	0.96	0.92, 1.01	–	–	0.97	0.92, 1.02	–	–
Vitamin D supplement use												
No supplement use	Ref.	–	Ref.	–	Ref.	–	Ref.	–	Ref.	–	Ref.	–
Supplement use	0.54	0.35, 0.82**	0.54	0.34, 0.86*	0.61	0.26, 1.40	0.56	0.22, 1.44	0.52	0.32, 0.84**	0.49	0.29, 0.85*
Sun exposure **(last 30 days)**												
0–1 h/d	Ref.	–	Ref.	–	Ref.	–	Ref.	–	Ref.	–	Ref.	–
1–3 h/d	0.58	0.44, 0.77***	0.59	0.44, 0.80**	0.52	0.32, 0.84**	0.56	0.32, 0.95*	0.60	0.43, 0.86**	0.61	0.42, 0.89*
>3 h/d	0.37	0.26, 0.55***	0.36	0.24, 0.55***	0.25	0.14, 0.46***	0.21	0.11, 0.42***	0.49	0.30, 0.82**	0.50	0.29, 0.89*
Skin colour												
Very light	Ref.	–	Ref.	–	Ref.	–	Ref.	–	Ref.	–	Ref.	–
Light colour/Medium light	0.48	0.26, 0.88*	0.47	0.24, 0.91*	0.84	0.21, 3.27	0.55	0.10, 3.09	0.41	0.21, 0.82*	0.44	0.21, 0.89*
Fairly dark	0.33	0.17, 0.62**	0.34	0.17, 0.67**	0.53	0.13, 2.12	0.32	0.05, 1.81	0.29	0.14, 0.60**	0.33	0.15, 0.70**
Dark or very dark	0.36	0.17, 7.66**	0.34	0.15, 0.75**	0.57	0.13, 2.52	0.33	0.05, 2.07	0.33	0.13, 0.81*	0.28	0.11, 0.75*
PTH (**pg/ml**)	1.00	1.00, 1.01*	1.00	0.99, 1.01	1.00	0.99, 1.01	1.00	0.99, 1.01	1.01	1.00, 1.01*	1.00	0.99, 1.01
Season of blood sample collection												
Summer	Ref.	–	Ref.	–	Ref.	–	Ref.	–	Ref.	–	Ref.	–
Fall	1.34	0.87, 2.07	1.18	0.74, 1.88	1.02	0.52, 1.98	0.68	0.32, 1.44	1.71	0.95, 3.09	1.69	0.90, 3.18
Winter	1.19	0.86, 1.65	1.04	0.73, 1.49	1.85	1.09, 3.12*	1.63	0.89, 3.00	0.91	0.60, 1.37	0.83	0.53, 1.32
Spring	1.84	1.32, 2.57***	1.75	1.22, 2.50**	2.71	1.53, 4.77**	2.91	1.52, 5.53**	1.49	0.99, 2.62	1.45	0.93, 2.27
Smoking status												
Not smoking	Ref.	–	–	–	Ref.	–	Ref.	–	Ref.	–	–	–
Smoking	1.19	0.91, 1.55	–	–	1.63	1.05, 2.52*	1.74	1.04, 2.90*	0.98	0.70, 1.37	–	–
	LR test for the model: 88.85, p < 0.001***				LR test for the model: 68.09, p < 0.001***				LR test for the model: 55.23, p < 0.001***			

25(OH)D. 25-hydroxyvitamin D. ng/ml, nanograms per milliliter. OR, Odds Ratio. CI, Confidence Interval. BMI, Body Mass Index. h/d, hours/day. μg/day, micrograms per day. PTH, Parathyroid hormone. Pg/ml, picogram per milliliter. LR test, Likelihood Ratio test. *P < 0.05, **P < 0.01, ***P < 0.001.

[a] Model 1: was adjusted for covariates having p values < 0.05 in crude analyses for the total sample which were age, BMI, activity level, vitamin D supplement use, sun exposure, skin colour, PTH and season of blood sample collection.

[b] Model 2: model 1 plus adjustment for smoking status which had a p value < 0.05 only in crude analyses for males.

[c] Model 3: model 1 plus adjustment for smoking status which had a p value < 0.05 only in crude analyses for females.

with the odds of having serum 25(OH)D < 20 ng/ml in males and in crude analyses but were excluded as there was no clinical significance (OR = 1).

4. Discussion

To our knowledge this is the first study that aims to identify factors associated with 25(OH)D concentration <20 ng/ml in Greek adults. HNNHS′ measurements showed that despite the ample sun in Greece, 36% of adults had insufficient serum 25(OH)D concentration (12–19.9 ng/ml) and 28.8% had deficient concentration (<12 ng/ml). In total, 64.8% had either deficient or insufficient concentration, i.e. concentration <20 ng/ml. Many factors that could help improve vitamin D status are modifiable, including weight status regulation, increase in physical activity, skin exposure to sunlight and supplement use. Despite the study's null findings between serum 25(OH)D concentration and vitamin D dietary intake, the latter was extremely low from almost the whole population, therefore efforts to increase intake are also required.

Vitamin D deficiency and insufficiency have been of concern recently with a worldwide review reporting that 37.3% of studies found mean values of 25(OH)D concentration <20 ng/ml with the subtotal for males being 22.3 ng/ml and for females 21.3 ng/ml [5]. In accordance to our study, high prevalence of deficiency has been reported in previous studies [5–9,11,25] with a review reporting 35–75% of adults from Mediterranean countries having serum 25(OH)D concentration <20 ng/ml (54% of Greek adults) [4]. These studies have taken place in more than 40 countries worldwide many of which in Europe [5,6]. Included studies were cross-sectional or cohort where vitamin D status was measured, whereas studies where status was estimated, rather than measured, were excluded. Most studies included a random sample.

In addition, other reports from Greece [7,8], Europe [25] and non-European counties [9,26–28] showed that the highest prevalence of serum 25(OH)D concentration <20 ng/ml was during March, in accordance to our study. Vitamin D deficiency was lower over summer months (June and July) as have been reported by others [25] and started to significantly decrease in May (Spring) during which sun exposure actually commences in Greece. In our study no blood samples were collected during August and September, therefore no comparisons can be made with vitamin D status in October. It can be inferred that vitamin D levels in serum start decreasing over September, since adults return to their usual work schedule, hence have lower sun exposure. Also, it must be underlined that direct comparisons cannot be made, since as a cross-sectional study, different serum samples were collected in each month. Similar seasonal trends have been also observed elsewhere [14].

Vitamin D food intake in our sample was low, a finding that seems reasonable as few foods contain vitamin D and there is no fortification law in Greece; a strategy that is reported to have a positive effect in other regions [29,30]. Our results are similar or lower compared to levels reported elsewhere [6,31,32]. In the United States, were milk and cereals are fortified, intakes are higher (3.9–7.0 μg/day) [33]. Higher intakes are also observed in European countries except Spain where there is also no fortification law and intake is 1.6–1.7 μg/day [6]. Although, dietary assessment methodologies vary between European countries, a notable discrepancy between intakes and recommendations exists [6].

Although, 0–1 or 1–3 h/day might seem adequate and Greece has adequate UVBR availability all year round [34] we have to consider that data is self-reported, that the majority of the skin might hypothetically be covered by clothing, especially over Winter months, hence little skin area might be exposed to the sun. Also, participants might have also included time exposed in the sun when behind a glass (e.g. inside a building or a car) that acts as a UVBR barrier [35]. Furthermore, for cultural or health reasons people might be avoiding sun exposure. Similar trends have been reported in sunny countries in the past [1,36,37].

Only approximately 6% of males and 11% of females reported consuming supplements, and <1% of males & 6.7% of females receiving ≥10 μg/day (data not shown). The significant sex difference could hypothetically be explained by the higher prevalence of osteoporosis in women compared to men as reported in previous findings (8.3% vs. 0.8% in adults) [15].

With regards to 25(OH)D low blood concentrations, our results are comparable with studies from Greece [7,8], Europe [11] and the rest of the world [3,38]. There were no significant differences in serum 25(OH)D concentration observed between males and females as in other reports [39].

Almost all subjects (>99%) of the population in this HNNHS study, were of Caucasian origin in contrast with other countries, who report a higher proportion is of non-Caucasian origin who differ physiologically and culturally [14], therefore results are not directly comparable. In this study only 7% of the sample had dark or very dark skin (Caucasian in nature) compared to 60% that has light or medium light and therefore associations could be influenced by sample size. In addition, as the vast majority of our volunteers is of Caucasian origin, we essentially only compared white skinned people who tan more or less easily. We could hypothesize that those with very fair skin tend to burn easily therefore also tend to protect their skin with clothing or use of more sunscreen, as shown by our study, than those who are of darker colour (higher skin type category). It has been shown that skin area exposure at the right time of day rather than long sun exposure, can result to optimal serum vitamin D levels [40]. Public health awareness programs can, therefore, be of great importance, and can help reduce deficiency prevalence found in Greece.

Other studies reported a positive effect of physical activity on 25(OH)D concentration adequacy which is hypothesized to be due to increased sun exposure [14] however there seems to be an effect irrespective of sun exposure as the same has been observed for indoor activity [41]. The main factors that influenced the odds of having 25(OH)D levels <20 ng/ml were sun exposure and season of blood collection. With increasing sun exposure, the odds of vitamin D deficiency were lower. Spring season of blood sample collection was associated with higher odds of vitamin D (25(OH)D) deficiency/insufficiency, probably because of the low levels of sun exposure during preceding months. As previously suggested [42], this seasonal difference in 25(OH)D concentrations could possibly be improved with the consumption of fortified products. Supplement use might not have been significantly associated with lower odds of deficiency due to the small sample size of males that were receiving supplements with content ≥10 μg/day (n = 2). This could hypothetically be explained by the lower prevalence of osteoporosis in males and/or lower degree of actively checking for osteoporosis in comparison to females, and hence reduced supplemental intake. Smoking as per other reports was associated with higher odds of serum 25(OH)D concentration <20 ng/ml and it is hypothesized that smoking impairs conversion of 25(OH)D to 1,25(OH_2)D [43].

In females, obesity was associated with an increased likelihood of low vitamin D status. The lipophilic nature of adipose tissue is hypothesized to act as sequester of vitamin D, rather than storage, and studies have reported increases in 25(OH)D concentration with weight reduction in obese individuals [14]. The exact mechanisms remain unclear [24]. Supplement use and sun exposure were associated with lower odds as was darker skin, as would be expected. In accordance with other studies, our biochemical data also show a negative correlation between serum PTH concentration and 25(OH)D concentration [44–46], therefore was accounted for in the model of final associations to decrease bias and confounding results, resulting from hormonal regulation. Supplement use was higher in females than in males, and time of blood sample collection was not significantly correlated with 25(OH)D levels in the blood. Seasonal difference found in supplement use especially by women taking ≥10 mcg/day vitamin D over Spring and Winter compared to Summer and Fall (13.6%) may be due to the perception that during Summer sunlight exposure is adequate for vitamin D synthesis.

It is finally noteworthy that in both sexes dietary intake was not significantly associated with the odds of having 25(OH)D blood levels <20 ng/ml probably because 94.6% had very low intakes. This is probably the reason for the disagreement between the results of our study and another recent one which showed that with higher intakes of vitamin D from food are associated with higher 25(OH)D levels [47].

Due to the nature of the study, some limitations should be considered. Firstly, no causal relationships can be drawn, since it is a cross-sectional study. Most participants lived in urban areas and therefore we were unable to explore differences between urban vs. semi-urban regions (0.37% from non-urban regions). However, in Greece, the vast majority of the population reside in metropolitan areas. In addition, we did not explore ethnic differences as <1% of the sample were of non-Caucasian race.

In conclusion, serum 25(OH)D deficiency is high in Greek adults of both sexes. Given vitamin D's possible link to several diseases, relevant actions and policies to correct deficiency and reduce prevalence, need to be taken. Food fortification and vitamin D supplements are two options towards that goal. Longer but safe sun

exposure could offer an additional effective and low-cost strategy for 25(OH)D deficiency prevention.

Statement of authorship

AZ conceptualized, designed and was the Principal Investigator of the HNNHS study. AZ and RM coordinated the design of the data collection instruments, coordinated and supervised data collection. GM supervised medical data collection. ID, DK, AVM and IB were involved in every step of the study and made substantial contributions to the design and methodology of data collection as well as the acquisition of data and training of field workers. TN coordinated mobile unit data collection. SMT and KA contributed to the mobile unit data collection and analysis. DBP coordinated sample collection methodology. ID conceptualized this study, carried out all statistical analyses and drafted the manuscript. EM supervised the preparation of the database and carried out the statistical analyses and together with MC revised the manuscript. GD, CG, EF, EMT, ET, TES, AV, ES, MC, AK, GK, SZ and AP contributed to parts of methodology. All the authors approved the final manuscript as submitted.

Contributors: EF, ET, TS, AV, ES, AT, GK, SZ, AP contributed to the writing of the protocols and the data collection on the field. All contributors approved the final manuscript as submitted.

Advisory Committee: GC, GD, GD, IM and ER acted as external advisory committee. All the Advisory Committee members approved the final manuscript as submitted.

Funding sources

The study was co-funded by Greece and the European Union (European Social Fund) under the Operational Program "Human Resources Development 2007–2013" and had no role in the design, analysis or writing of this article.

Declaration of Competing Interest

RM reports grants from NIH/NHLBI, grants from Unilever R&D, grants from Bill and Melinda Gates Foundation, personal fees from World Bank and personal fees from Bunge.

Appendix A

HNNHS Contributors

Evangelia Fappa[1], Eleni-Maria Theodoraki[1], Eirini Trichia[1], Theodora-Eirini Sialvera[1], Aggeliki Varytimiadi[1], Eleni Spyreli[1], Antonis Koutelidakis[2], George Karlis[1], Stauroula Zacharia[1], Anna Papageorgiou[1].

HNNHS Advisory Committee

George P. Chrousos[3], Georgios Dedoussis[4], George Dimitriadis[5], Yannis Manios[4], Eleftheria Roma[3].

[1] Department of Food Science and Human Nutrition, Agricultural University of Athens, Iera Odos 75, 118 55 Athens, Greece.

[2] Department of Food Science and Nutrition, University of Aegean, Mytilini, Greece.

[3] First Department of Pediatrics, Medical School, National and Kapodistrian University of Athens, Mikras Asias 75, 115 27 Athens, Greece.

[4] Department of Nutrition and Dietetics, School of Health Science and Education Harokopio University, Athens, Eleftheriou Venizelou 70, 176 76 Athens, Greece.

[5] 2nd Department of Internal Medicine, Research Institute and Diabetes Center, National and Kapodistrian University of Athens, "Attikon" University Hospital, Haidari, Greece.

References

[1] van Schoor N, Lips P. Global overview of vitamin D status. Endrocrinol Metab Clin North Am 2017;46(4):845–70. https://doi.org/10.1016/j.ecl.2017.07.002.
[2] Holick MF. The vitamin D deficiency pandemic: approaches for diagnosis, treatment and prevention. Rev Endocr Metab Disord 2017;18(2):153–65. https://doi.org/10.1007/s11154-017-9424-1.
[3] Palacios C, Gonzalez L. Is vitamin D deficiency a major global public health problem? J Steroid Biochem Mol Biol 2014;144(Pt A):138–45. https://doi.org/10.1016/j.jsbmb.2013.11.003.
[4] Manios Y, Moschonis G, Lambrinou CP, Tsoutsoulopoulou K, Binou P, Karachaliou A, et al. A systematic review of vitamin D status in southern European countries. Eur J Nutr 2017;57:2001–36. https://doi.org/10.1007/s00394-017-1564-2.
[5] Hilger J, Friedel A, Herr R, Rausch T, Roos F, Wahl DA, et al. A systematic review of vitamin D status in populations worldwide. Br J Nutr 2014;111:23–45. https://doi.org/10.1017/S0007114513001840.
[6] Spiro A, Buttriss JL. Vitamin D: an overview of vitamin D status and intake in Europe. Nutr Bull 2014;39:322–50. https://doi.org/10.1111/nbu.12108.
[7] Katrinaki M, Kampa M, Margioris A, Castanas E, Malliaraki N. Vitamin D levels in a large Mediterranean cohort: reconsidering normal cut-off values. Hormones 2016;15:205–23. https://doi.org/10.14310/horm.2002.1674.
[8] 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;29:409–13.
[9] Grigoriou EV, Trovas G, Papaioannou N, Makras P, Kokkoris P, Dontas I, et al. Serum 25-hydroxyvitamin D status, quantitative ultrasound parameters, and their determinants in Greek population. Arch Osteoporos 2018;13:111. https://doi.org/10.1007/s11657-018-0526-5.
[10] 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;25:198–203. https://doi.org/10.1007/s00774-006-0746-4.
[11] 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;103:1033–44. https://doi.org/10.3945/ajcn.115.120873.
[12] Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab 2011;96:53–8. https://doi.org/10.1210/jc.2010-2704.
[13] 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. https://doi.org/10.1136/bmj.g2035.
[14] Scientific Advisory Committee on Nutrition. Vitamin D and health report. https://www.gov.uk/government/publications/sacn-vitamin-d-and-health-report; 2018.
[15] Magriplis E, Dimakopoulos I, Karageorgou D, Mitsopoulou AV, Bakogianni I, Micha R, et al. Aims, design and preliminary findings of the hellenic national nutrition and health Survey (HNNHS). BMC Med Res Methodol 2019;19:37. https://doi.org/10.1186/s12874-018-0655-y.
[16] Center for Disease Control. Behavioral risk factor surveillance system survey questionnaire. http://www.cdc.gov/brfss/index.htm; 2013.
[17] Cobas Elecsys® vitamin D total. http://www.cobas.com/home/product/clinical-and-immunochemistry-testing/elecsys-vitamin-d-total-assay.html; 2013.
[18] US Department of Agriculture Automated. AMPM - multiple-pass method. https://www.ars.usda.gov/northeast-area/beltsville-md-bhnrc/beltsville-human-nutrition-research-center/food-surveys-research-group/docs/ampm-usda-automated-multiple-pass-method/; 2013.
[19] European Food Safety Authority. EU menu declaration. http://wwwefsaeuropaeu/en/datexfoodcdb/datexeumenuhtm; 2014.
[20] Rooney MR, Harnack L, Michos ED, Ogilvie RP, Sempos CT, Lutsey PL. Trends in use of high-dose vitamin D supplements exceeding 1000 or 4000 international units daily, 1999–2014. JAMA 2017;20(317):2448–50. https://doi.org/10.1001/jama.2017.4392.
[21] Humayun Q, Iqbal R, Azam I, Khan AH, Siddiqui AR. Baig-Ansari N. Development and validation of sunlight exposure measurement questionnaire (SEM-Q) for use in adult population residing in Pakistan. BMC Public Health 2012;12:421. https://doi.org/10.1186/1471-2458-12-421.
[22] McCarty CA. Sunlight exposure assessment: can we accurately assess vitamin D exposure from sunlight questionnaires? Am J Clin Nutr 2008;87. 1097S-101S.
[23] Center for Disease Control. National health and nutrition examination survey. http://www.cdc.gov/nchs/nhanes.htm; 2016.
[24] Institute of Medicine. Dietary reference intakes for calcium and vitamin D. Washington, DC: The National Academy Press; 2018. https://www.ncbi.nlm.nih.gov/books/NBK56070/pdf/Bookshelf_NBK56070.pdf.
[25] Manios Y, Moschonis G, Lambrinou CP, Mavrogianni C, Tsirigoti L, Hoeller U, et al. Associations of vitamin D status with dietary intakes and physical activity levels among adults from seven European countries: the Food4Me

study. Eur J Nutr 2017;57:1357–68. https://doi.org/10.1007/s00394-017-1415-1.
[26] Kasahara AK, Singh RJ, Noymer A. Vitamin D (25OHD) serum seasonality in the United States. PLoS One 2013;8:e65785. https://doi.org/10.1371/journal.pone.0065785.
[27] Bolland MJ, Chiu WW, Davidson JS, Grey A, Bacon C, Gamble GD, et al. The effects of seasonal variation of 25-hydroxyvitamin D on diagnosis of vitamin D insufficiency. N Z Med J 2008;121:63–74.
[28] Levis S, Gomez A, Jimenez C, Veras L, Ma F, Lai S, et al. Vitamin d deficiency and seasonal variation in an adult South Florida population. J Clin Endocrinol Metab 2005;90:1557–62. https://doi.org/10.1210/jc.2004-0746.
[29] Raulio S, Erlund I, Mannisto S, Sarlio-Lahteenkorva S, Sundvall J, Tapanainen H, et al. Successful nutrition policy: improvement of vitamin D intake and status in Finnish adults over the last decade. Eur J Public Health 2017;27:268–73. https://doi.org/10.1093/eurpub/ckw154.
[30] Allen RE, Dangour AD, Tedstone AE, Chalabi Z. Does fortification of staple foods improve vitamin D intakes and status of groups at risk of deficiency? A United Kingdom modeling study. Am J Clin Nutr 2015;102:338–44. https://doi.org/10.3945/ajcn.115.107409.
[31] Bailey RL, Dodd KW, Goldman JA, Gahche JJ, Dwyer JT, Moshfegh AJ, et al. Estimation of total usual calcium and vitamin D intakes in the United States. J Nutr 2010;140:817–22. https://doi.org/10.3945/jn.109.118539.
[32] Freisling H, Fahey MT, Moskal A, Ocke MC, Ferrari P, Jenab M, et al. Region-specific nutrient intake patterns exhibit a geographical gradient within and between European countries. J Nutr 2010;140:1280–6. https://doi.org/10.3945/jn.110.121152.
[33] Moore C, Murphy MM, Keast DR, Holick MF. Vitamin D intake in the United States. J Am Diet Assoc 2004;104:980–3. https://doi.org/10.1016/j.jada.2004.03.028.
[34] 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;8:533. https://doi.org/10.3390/nu8090533.
[35] Duarte I, Rotter A, Malvestiti A, Silva M. The role of glass as a barrier against the transmission of ultraviolet radiation: an experimental study. Photodermatol Photoimmunol Photomed 2009;25:181–4. https://doi.org/10.1111/j.1600-0781.2009.00434.x.
[36] Gannage-Yared MH, Chemali R, Yaacoub N, Halaby G. Hypovitaminosis D in a sunny country: relation to lifestyle and bone markers. J Bone Miner Res 2000;15:1856–62. https://doi.org/10.1359/jbmr.2000.15.9.1856.
[37] Mishal AA. Effects of different dress styles on vitamin D levels in healthy young Jordanian women. Osteoporos Int 2001;12:931–5. https://doi.org/10.1007/s001980170021.
[38] Schleicher RL, Sternberg MR, Lacher DA, Sempos CT, Looker AC, Durazo-Arvizu RA, et al. The vitamin D status of the US population from 1988 to 2010 using standardized serum concentrations of 25-hydroxyvitamin D shows recent modest increases. Am J Clin Nutr 2016;104:454–61. https://doi.org/10.3945/ajcn.115.127985.
[39] Verdoia M, Schaffer A, Barbieri L, Di Giovine G, Marino P, Suryapranata H, et al. Impact of gender difference on vitamin D status and its relationship with the extent of coronary artery disease. Nutr Metab Cardiovasc Dis 2015;25:464–70. https://doi.org/10.1016/j.numecd.2015.01.009.
[40] Webb AR, Kazantzidis A, Kift RC, Farrar MD, Wilkinson J, Rhodes LE. Meeting vitamin D requirements in white Caucasians at UK latitudes: providing a choice. Nutrients 2018;10(4):497. https://doi.org/10.3390/nu10040497 (Accessed 2019).
[41] 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;26:881–91. https://doi.org/10.1007/s10552-015-0563-y.
[42] Manios Y, Moschonis G, Lyritis GP. Seasonal variations of vitamin D status in Greek postmenopausal women receiving enriched dairy products for 30 months: the Postmenopausal Health Study. Eur J Clin Nutr 2011;65:412–4. https://doi.org/10.1038/ejcn.2010.270.
[43] Mulligan JK, Nagel W, O'Connell BP, Wentzel J, Atkinson C, Schlosser RJ. Cigarette smoke exposure is associated with vitamin D3 deficiencies in patients with chronic rhinosinusitis. J Allergy Clin Immunol 2014;134:342–9. https://doi.org/10.1016/j.jaci.2014.01.039.
[44] Chapuy MC, Preziosi P, Maamer M, Arnaud S, Galan P, Hercberg S, et al. Prevalence of vitamin D insufficiency in an adult normal population. Osteoporos Int 1997;7:439–43.
[45] Looker AC, Pfeiffer CM, Lacher DA, Schleicher RL, Picciano MF, Yetley EA. Serum 25-hydroxyvitamin D status of the US population: 1988-1994 compared with 2000–2004. Am J Clin Nutr 2008;88:1519–27. https://doi.org/10.3945/ajcn.2008.26182.
[46] Sai AJ, Walters RW, Fang X, Gallagher JC. Relationship between vitamin D, parathyroid hormone, and bone health. J Clin Endocrinol Metab 2011;96:E436–46. https://doi.org/10.1210/jc.2010-1886.
[47] Livingstone KM, Celis-Morales C, Hoeller U, Lambrinou CP, Moschonis G, Macready AL, et al. Weekday sunlight exposure, but not vitamin D intake, influences the association between vitamin D receptor genotype and circulating concentration 25-hydroxyvitamin D in a pan-European population: the Food4Me study. Mol Nutr Food Res 2017;61. https://doi.org/10.1002/mnfr.201600476.