

Association of serum vitamin D status with dietary intake in adults with overweight and obesity

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Abstract

Vitamin D deficiency is a widespread problem throughout the world, often resulting from impaired synthesis in the skin and insufficient intake. The present study aimed to provide an in-depth analysis of vitamin D intake in individuals who are normal weight, overweight, and obese in relation to plasma vitamin D levels in these individuals. Materials and methods: There were 264 participants in the study. Nutrition was evaluated using the 24-hour memory recall approach. Weight and body composition were determined by expert bio-electrical impedance using Tanita equipment (420 BC MA), while nutritional status was evaluated using anthropometric indicators, including morphological indicators and anthropometric indices. Results: The average daily intake of vitamin D was determined to be 7.6 µg for normal-weight participants, 6.6 µg for individuals who are overweight, and 6.0 µg for subjects who are obese, based on BMI categories. When comparing people who consumed more than 10 µg of vitamin D, the group with adequate vitamin D consumption had the largest relative proportion (37.3%). Those with vitamin D deficiency and obesity class I had the lowest average daily intake of vitamin D (3.3 mcg). The correlation between plasma levels of vitamin D and the main indicators of dietary intake in the subjects, separated by sex and anthropometric parameters, revealed the existence of a weak correlation between plasma levels of vitamin D and individual indicators of dietary intake, such as total fat (g; E%), MUFAs (d; E%), PUFAs (d; E%), magnesium, and retinol. Conclusion: Vitamin D deficiency affects many groups worldwide, including Bulgaria's elderly citizens. In order to address this severe issue, health experts must design health policies and guidelines based on current and future scientific facts, which will only be available through new studies.

Keywords

vitamin D, intake, 25(OH)D, obesity

Introduction

Worldwide, there is a high frequency of vitamin D deficiency (Holick and Chen 2008; Prentice 2008; Wahl et al. 2012; Hilger et al. 2014), and this condition is becoming more common in prosperous countries. Conditions where cutaneous production and intake are impaired are frequently associated with vitamin D deficiency (Webb 2006). According to current classifications, serum 25(OH)D levels between 50 and 125 nmol/l indicate vitamin D sufficiency, 25 to 50 nmol/l indicates vitamin D insufficiency, and 25 nmol/l indicates vitamin D deficiency.

There was a 21.3% incidence of vitamin D deficiency and a 54.5% prevalence of vitamin D insufficiency in a nationwide representative survey of Bulgarians aged 20–89 years, based on serum 25(OH)D (Borissova et al. 2012a). A significantly greater incidence of obese patients with 25(OH)D < 25 nmol/l was reported (57.8% vs. 42.2%, $p < 0.02$) when comparing individuals with 25(OH)D < 25 nmol/l with those with higher vitamin D levels (Borissova et al. 2012b). Foods naturally contain a limited quantity of vitamin D, with the exception of fatty ocean fish (Wu et al. 2009). Vitamin D is found in very few foods, mostly as the highly accessible form of cholecalciferol (vitamin D3). Moreover, some plants synthesise it as ergocalciferol (vitamin D2). When assessing vitamin D status, supplementation in various forms should be taken into account, as it can be added to consumption (Dimakopoulos et al. 2019; Dimakopoulos et al. 2020).

Studies on vitamin D dietary intake, its interactions with other macro- and micronutrients from dietary intake, and its influence on anthropometric and metabolic parameters in overweight or obese adults have not been carried out in Bulgaria. There is no Bulgarian data on how vitamin D dietary intake affects the serum levels of 25(OH) vitamin D in various forms and severity of obesity. This research addresses the lack of information regarding vitamin D intake in individuals with varying degrees and phenotypic manifestations of obesity. It also highlights the significance of dietary intake on vitamin D status in individuals with varying body mass indexes during Bulgaria's winter months, when UVB sunlight fails to impact vitamin D production.

The purpose of this study is to evaluate the vitamin D status of Bulgarian adults by comparing serum 25(OH)D concentration to total vitamin D intake (from diet and dietary supplements) and quantity derived from sun exposure.

Materials and methods

Study design and procedure

A cross-sectional observational study was conducted, which included 264 people aged 19 to 60 in the city of Sofia. The sample was not representative of the capital city, including 155 women (58.9%) and 109 men (41.3%). The age

groups in the sample were determined in accordance with Ordinance No. 1 on the physiological norms for nutrition of the population in Bulgaria and are, respectively, 19–<30 years (42 persons) and 30–<60 years (222 persons). As the control group, 72 participants in the study had a normal body weight (BMI = 18.5 to 24.9 kg/m²), 65 were individuals who are overweight (BMI = 25.0 to 29.9 kg/m²), and 127 were obese in various degrees (BMI > 30.0 kg/m²) (Nikolova et al. 2019; Nikolova et al. 2023).

Each participant had a thorough medical history taken, underwent a physical examination, and had medical data collected. Anthropometric indicators and indices were used to assess nutritional status, and Tanita 420 BC MA equipment was utilised to measure professional bioelectrical impedance and determine weight and body composition (Nikolova et al. 2019; Nikolova et al. 2023).

After an overnight fast, blood samples for serum 25(OH)D were taken between 7:00 and 10:00 in the morning. Serum aliquots were then kept at -20 °C until additional analysis. Using electro-hemi-luminescent detection (ECLIA) on an Elecsys 2010 analyser from Roche Diagnostics in Switzerland, the serum concentration of 25-(OH)-vitamin D was determined. A coefficient of variation between 1.7% and 7.8% can be used to characterise the intra-assay error. The correlation with liquid chromatography and mass spectrometry (LC-MS/MS) is characterised by a Pearson's $r = 0.894$. Subjects with serum 25(OH)D < 25.0 nmol/l were defined as deficient, those with levels between 25.0 and 49.9 nmol/l as insufficient, and ≥ 50 nmol/l as sufficient (Borissova et al. 2012a; Borissova et al. 2012b). Additionally, levels ≥ 75.0 nmol/l were defined as optimal for bone health (Holick and Chen 2008).

The study was carried out in 2014 and 2015 between January and April to minimise seasonal variations in vitamin D levels. The winter season in Bulgaria's latitude results in shorter days, the wearing of bulkier clothing that covers nearly the entire skin, and an angle of incidence of UVB rays that is useless for the skin's ability to synthesise vitamin D. This lessens the correlation between serum vitamin D levels and the skin's capacity to produce vitamin D efficiently when exposed to UVB radiation. In order to identify the greatest number of normal-weight, overweight, and obese individuals with vitamin D deficiency, as well as to determine potential correlations with the parameters under investigation, the risky winter season is used.

The 2014 and 2015 winter seasons were used to evaluate nutrition. A retrospective memory playback of the food consumed over the previous 24 hours was put into place and called the 24-hour memory playback. Participants in the study had their dietary intake—quantity of food consumed, energy consumed, and nutrients consumed—analysed twice a week on non-consecutive days, including one workday and one rest day. Through the use of active participant interviewing, it was possible to calculate the amount of food ingested using measurements

or kitchen measures. Additionally, an examination of the individuals' supplementary vitamin and/or mineral supplementation was conducted. A computer program intended to estimate dietary intake was used to process the dietary data of each study participant. The chemical makeup of Bulgarian meals and beverages is stored in a database that the system uses.

The Bulgarian database on the composition of foods was used to analyse the vitamin D content in Bulgarian foods. This makes it possible for the first time to make a detailed analysis of the dietary intake of vitamin D for the territory of Bulgaria, a region in Europe, in relation to detailed anthropometric status, body composition, and distribution, as well as in people with varying BMI.

Inclusion and exclusion criteria for the study participants

The age range of participants (18–60 years old) and their expressed desire to take part in the study constituted inclusion criteria. The age range was established to reduce the possibility of further inaccuracy arising from alterations in body composition triggered by sarcopenia associated with ageing. The following were excluded: immobility, severe or chronic sickness, pharmacological therapy that alters body weight and composition, and other conditions known to cause morbid obesity. Conditions like heart failure (NYHA III and IV), respiratory failure, pancreatitis, liver cirrhosis, chronic kidney disease (stages III–V), severe fractures, myasthenia gravis, and others were among the exclusion criteria. Within the period of three months prior to the test, glucocorticoids, immunosuppressants, antipsychotics, vitamins, multivitamins (particularly vitamin D), and other medicines were prohibited. Furthermore, the study did not include participants who were on short-term vacations in sunny regions or who used tanning beds. Also, the duration of the exclusion period for certain medications and vitamin supplements is not stated. Selected from the general population, participants were referred by their general practitioners to get nutritional guidance for maintaining a healthy lifestyle or, for those who were overweight or obese, to be motivated to reduce weight (Nikolova et al. 2018).

Data analysis

IBM SPSS Statistics 23.0 was used to enter and process the data. A significance threshold of $p < 0.05$ is used to reject

the null hypothesis. A variety of descriptive and evaluation methods were used, including descriptive analysis (which involved breaking down the frequency distribution of the considered signs into groups), variation analysis of quantitative variables (which involved calculating mean values, standard deviation, standard error of the mean, and 95% confidence interval of the mean), indicators of scattering of quantitative signs, and graphical analysis to visualise the results. Hypothesis testing techniques and the subsequent standards were applied in order to demonstrate statistical dependence, which was shown in

tabular form: The student's T-test is parametric, with separate samples. T-test: this statistical test is used to examine hypotheses regarding differences between two independent samples when the sample's values follow a normal distribution (Gauss-Laplace); the Mann-Whitney method, Fisher's exact test, the exact criterion, and the χ^2 (chi-square) test are examples of non-parametric methods. They are used to compare mean values in two groups of a quantitative variable when the distribution is not normal. Multiple logistic stepwise regressions, single-factor logistic regressions, and non-linear regressions were also employed in the regression study.

Ethical considerations

This work was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving human subjects. The study was approved by the Scientific Ethics Committee of the Medical University of Sofia, Bulgaria, No. 2553/3.06.2024 year (Protocol No. 10/27.05.2024).

Results

According to the vitamin D status, 88 participants (33.3%) had deficiency ($25(\text{OH})\text{D} < 25.0 \text{ nmol/l}$), 106 (40.2%) had insufficiency ($25(\text{OH})\text{D}$ between 25.0 and 49.9 nmol/l), 49 (18.6%) had levels between 50 and 74.9 nmol/l, and only 21 participants (8.0%) had optimal levels (above 75.0 nmol/l). Therefore, only $\frac{1}{4}$ of the whole study sample (26.6%) had vitamin D levels $\geq 50.0 \text{ nmol/l}$. Table 1 displays the vitamin D level distribution based on BMI categories.

The relative rate of people distributed according to dietary reference intervals (dietary reference intakes) for vitamin D in the groups of sufficiency, insufficiency, and deficiency is shown in Fig. 1.

Table 1. The distribution of vitamin D levels according to the BMI categories.

Serum 25(OH) D	Normal weight	Overweight	Obesity class I	Obesity class II-III
Deficiency ($\leq 25.0 \text{ nmol/l}$)	18.1% ^a	27.7% ^{ac}	39.0% ^{bc}	54.0% ^b
Insufficiency (25.0–49.9 nmol/l)	41.7% ^a	43.1% ^a	39.0% ^a	36.0% ^a
Sufficiency (50.0–74.9 nmol/l)	23.6% ^a	23.1% ^{ac}	15.6% ^{ac}	10.0% ^{bc}
Optimal sufficiency ($\geq 75.0 \text{ nmol/l}$)	16.7% ^a	6.2% ^{bc}	6.5% ^{ac}	0% ^d

* The same uppercase letter in the horizontal line represents no significant difference, while different letters suggest significant differences between BMI subgroups ($p \leq 0.05$).

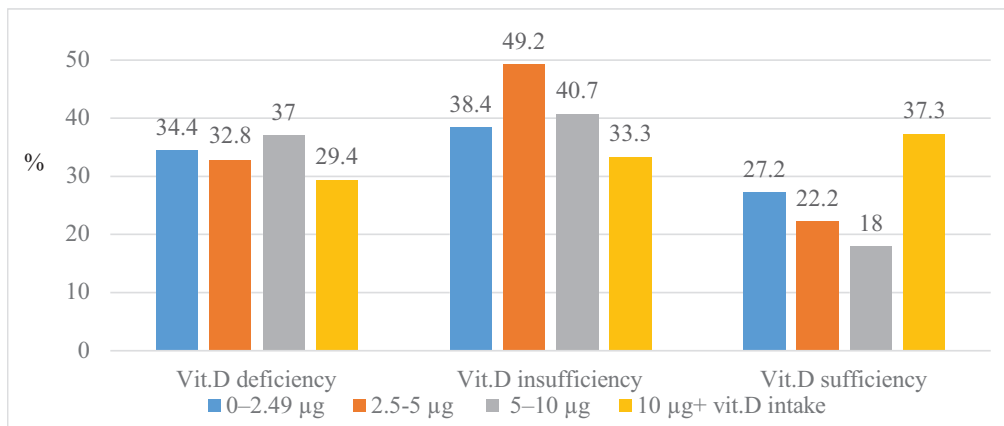


Figure 1. The relative rate of individuals distributed in % according to dietary reference intervals for vitamin D in the groups of sufficiency, insufficiency, and deficiency.

Subjects with serum 25(OH)D < 25.0 nmol/l were defined as vitamin D deficient, those with levels between 25.0 and 49.9 nmol/l as vitamin D insufficient, and \geq 50 nmol/l as vitamin D sufficient. The four dietary intake periods for vitamin D intake were described by the various categories of vitamin D deficiency, insufficiency, and sufficiency: The lower reference nutrient intake for dietary intake of vitamin D (LRNI, as determined by the Institute of Medicine (IOM, 2011)) is 0–2.49 μ g; the estimated average requirement for dietary intake of vitamin D (EAR, as determined by the Institute of Medicine (IOM, 2011)) is 2.5–5.0 μ g, 5–10 μ g, and > 10 μ g.

The relative rate of individuals with vitamin D intake above 10 μ g is highest in the group of vitamin D sufficiency (37.3%). There is no statistically significant difference between the relative rate of individuals in % distributed in the dietary reference intervals for vitamin D, respectively, in the groups of sufficiency, insufficiency, and deficiency, $p > 0.05$, as well as among the various groups. A statistically significant difference was found only among the percentages of individuals with dietary reference intervals for vitamin D 2.5–5 μ g in the groups with insufficiency (49.2%) and sufficiency (18%), $p = 0.0003$.

In the BMI groups, average daily vitamin D intake was found to be 7.6 μ g/day in normal-weight subjects, 6.6 μ g/day in subjects who are overweight, and 6.0 μ g/day in subjects who are obese. Individuals with vitamin D deficiency and Class I obesity had the lowest average daily intake of vitamin D—3.3 μ g, which is two times lower in comparison to those with overweight (6.7 μ g) and 2.3 times compared to people with Class II and Class III obesity (7.4 μ g) and vitamin deficiency, respectively (Fig. 2). No statistically significant differences were found in the average daily intake of vitamin D in groups of sufficiency, insufficiency, and deficiency related to vitamin D according to BMI, $p > 0.05$, n.s.

Subjects with serum 25(OH)D < 25.0 nmol/l were defined as vitamin D deficient, those with levels between 25.0 and 49.9 nmol/l as vitamin D insufficient, and \geq 50 nmol/l as vitamin D sufficient. Normal body weight is defined as a BMI = 18.5 to 24.9 kg/m²; overweight is defined as BMI = 25.0 to 29.9 kg/m²; and obesity is defined as BMI > 30.0 kg/m².

Spearman's rank correlation coefficients, determining the relationship between plasma levels of vitamin D and core indicators of food intake (24-h recall) in the groups of subjects, differentiated by sex and anthropometric indices, are shown in Tables 2, 3.

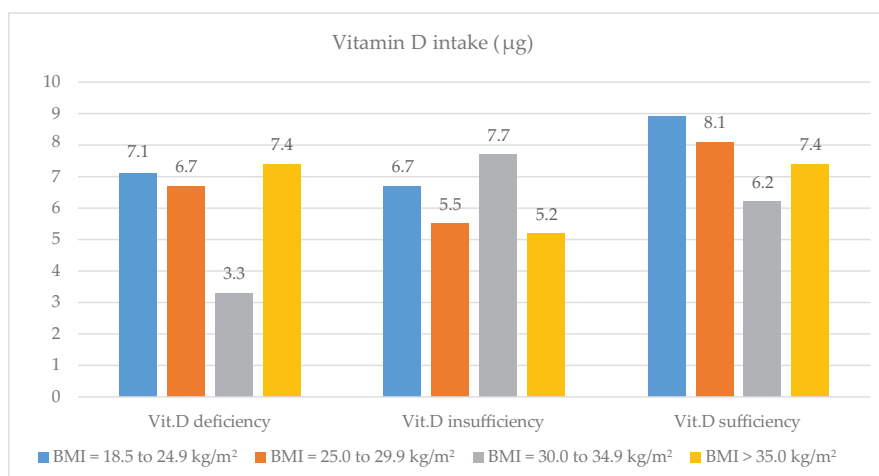


Figure 2. Average daily intake of vitamin D (μ) in the groups of subjects with sufficiency, insufficiency, and deficiency of vitamin D according to BMI, $p > 0.05$, n.s.

Table 2. Relationship between plasma levels of vitamin D and core measures of dietary intake in the subjects, differentiated by sex and anthropometric indices (Spearman's coefficients).

Measure	Total	Men	Women	Normal weight	(Overweight and Obesity)		
					Excess weight	Overweight	Obesity (Class I, II, III)
Energy (kcal)	0.082	0.113	0.037	-0.055	0.112	0.056	0.156
Protein, E%	0.034	0.027	0.032	0.083	0.093	0.165	0.065
Fat, E%	0.123*	0.103	0.142	0.262*	0.043	0.098	0.011
SFA, E%	0.058	0.063	0.050	0.164	0.005	0.089	-0.034
MUFAs, E%	0.138*	0.078	0.177*	0.231	0.051	0.063	0.037
PUFAs, E%	0.036	0.136	-0.008	0.080	0.037	0.050	0.040
Carbohydrates, E%	-0.126*	-0.047	-0.175*	-0.209	-0.127	-0.186	-0.106
Alcohol, E%	0.022	-0.067	0.093	-0.090	0.076	0.046	0.101
Protein (g)	0.095	0.135	0.049	0.024	0.150*	0.182	0.165
Animal protein (g)	0.086	0.079	0.077	0.089	0.131	0.173	0.139
Animal protein, (E%)	0.021	-0.065	0.063	0.080	0.047	0.073	0.041
Fat (g)	0.151*	0.168	0.131	0.094	0.135	0.073	0.182*
SFA (g)	0.119	0.142	0.089	0.118	0.097	0.089	0.118
MUFAs (g)	0.163**	0.155	0.164*	0.114	0.124	0.063	0.166
PUFAs (g)	0.097	0.191*	0.011	-0.005	0.124	0.027	0.191*
Cholesterol (mg)	-0.002	0.024	-0.067	-0.166	0.071	0.151	0.064
Carbohydrates (g)	-0.016	0.042	-0.103	-0.217	0.028	-0.035	0.064
Fibres (g)	0.089	0.213*	-0.021	0.009	0.120	0.088	0.149

** p < 0.01; * p < 0.05.

Table 3. Relationship between plasma levels of vitamin D and dietary vitamins and minerals in subjects differentiated by sex and anthropometric indices (Spearman's coefficients).

Measure	Total	Men	Women	Normal weight	(Overweight and Obesity)		
					Excess weight	Overweight	Obesity (Class I, II, III)
Vitamin D (µg)	0.119	0.080	0.150	0.150	0.080	0.076	0.077
Vitamin A (µg PE)	0.007	0.037	-0.027	-0.211	0.135	0.141	0.134
Vitamin E (mg α TE)	0.036	0.183	-0.098	-0.029	0.102	0.025	0.159
Vitamin B1 (mg)	0.113	0.149	0.074	0.006	0.194**	0.208	0.197*
Vitamin B2 (mg)	0.062	0.140	-0.018	-0.066	0.107	0.063	0.131
Niacin (mg)	0.091	0.113	0.059	0.039	0.132	0.108	0.156
Vitamin B6 (mg)	0.052	0.122	0.006	-0.083	0.082	-0.071	0.155
Folate (µg)	0.034	-0.006	0.064	-0.025	0.046	0.079	0.032
Vitamin B12 (µg)	0.045	0.223*	-0.042	-0.098	0.117	0.100	0.143
Vitamin C (mg)	0.041	-0.011	0.074	0.027	0.020	-0.036	0.033
Added sugar (g)	-0.026	-0.039	-0.022	-0.099	-0.020	-0.097	0.008
Alcohol (g)	-0.004	-0.085	0.073	-0.127	0.045	0.038	0.060
Retinol (µg)	0.153*	0.171	0.138	0.077	0.143*	0.204	0.081
Beta carotene (µg)	-0.007	0.019	-0.041	-0.065	0.010	0.026	0.024
Pantothenic acid (µg)	0.017	0.050	-0.003	-0.134	0.043	0.214	0.015
Biotin (mg)	0.013	0.114	-0.029	-0.148	0.041	0.102	0.036
Se (µg)	-0.004	0.017	-0.017	-0.129	0.031	0.240	0.010
Na (mg)	0.013	0.005	-0.024	-0.003	0.046	0.125	0.055
K (mg)	0.068	0.130	0.017	0.053	0.059	0.012	0.095
Ca (mg)	0.100	0.163	0.054	-0.008	0.108	0.034	0.139
P (mg)	0.115	0.161	0.065	0.009	0.153*	0.165	0.161
Mg (mg)	0.138*	0.200*	0.087	0.005	0.161*	0.155	0.173
Fe, total (mg)	0.105	0.122	0.084	0.025	0.107	0.181	0.105
Fe, heme (mg)	0.003	-0.019	-0.002	-0.176	0.124	0.161	0.124
Cu (µg)	0.039	0.083	-0.008	-0.205	0.092	0.132	0.117
Zn (mg)	0.083	0.190*	-0.032	-0.004	0.121	0.187	0.121
Mn (µg)	0.110	0.182	0.024	0.094	0.114	0.104	0.117

** p < 0.01; * p < 0.05.

Regarding the entire sample as well as the subgroups by sex and BMI, plasma levels of vitamin D correlated significantly with a small number of dietary intake measures. The correlation varied from weak to moderate, being directly proportional (except for that with average daily carbohydrate intake (g), which was inversely proportional).

For the entire sample, the established directly proportional correlations between plasma vitamin D levels were as follows: with the average dietary intake of fat and MUFAs (g and E%), with the average intake of carbohydrates (E%), and with magnesium and retinol. In men, plasma levels of vitamin D correlated significantly with the

average daily dietary intake of PUFAs, dietary fibre, zinc, magnesium, and vitamin B12. In the group of women, only weak correlations with the average dietary intake of MUFAs (g; E%) and carbohydrates (E%) were observed. When the sample was broken down by BMI, plasma vitamin D levels correlated significantly with very few of the dietary intake measures. In subjects with a normal BMI, only a moderately proportional correlation between plasma vitamin D levels and mean total fat intake (E%) remained stable. No significant relationship was found in the overweight group. In excess-weight individuals (total overweight and all obesity groups), a correlation with the average daily intake of protein (g), phosphorus, vitamin B1, and, identically for the entire sample, magnesium and retinol appeared. In the obese group, plasma vitamin D levels correlated significantly only with the mean dietary intake of fat (g), PUFAs (g), and vitamin B1.

In the initial multivariate regression analysis, including all indicators from Tables 2, 3, an adjusted coefficient of $R^2 = 0.225$ without statistical significance was achieved, $p = 0.054$. In the backward step-wise analysis, the average daily intake of animal protein (g), average daily intake of total fat (g), cholesterol (mg), dietary fibre (g), and selenium (μg) were the predictors with the greatest predictive value regarding plasma levels of vitamin D ($p = 0.012$; $R^2 = 0.310$) (see Table 4). It was not possible to increase the relevance of the data or obtain new information by adjusting for age, sex, or BMI. Except for mean animal protein intake, there was a unidirectional relationship between the predictors in the model and the levels of vitamin D. About 30% of the factors impacting fluctuations in vitamin D levels are included in the regression model, as can be seen from the coefficient of determination (R^2).

Table 4 presents the classification table of regression coefficients from the multiple logistic regression model analysing blood levels of vitamin D and the dietary intake characteristics that were evaluated. The results indicate that the factors with the highest predictive value are related to the level of 25(OH)D ($p = 0.012$; $R^2 = 0.310$).

Table 4. The classification table.

Predictors	Non-standardised coefficients		Standardised coefficients	P
	B	Std. Error	Beta	
Animal protein (g)	-37.460	8.177	-0.691	0.000
Fat (g)	78.065	22.435	0.485	0.001
Cholesterol (mg)	0.187	0.066	0.237	0.005
Dietary fibre (g)	0.332	0.076	0.574	0.000
Se (μg)	0.792	0.219	0.306	0.000
Alcohol (ml)	-0.15	0.007	-0.167	0.028
Alcohol, E%	-0.617	0.268	-0.430	0.022
Constant	-0.038	0.019	-0.575	0.044

Discussion

There are currently several genetic variations that impact vitamin D status close to genes involved in hydroxylation, cholesterol production, and vitamin D transport. People who have a much higher risk of vitamin D deficiency than

the general population are identified by genetic variation at these loci. The available genetic variations influencing vitamin D production or absorption in overweight and obese people require further studies (Voltan et al. 2023).

Obesity frequently results in unhealthy eating patterns, which may reduce vitamin D intake. Obese people frequently choose yo-yo diets, which alternate between times of extreme nutritional restriction and periods of overindulgence in high-energy, low-biodensity foods. They follow alternative eating patterns, time-restricted eating (TRE), vegan-vegetarian regimens, fasting treatments, fasting days, and monotonous and unnecessary restrictive diets, which essentially result in a limited intake of not only energy and macronutrients but also micronutrients, particularly vitamin D. It ought to point out that food sources contribute just a tiny portion of overall vitamin D intake (Wamberg et al. 2015). Some authors report no difference in dietary intake of vitamin D in individuals of normal weight versus individuals who were overweight (Walsh et al. 2016; Walsh et al. 2017). However, in two consecutive studies in northern Norway (Tromsø 5 and Tromsø 4), a lower dietary intake of vitamin D was found in men, who are obese, but not in women when compared with non-obese individuals (Kamycheva et al. 2003; Kamycheva et al. 2004). Furthermore, this link does not always indicate causality. Instead, while it cannot be totally ruled out as a contributing factor, diet is considered to be an insignificant variable in describing deficient vitamin D status in overweight and obese people. Due to inadequate UVB sun exposure, vitamin D is not produced in sufficient amounts in the skin during the winter months. As a result, the body must primarily obtain vitamin D from nutrition.

The dietary record (DR) method, considered the gold standard in dietary assessment, and the 24-hour recall method are not the most appropriate instruments to measure vitamin D intake (Głąbska et al. 2022; Voltan et al. 2023); instead, a longer period of time is required to collect data due to the significant daily variation in vitamin D intake, which depends on various factors such as the number of fish consumed and the variety of fortified foods (Verbek and Vackier 2003). The average daily dietary intake of vitamin D in the groups with varying BMIs was determined to be as follows: 6.6 $\mu\text{g}/\text{day}$ in subjects who are overweight, 6.0 $\mu\text{g}/\text{day}$ in subjects who are obese, and 7.6 $\mu\text{g}/\text{day}$ in normal-weight subjects. Less than 5 μg of vitamin D is consumed on average per day in the European Union, according to several studies (Lichthammer et al. 2015; Hribar et al. 2021a, 2021b). The range of the average daily dietary intake across nations is 1.1 to 6 $\mu\text{g}/\text{day}$ (Jenab et al. 2009; Freisling et al. 2010; Roman et al. 2011; Kiely and Black 2012; Novakovic et al. 2013; Spiro and Buttriss 2014; Rip-pin et al. 2017). The maximum intake is found in Northern Europe (up to 14 $\mu\text{g}/\text{day}$), while the lowest intake is found in Southern Europe (Elmadfa et al. 2009; Jenab et al. 2009; Roman et al. 2011; Kiely and Black 2012; Lips et al. 2019). A possible explanation for the higher vitamin D intake in Northern Europe is a larger consumption of fish and foods fortified with the vitamin. The average daily dietary intake of vitamin D was found to be very low in all four countries

studied by Lichthammer et al. (2015) of individuals aged 15–75 years from four Central and Eastern European countries. Austria had the lowest daily dietary intake (2.2 µg), followed by Slovenia (2.6 µg), Poland (3.8 µg), and Hungary (4.1 µg). Another study, the National Cross-sectional Study of Food Consumption (SI. Menu), carried out in Slovenia, reported lower average daily dietary intakes of vitamin D for the different population groups studied compared to the intake found in our study: 2.7 µg for adolescents (n = 468; 10–17 years), 2.9 µg for adults (n = 364; 18–64 years), and 2.5 µg for the elderly (n = 416; 65–74 years) (Hribar et al. 2021a). The average daily calorie intake has been recognised as one of the most important factors influencing vitamin D intake in all population groups. Sex also appeared to be an important predictor of vitamin D dietary intake in adolescents and adults, with men consuming more vitamin D; However, in our study, we found that BMI was the determinant of lower levels of vitamin D intake with the food (Hribar et al. 2021a).

It has been demonstrated that there is no correlation between average daily dietary vitamin D intake and plasma vitamin D levels, indicating that dietary vitamin D intake has not been a major factor in circulating vitamin D concentration. The relationship between serum levels of vitamin D and core measures of dietary intake in the subjects, differentiated by sex and anthropometric indices, has demonstrated the presence of a weak correlation between plasma levels of vitamin D and single measures of dietary intake of the following macronutrients: total fat (g; E%), MUFAs (g; E%) and PUFAs (g; E%), carbohydrates (E%), proteins (g), and fibres (g). The average daily intake of animal protein (g), total fat (g), cholesterol (mg), dietary fibre (g), and selenium (mcg) had the highest predictive value for plasma levels of vitamin D, according to multivariate regression analysis. Similar findings were also reported in a cross-sectional study that examined the relationship between vitamin D serum levels and dietary nutrient intake in 289 healthy people in Mashhad, Iran, between the ages of 30 and 50 years (Sharifan et al. 2023; Qasrawi et al. 2024). The results of the XGBoost model identified the six most significant factors affecting serum vitamin D levels as follows: dietary carbohydrates (21.54%), total dietary protein (16.60%), poly-saturated fat (16.60%), lactose (15.61%), total daily nitrogen (15.01%), and total sugar (14.62%). Vitamin D levels were predicted with 89% accuracy. Dietary macronutrients and micronutrients may influence vitamin D levels by affecting the rate of absorption and/or bioavailability (Maurya and Aggarwal 2017; Roth et al. 2018; Marwaha and Dabas 2019). However, the evidence in this field of study is inconsistent (Borel et al. 2015). According to a small-scale intervention trial, the vitamin D levels were considerably higher than at baseline after 5 weeks of low-carbohydrate dieting (Newton et al. 2012). The results of our study showed a negative correlation between the plasma vitamin D levels and the overall study group as well as the female group's total carbohydrate intake (E%). Fibre (g) and plasma vitamin D levels were also found to be significantly positively correlated in men; in the regression analysis, fibre (g) is one of the major predictors influencing

the serum levels of 25(OH) vitamin D. A plausible explanation for the established dependence could be related to the fermentation of certain dietary carbohydrates, like starches and soluble fibres, in the gastrointestinal tract. This process aids in boosting bacterial growth, which in turn encourages the synthesis of short-chain fatty acids (SCFAs), which are thought to aid in improved absorption of vitamin D (Raimundo 2013; Drabińska et al. 2018; Sharifan et al. 2023).

The KNHANES study examined the macronutrient composition of Korean women's diets and found a correlation between lower levels of vitamin D and lower dietary protein consumption. Therefore, in order to better control vitamin D deficiency in this population, it has been suggested to place an emphasis on a high-quality protein diet. However, comparing vitamin D-deficient people to non-deficient people, there was no statistically significant change in macronutrient consumption (Chun et al. 2020). Additionally, it has been demonstrated that vegetative proteins affect the gut microbiota and, in turn, the absorption of vitamin D, which in consequence affects vitamin D status (De Filippis et al. 2016). Our findings, which indicated that daily protein consumption was the second most significant factor in predicting vitamin D deficiency, are in line with previous evidence. Regression analysis results indicated that animal protein intake (g) was one of the dietary factors that predicted vitamin D status. In our study, a positive relationship was found only between the plasma levels of vitamin D and the total protein intake in grams in the overweight group (overweight and obese).

Multiple prior research investigations have focused on the impact of dietary fat on serum vitamin D levels. It makes perfect sense to believe that since vitamin D is fat-soluble, consuming more fat will enhance vitamin D absorption. Serum vitamin D levels are raised when vitamin D supplementation is combined with a high-fat meal (more than 15 g of fat), according to a double-blind randomised controlled trial (Raimundo et al. 2015). There was no correlation between serum vitamin D levels and the overall amount of fat consumed daily, according to another study. On the other hand, it was discovered that while menus high in polyunsaturated fatty acids (PUFAs) had the opposite impact, meals high in monounsaturated fatty acids (MUFAs) improved the effect of additional vitamin D intake on serum vitamin D levels in healthy individuals (Niramitmahapanya et al. 2011). Following the Mediterranean diet is linked to higher levels of vitamin D, as shown by another prospective cohort study on healthy, obese, or overweight people. The impact of MUFA-rich oils on vitamin D absorption—such as olive oil, which is included in diets—was used to explain this link (Dawson-Hughes et al. 2015; Barrea et al. 2020; Zupo et al. 2020; Dalamaga et al. 2021). Similar to previous studies, we have found that there is a correlation between the plasma levels of vitamin D and fat (E%) for the entire group of subjects studied, including those with normal weight, obesity, and fat (g) in the general group. In addition to MUFAs (g and E%) in the overall group, female sex, and PUFAs (g) in the male group, this correlation is used to calculate the vitamin D status in the regression model. In the regression model, fat (g) was another important predictor of

vitamin D status. It is unclear how exactly dietary fat, both in terms of amount and type, affects the bioavailability or absorption of vitamin D. According to a theory, there is very little dietary fat—mostly high in the MUFA:PUFA ratio—that improves the absorption of vitamin D by solubilising the vitamin when compared to the amount of fat taken daily. More fat, particularly polyunsaturated fatty acids (PUFA), can enlarge the micelles that contain vitamin D, which makes it harder for the vitamin to get through the unstirred water layer lining the intestinal mucosa and reduces vitamin D absorption (Nirarnitmahapanya et al. 2011; Goncalves et al. 2013; Madsen et al. 2013; Dawson-Hughes et al. 2015). Magnesium and retinol, but not vitamin A, are consistent with new scientific findings on the roles of vitamin A, zinc (Zn), and magnesium (Mg) in vitamin D activation and function (Pérez Rodrigo et al. 2015; Vranić et al. 2019). It has been established that there is a link between magnesium and plasma vitamin D levels and that magnesium plays a role in the activation of gene expression and vitamin D function. Vitamin D deficiency is also associated with obesity (Christakos et al. 2016).

Limitation

There are several limitations on this research. Firstly, food composition databases—rather than laboratory analyses—were required to estimate the vitamin D content of food. The national food composition data for Bulgaria do not include the amount of vitamin D in certain foods, resulting in the application of the extra food composition databases, which is a methodological limitation. Secondly, a significant study limitation is that our estimation of the daily dietary intake of vitamin D does not consider the consumption of food supplements or foods fortified with the vitamin D. Thirdly, given that the small sample is limited to Sofia, it is possible that the results are not indicative for all Bulgarians. The population under study was surveyed over one winter season, so seasonal variations in dietary patterns and vitamin D levels are plausible, with information lacking on what the subjects' plasma vitamin D levels were prior to the 2014 and 2015 winter seasons. Nevertheless, our study has certain strengths. The strength of our study comes from the two approaches we applied to measure the dietary intake of vitamin D: 24-hour recalls and a 25-item food frequency questionnaire (FFQ), which is only differentiated with regard to vitamin D-containing food sources using nationally validated questionnaires for food intake frequency in Bulgaria. It should be recalled, though, that dietary intake analysis is still based on recollection and is therefore biased, even when it is done using proven methodologies. The fact that the sample was taken from a particular and growingly representative subset of obese individuals, including those with sarcopenic obesity in the 19–60 age range, is another strength of the study. Since the blood sample was taken in the winter, we can clearly see how nutrition has a significant impact on serum 25(OH)D.

The findings in this paper provide a good starting point for future studies on the dietary intake of vitamin D and other vitamins and minerals related to vitamin D status (fat-soluble vitamins A, K2, and E; water-soluble vitamins B1, B12, and C; minerals Ca, Mg, P, Zn, and Se) among a larger nationally representative sample of adults with various pathological phenotypic manifestations of overweight and children and adolescent populations suffering from overweight, obesity, and sarcopenic obesity, considering the comprehensive vitamin and mineral, anthropometric, clinical, and cardio-metabolic statuses of the individuals in these risk groups. Such studies will also examine the effect of the overall dietary pattern on plasma vitamin D levels.

Conclusion

Numerous populations worldwide, including the elderly population in Bulgaria, suffer from vitamin D deficiency. Studies are required to address this significant problem with new scientific knowledge and help establish health policies and guidelines that health professionals will apply in the future. In conclusion, the most significant predictors of vitamin D levels, as demonstrated by our findings, were the average daily intake of animal protein (g), total fat (g), cholesterol (mg), dietary fibre (g), and selenium (mcg). This study provides crucial data that may be applied in clinical practice to reduce the prevalence of vitamin D deficiency through dietary treatments. To clarify the exact link between vitamin D levels and dietary intakes of macro- and micronutrients, more clinical trials and prospective research are needed.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

Clinical trials: This work was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving human subjects. The study was approved by the Scientific Ethics Committee of the Medical University of Sofia, Bulgaria, No. 2553/ 3.06.2024 year (Protocol No. 10/27.05.2024).

The authors declared that no experiments on humans or human tissues were performed for the present study.

The authors declared that no informed consent was obtained from the humans, donors or donors' representatives participating in the study.

The authors declared that no experiments on animals were performed for the present study.

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Data availability

All of the data that support the findings of this study are available in the main text.

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