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Vitamin D levels and cardiometabolic risk factors in Portuguese adolescents☆

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ABSTRACT

Background: Growing evidence suggests a possible association between low vitamin D levels and increased cardiovascular risk. However, research regarding the period of adolescence is scarce. We aimed to evaluate the association of vitamin D, intake and serum 25(OH)D levels, with cardiometabolic risk factors in 13-year-old adolescents.

Methods: We conducted a cross-sectional analysis of 1033 adolescents evaluated at 13 years old as part of the population-based cohort EPITeen. Vitamin D intake was assessed by a food frequency questionnaire. Serum 25(OH)D levels were assessed for a subsample of 514 participants. Metabolic syndrome (MetS) features were defined according to the National Cholesterol Education Program Adult Treatment Panel III definition modified for age. Logistic regression was fitted to estimate the association between vitamin D status and cardiometabolic risk factors, adjusting for sex, parental education, BMI, physical activity and season.

Results: Mean (SD) vitamin D levels, 4.61 (2.50) μg for intake and 16.52 (5.72) ng/mL for serum, were below the recommendations. The prevalence of MetS was 13.2%. Total cholesterol and LDL levels significantly decreased with 25(OH)D serum increase. After adjustment, no association was found between vitamin D levels and MetS. Regarding MetS features, an increased odds of high BMI was observed for those with a lower intake (OR 1.87 95% CI 1.04–3.35).

Conclusions: Although a significant increase in total and LDL cholesterol was observed for lower 25(OH)D levels, and an increased odds of high BMI was observed for those with a lower vitamin D intake, no significant association was observed between vitamin D levels and metabolic syndrome.

1. Introduction

For decades, a great deal of attention to vitamin D has been placed on understanding its role on bone health [1–3]. Vitamin D actions are mediated through binding to a vitamin D receptor (VDR) located primarily in the nuclei of target cells. The acknowledgement that VDR is found fairly present in tissues not involved with calcium and phosphate homeostasis suggests that vitamin D might play a more general role than it was previously thought [4–6]. Furthermore, the increase from 5 to 10 μg proposed by the Institute of Medicine concerning the recommendations for adequate intake of vitamin D of Estimated Average Requirement [7] highlights the growing confidence of its relevance for public health.

Recent studies have suggested that vitamin D levels play an important role in cardiovascular diseases (CVDs) [8–11]. The mechanisms by which vitamin D may be linked to CVDs involve the blunting effect of advanced glycation end products on endothelial cells, which are related to arterial stiffness and endothelial function [12,13]. Additionally, vitamin D may also exert protective effects on the vessel walls by inhibition of macrophage to foam cell formation and via its anti-inflammatory effects [12,13]. Also, vitamin D has been associated with the downregulation of the renin-angiotensin-aldosterone system [12,13], with a lipid-lowering effect [14], decreased insulin resistance, increased insulin secretory response to glucose and improved glucose homoeostasis [15].

CVDs remain the leading cause of death and disability worldwide [16]. Although these events occur most frequently during or after the fifth decade of life, longitudinal studies have shown that risk factors start in

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childhood persisting into adulthood [17,18], and predict the occurrence of CVDs later in life [19].

The "obesogenic" environment that has been observed in the majority of western societies during the last decades has contributed to the increase in metabolic/physiologic changes at early ages [20]: raised blood pressure (hypertension), overweight/obesity, impaired glucose and raised blood lipids (dyslipidemia). The clustering of these risk factors is called Metabolic Syndrome (MetS) [21]. Since these are the precursors of CVD, it is of greater interest to measure the effect of vitamin D in these risk factors at early stages [15].

Despite the emerging evidence suggesting an association between low vitamin D levels and cardiometabolic risk factors [22,23], IOM reports that available evidence is still insufficient to link health benefits other than skeletal health to particular levels of vitamin D intake or 25(OH)D serum measures [7]. One of the possible reasons for the differences between studies may lie in methodological differences regarding the measurement of exposure, once vitamin D can be obtained not only through diet, but also through UVB exposure [6].

Even though studies have suggested an association between lower vitamin D levels and the prevalence of MetS, few studies have explored the effect of both dietary and serum vitamin D on cardiovascular risk factors [24], and, to best of our knowledge, none has addressed the period of adolescence.

The present study aimed to examine the association of vitamin D, both intake and serum 25(OH)D levels, with cardiometabolic risk factors in 13-year-old adolescents.

2. Methods

Participants were adolescents from the *Epidemiological Health Investigation of Teenagers* in Porto (EPITeen). As reported elsewhere [25], the EPITeen evaluated adolescents born in 1990, who were enrolled at public and private schools in Porto, Portugal, during the 2003/2004 school year. Data were collected using two self-administered questionnaires: one fulfilled at home, comprising information on demographic and social characteristics, family and individual history of disease and a FFQ to evaluate food intake; and another fulfilled at school, which included information on physical activity, smoking and alcohol intake. A physical examination was performed at school, by a team of experienced nurses, nutritionists and physicians, comprising anthropometric assessment, blood pressure measurement and a venous blood sample drawn after a 12-hour overnight fast.

The Ethics Committee of Hospital S. João approved the study and written consent was obtained from both legal guardians and adolescents.

2.1. Participants

We identified 2786 eligible participants, of whom 2159 agreed to participate and provided information at least for part of the planned assessment, resulting in an overall participation rate of 77.5%, similar in public (77.7%) and private (77.0%) schools ($p = 0.709$).

Of the 2159 participants, 247 did not return the home questionnaire and 297 did not fill in the FFQ or were excluded because no information was provided on more than 10% of food items. A further 93 participants were not considered for the current analysis because their total energy intake was more than 3 times the interquartile range or their intake of fruit or vegetables was more than 1.5 times the interquartile range. Additionally, 80 participants did not perform the anthropometric or blood pressure measurements; other 409 did not undergo blood collection. Thus, the analysis on vitamin D intake was based on the information of 1033 participants. For budgetary constraints, we evaluated serum vitamin D levels for half of the sample with intake information (n = 514).

Participants included in the analysis were compared to those not included. Non included participants were mostly from public schools, with less educated parents, and a higher proportion spent their time on activities with higher level of physical activity. No significant differences were found regarding sex, BMI and vitamin supplementation. Additionally, characteristics of participants from the subsample (n = 514) were compared to those without serum vitamin D assessment (n = 519). Participants with data on serum vitamin D presented higher LDL cholesterol levels, lower insulin and HOMA levels, but no significant differences were observed regarding sex, vitamin D supplementation, BMI, waist circumference, total cholesterol, HDL cholesterol, triglycerides and glucose.

2.2. Evaluation of vitamin D intake

Vitamin D intake was evaluated using a food frequency questionnaire (FFQ) regarding the previous 12 months, fulfilled by the adolescents with the help of their parents or legal guardians. The FFQ was designed according to *Willett* and colleagues [26] and adapted for the Portuguese population, according to dietary data available for our country, and validated for the adult population by comparison with four 7 daily food records (each one in a different season of the year) [27]. The FFQ comprised a frequency section with nine possible responses ranging from never to six or more times daily. It was adapted for adolescents by including foods more frequently eaten by this age group [28], comprising ninety-one food items or beverage categories. It also included an open-ended section for foods not listed in the questionnaire, but eaten at least once a week.

Seasonal variation of food consumption was also considered according to participants' replies. To estimate nutrient intake, we used the software *Food Processor Plus®* (ESHA Research, Salem, OR, USA) based on values from the US Department of Agriculture. Values for Portuguese food were added, based on the Portuguese tables of food composition, typical recipes and data from previous studies [27].

Vitamin D supplements were not considered to quantify vitamin D intake.

2.3. Evaluation of serum vitamin D

Serum 25(OH)D was determined using DiaSorin LIAISON®, which is a direct competitive chemiluminescence immunoassay for human serum or plasma intended for use on the DiaSorin LIAISON® automated analyzer. The assay uses magnetic particles (solid phase) coated with antibody against 25(OH)D and 25(OH)D conjugated to an isoluminol derivative (tracer). During the first incubation phase (10 min), 25(OH)D is dissociated from binding protein by buffer containing 10% ethanol and then binds to the anti-25(OH)D antibody on the solid phase. After a second 10 min incubation with the tracer, the unbound material is washed off and starter reagents are added to generate a flash chemiluminescent signal which is measured by a photomultiplier and is inversely related to 25(OH)D concentration [29].

2.4. Cardiometabolic risk factors

Weight and height were obtained with the subject in light indoor clothes and no shoes. Weight was measured in kilograms, to the nearest tenth, using a digital scale (Tanita TBF-300, Tanita Corporation of America, Inc., Illinois, USA) and height was measured in centimeters, to the nearest tenth, using a portable stadiometer. Adolescents were classified according to the age- and sex-specific BMI based on the Centers for Disease Control and Prevention's 2000 BMI-for-age growth charts for the United States [30]. Based on this data we classified those above the 95th percentile as obese and those between the 85th and 95th percentiles as overweight.

Waist circumference was measured to the nearest centimeter, midway between the lower limit of the rib cage and the iliac crest, with the subject standing, using a flexible and non-distensible tape and avoiding exertion of pressure on the tissues.

Blood pressure was measured with a mercury sphygmomanometer using the auscultatory method, following the recommendations of the American Academy of Pediatrics [31]. After 10 min of rest, two blood pressure measurements were taken, separately by at least 5 min. A third

measure was taken when the difference between the first two was higher than 5 mm Hg. The average of the two closest measurements was used in this analysis. High blood pressure was considered if systolic or diastolic blood pressure above the 90th percentile for age, sex, and height [31].

Serum glucose, cholesterol, triglycerides and high-density lipoprotein (HDL)-cholesterol were determined using automatic standard routine methods. Serum insulin was measured using a 125I-labelled insulin radioimmunoassay method. Insulin resistance was estimated according to the homeostatic model assessment (HOMA), as the product of fasting glucose (mmol/L) and insulin (μUI/mL) divided by a constant 22.5 [32].

Metabolic syndrome features was defined according to the National Cholesterol Education Program Adult Treatment Panel III definition modified for age [33]: waist circumference ≥75th percentile [34] for age and sex; HDL cholesterol <50 mg/dL; triglycerides ≥100 mg/dL; systolic or diastolic blood pressure ≥90th percentile for age, sex, and height; and fasting glucose ≥110 mg/dL [31]. The presence of the metabolic syndrome phenotype was defined as having 3 or more of these 5 characteristics [33].

2.5. Covariates

Parental educational level was measured as the number of successfully completed years of formal schooling and adolescents were classified according to the parent with the highest educational level.

Vitamin supplementation was assessed asking parents the question "Did your child take supplements in the past 12 months?". If "yes" information was gathered on commercial name and dosage of supplementation. It was not possible to confirm the composition for a minority of the supplements reported (<5%), but for the supplements with information a high dose of vitamin D was present. Thus, participants were classified according to whether or not having taken supplements, without considering the specific dose, assuming that all participant with supplements had high amount.

Leisure-time physical activity was classified according to a four-choice question of subjective intensity categories (mainly sitting, mainly standing, active or very active).

Season during which participants were evaluated was combined into two categories: November–February and March–July.

2.6. Statistical analysis

Proportions were compared using the chi-square test or Fisher's exact test, as appropriate. Quantitative variables were compared using the student *t*-test and one-way ANOVA, the results being presented as mean (standard deviation).

Table 1
Vitamin D levels - intake (μg) and serum (ng/mL) - according to participants' characteristics.

Variable	n	%	Vit D intake Mean (SD), μg/day	*p*	n	%	Vit D serum Mean (SD), ng/mL	*p*
Overall	1033		4.61 (2.50)		514		16.52 (5.72)	
Sex								
Girls	554	53.6	4.51 (2.56)	0.164	270	52.5	15.71 (5.06)	<0.001
Boys	479	46.4	4.72 (2.44)		244	47.5	17.76 (6.15)	
Vitamin supplementation								
No	779	73.0	4.52 (2.44)	0.016	382	74.3	16.52 (5.92)	0.685
Yes	205	27.0	5.00 (2.76)		112	21.8	16.77 (5.28)	
Parental education (years)								
0–6	240	23.5	4.63 (2.50)	0.484	94	18.3	16.71 (5.85)	0.014
7–9	218	21.3	4.80 (2.78)		89	17.3	14.78 (5.07)	
10–12	281	27.5	4.60 (2.40)		148	28.8	16.72 (5.72)	
>12	284	27.8	4.44 (2.40)		183	35.6	17.11 (5.82)	
Physical activity								
Mainly sitting	281	27.2	4.42 (2.34)	0.085	149	29.0	16.46 (5.58)	0.061
Mainly standing	234	22.7	4.60 (2.72)		103	20.0	15.86 (6.57)	
Active	306	29.6	4.61 (2.39)		162	31.5	16.34 (5.32)	
Very active	164	15.9	5.04 (2.67)		73	14.2	18.15 (5.68)	
Season								
March–July	643	62.2	4.46 (2.44)	0.016	343	66.7	17.24 (5.94)	<0.001
November–February	390	37.8	4.85 (2.56)		171	33.3	15.09 (4.96)	
BMI ≥95th percentile[a]								
No	928	89.8	4.68 (2.55)	0.004	466	90.7	16.45 (5.68)	0.370
Yes	105	10.2	3.94 (1.94)		48	9.3	17.23 (6.16)	
Waist circumference ≥75th percentile[a]								
No	764	74.0	4.76 (2.62)	0.001	374	72.8	16.70 (5.74)	0.252
Yes	269	26.0	4.19 (2.10)		140	27.2	16.05 (5.66)	
High blood pressure[b]								
No	686	66.4	4.58 (2.38)	0.536	345	67.1	16.63 (5.70)	0.550
Yes	347	33.6	4.68 (2.74)		169	32.9	16.31 (5.78)	
Fasting glucose ≥110 mg/dL								
No	1027	99.4	4.62 (2.51)	0.512	511	99.4	16.53 (5.72)	0.874
Yes	6	0.6	3.94 (1.32)		3	0.6	16.00 (6.08)	
HDL cholesterol <50 mg/dL								
No	482	46.7	4.87 (2.72)	0.002	245	47.7	16.15 (5.59)	0.159
Yes	551	53.3	4.38 (2.27)		269	52.3	16.86 (5.82)	
Triglycerides ≥100 mg/dL								
No	914	88.5	4.58 (2.50)	0.317	462	89.9	16.65 (5.86)	0.151
Yes	119	11.5	4.82 (4.82)		52	10.1	15.44 (4.20)	
Metabolic syndrome[c]								
No	897	86.8	4.64 (2.52)	0.263	454	88.3	16.57 (5.76)	0.642
Yes	136	13.2	4.38 (2.36)		60	11.7	16.20 (5.42)	

[a] Data are according to age and sex.
[b] Defined as systolic or diastolic blood pressure ≥ the 90th percentile for age, sex, and height [31].
[c] Defined according to the definition of *de Ferranti* et al. [33].

The best transformation for skewed variables (triglycerides, insulin and HOMA-IR) was performed, and means were then retransformed to the original scale to improve data interpretation. Using linear regression models, crude and adjusted means and 95% confidence intervals (95% CI) of anthropometric and metabolic characteristics were calculated according to quartiles of both vitamin D intake and serum levels. Logistic regression models were fitted to estimate odds ratio and 95% CIs for the occurrence of selected cardiometabolic risk factors, according to quartiles of both vitamin D intake and serum levels. All models were further adjusted for sex, parental education, BMI, physical activity and season.

Statistical significance was considered with an alpha critical value of 0.05. Statistical analyses were performed using the Statistical Package for the Social Sciences (IBM® SPSS® Statistics), version 21.0.

3. Results

Table 1 presents the mean of vitamin D intake per day and serum 25(OH)D levels according to demographic and clinical characteristics of the participants. The prevalence of metabolic syndrome was 13.2%. The most prevalent MetS feature was low HDL cholesterol, with 53.3% of participants showing values below 50 mg/dL. In contrast, the component with the lowest prevalence was high glucose levels (0.6% for both sample and subsample). We observed very low levels of vitamin D from both intake (4.61 (2.50) μg/day) and serum (16.52 (5.72) ng/mL). Vitamin D intake was lower in those who reported not to use vitamin supplements, in obese or abdominally obese, in those who had low HDL cholesterol and in those who were evaluated between March and July. Serum levels of vitamin D were lower in girls, in participants whose parents presented a lower educational level and in those who were evaluated between November and February.

The crude mean (95% CI) of cardiometabolic risk factors according to quartiles of vitamin D levels is shown in Table 2. Decreasing levels of BMI and waist circumference were observed with vitamin D intake increase. Regarding serum vitamin D, decreasing values of total cholesterol, LDL and triglycerides were found with increasing levels of vitamin D, the same trend was found for systolic and diastolic blood pressure levels, but not reaching statistical significance. After adjusting for sex, parental education, BMI, physical activity and season, no significant differences were observed for all of the features concerning intake of vitamin D, but the decrease on total and LDL cholesterol levels with increasing serum vitamin D levels remained significant (Table 3).

Considering the occurrence of MetS and its features, no association was observed with vitamin D levels, however, we found a higher odds of high adiposity among those with lower vitamin D intake and a higher odds of higher triglycerides among those with lower serum vitamin D levels (Table 4).

4. Discussion

In this sample we found very low levels of vitamin D intake (4.61 (2.50) μg/day) and 25(OH)D (16.52 (5.72) ng/mL), reinforcing the descriptions of the worldwide high prevalence of vitamin D deficiency [35–37], also observed in adolescents from other European countries [38–40]. Despite the low vitamin D levels found, a significant increase of total and LDL cholesterol and triglycerides was observed among those with lower levels of serum vitamin D. Moreover, an increased odds of high BMI was observed for those with a lower intake.

Table 2
Mean (95% CI) of selected anthropometric and metabolic characteristics according to quartiles of vitamin D (intake and serum).

	Quartiles of vitamin D intake, μg/day				
Variable	I (<2.88)	II (2.88–4.10)	III (4.11–5.60)	IV (>5.60)	*P-trend*
Anthropometrics					
BMI	21.6 (21.1–22.1)	21.2 (20.8–21.6)	20.9 (20.5–21.3)	20.7 (20.3–21.1)	0.023
Waist circumference	73.5 (72.5–74.6)	72.9 (71.8–74.0)	72.8 (71.7–73.9)	71.6 (70.7–72.6)	0.088
Blood pressure, mm Hg					
Systolic	113.3 (111.9–114.8)	113.8 (112.4–115.2)	112.1 (110.8–113.4)	112.6 (111.2–114.0)	0.301
Diastolic	67.6 (66.6–68.6)	67.6 (66.6–68.5)	68.0 (66.9–69.1)	68.3 (67.4–69.3)	0.680
Lipids, mg/dL					
Total cholesterol	166.7 (162.8–170.6)	165.5 (161.7–169.3)	166.9 (163.0–170.8)	165.7 (161.9–169.4)	0.940
LDL cholesterol	105.3 (102.1–108.5)	103.7 (100.6–106.7)	103.6 (100.6–106.6)	103.4 (100.4–106.4)	0.827
Triglycerides	61.6 (58.6–64.7)	59.2 (56.3–62.2)	58.4 (55.6–61.4)	60.2 (57.2–63.2)	0.506
HDL cholesterol	48.1 (46.7–49.5)	48.9 (47.6–50.2)	50.6 (49.2–52.0)	49.1 (47.6–50.6)	0.088
Diabetes-related measures					
Fasting glucose, mg/dL	84.2 (83.0–85.3)	84.6 (83.5–85.8)	85.3 (84.1–86.5)	86.1 (84.9–87.3)	0.113
Fasting insulin, μU/mL	6.2 (5.6–7.0)	6.2 (5.6–7.0)	6.4 (5.7–7.1)	6.1 (5.4–6.8)	0.961
Homa–IR	1.2 (1.2–1.4)	1.3 (1.2–1.4)	1.3(1.2–1.4)	1.2 (1.2–1.4)	0.972
	Quartiles of serum vitamin D, ng/mL				
	I (<13.0)	II (13.0–16.0)	III (17.0–20.0)	IV (>20.0)	*P-trend*
Anthropometrics					
BMI	21.3 (20.8–21.8)	21.0 (20.4–21.6)	20.7 (20.0–21.3)	21.1 (20.0–21.3)	0.557
Waist circumference	73.3 (72.0–74.6)	72.3 (70.7–73.8)	72.3 (70.8–73.8)	72.8 (71.2–74.2)	0.708
Blood pressure, mm Hg					
Systolic	113.7 (112.0–115.4)	113.4 (111.3–115.5)	112.4 (110.4–114.4)	111.3 (109.3–113.4)	0.309
Diastolic	68.2 (66.8–69.8)	68.2 (66.8–69.8)	67.5 (66.0–69.0)	67.0 (65.6–68.4)	0.566
Lipids, mg/dL					
Total cholesterol	172.2 (167.7–176.8)	166.0 (160.2–171.8)	169.8 (164.2–175.4)	157.8 (152.2–163.3)	0.001
LDL cholesterol	109.8 (106.1–113.4)	104.0 (99.4–108.7)	106.4 (102.0–111.0)	98.4 (93.8–102.8)	0.002
Triglycerides	61.0 (57.7–64.6)	61.5 (57.2–66.1)	57.1 (53.2–61.2)	54.0 (50.4–57.8)	0.022
HDL cholesterol	49.4 (47.7–51.0)	48.2 (46.2–50.4)	51.2 (49.2–53.3)	47.8 (45.8–49.9)	0.099
Diabetes-related measures					
Fasting glucose, mg/dL	83.9 (82.6–85.3)	84.5 (82.8–86.3)	85.9 (84.2–87.6)	84.5 (82.8–86.2)	0.356
Fasting insulin, μU/mL	6.2(5.5–7.1)	6.4(5.4–7.4)	5.8(5.0–6.8)	5.4(4.6–6.3)	0.437
Homa–IR	1.2(1.1–1.4)	1.3(1.1–1.5)	1.2(1.0–1.4)	1.1(1.0–1.3)	0.540

Table 3
Adjusted mean (95% CI) of selected anthropometric and metabolic characteristics according to quartiles of vitamin D (intake and serum).

	Quartiles of vitamin D intake, μg/day				
Variable	I (<2.88)	II (2.88–4.10)	III (4.11–5.60)	IV (>5.60)	*P-trend*
Anthropometrics					
BMI[a]	21.4 (21.0–21.9)	21.1 (20.6–21.6)	20.8 (20.4–21.2)	20.6 (20.2–21.0)	0.078
Waist circumference[a]	73.4 (72.3–74.5)	73.0 (71.8–74.0)	72.6 (71.6–73.8)	71.5 (70.4–72.6)	0.081
Blood Pressure, mm Hg					
Systolic[b]	113.3 (112.0–114.6)	114.1 (112.8–115.4)	112.7 (111.4–114.0)	113.2 (112.0–114.6)	0.528
Diastolic[b]	67.0 (66.0–68.0)	67.1 (66.1–68.2)	68.1 (67.2–69.2)	68.4 (67.4–69.4)	0.104
Lipids, mg/dL					
Total cholesterol[b]	167.5 (163.6–171.4)	166.1 (162.2–170.0)	167.6 (163.8–171.6)	165.9 (162.0–169.7)	0.883
LDL cholesterol[b]	104.8 (101.6–108.2)	103.4 (100.2–106.6)	103.5 (100.4–106.7)	103.5 (100.4–106.6)	0.904
Triglycerides[b]	61.0 (58.0–64.0)	58.6 (55.7–61.6)	59.1 (56.2–62.2)	60.3 (56.8–62.8)	0.688
HDL cholesterol[b]	49.4 (48.0–50.8)	49.9 (48.6–51.2)	51.2 (49.8–52.6)	49.2 (47.9–50.6)	0.169
Diabetes-related measures					
Fasting glucose, mg/dL[b]	84.2 (82.9–85.4)	85.0 (83.8–86.2)	85.7 (84.5–86.9)	86.0 (84.9–87.2)	0.132
Fasting insulin, μU/mL[b]	6.0 (5.4–6.8)	6.4 (5.6–7.0)	6.6 (6.0–7.4)	6.4 (5.6–7.0)	0.696
Homa –IR[b]	1.2 (1.1–1.4)	1.3 (1.2–1.4)	1.4 (1.2–1.6)	1.3 (1.2–1.5)	0.559
	Quartiles of serum vitamin D, ng/mL				
	I (<13.0)	II (13.0–16.0)	III (17.0–20.0)	IV (>20.0)	*P-trend*
Anthropometrics					
BMI[a]	21.1 (20.6–21.6)	21.1 (20.4–21.8)	20.6 (20.0–21.3)	21.2 (20.5–21.8)	0.662
Waist circumference[a]	73.1 (71.8–74.4)	72.7 (71.1–74.4)	72.3 (70.7–73.9)	72.7 (71.0–74.3)	0.885
Blood Pressure, mm Hg					
Systolic[b]	113.8 (112.2–115.5)	113.7 (111.6–115.8)	112.7 (110.7–114.7)	111.3 (109.2–113.4)	0.224
Diastolic[b]	67.4 (66.2–68.7)	67.7 (66.2–69.2)	66.8 (65.4–68.4)	66.3 (64.8–67.8)	0.516
Lipids, mg/dL					
Total cholesterol[b]	172.6 (167.7–177.5)	167.0 (161.0–173.0)	169.3 (163.4–175.2)	158.6 (153.0–164.5)	0.003
LDL cholesterol[b]	109.4 (105.5–113.4)	103.7 (98.8–108.6)	105.3 (100.6–110.1)	97.9 (93.0–102.8)	0.003
Triglycerides[b]	59.1 (55.7–62.8)	57.6 (57.9–66.7)	56.8 (53.0–60.9)	54.6 (50.4–59.1)	0.067
HDL cholesterol[b]	50.2 (48.6–52.0)	49.6 (47.4–51.6)	52.0 (49.9–54.0)	49.0 (46.9–51.0)	0.179
Diabetes-related measures					
Fasting glucose, mg/dL[b]	84.6 (83.0–86.0)	85.4 (83.5–87.2)	86.0 (84.2–87.8)	85.2 (83.4–87.0)	0.642
Fasting insulin, μU/mL[b]	6.0 (5.4–7.0)	6.6 (5.6–7.8)	6.0 (5.2–7.1)	5.9 (5.0–6.8)	0.706
Homa –IR[b]	1.2 (1.1–1.4)	1.4 (1.2–1.6)	1.2 (1.0–1.5)	1.2 (1.0–1.4)	0.736

[a] Adjusted for sex, parental education, physical activity and season.
[b] Adjusted for sex, parental education, BMI, physical activity and season.

Although, in general, the differences were not statistically significant, our results show that those with lower vitamin D levels present a worse cardiometabolic profile. A possible reason for this lack of significant association might be due to the low vitamin D levels observed among participants that might have smoothed out differences, since not even the reference class presented desirable values.

A greater odds was observed for obesity among adolescents with lower vitamin D intake. The same trend was also observed for serum 25(OH)D levels, but without reaching statistical significance. Despite having been often suggested the sequestration within adipose tissue as an explanation for the lower levels of vitamin D in subjects with a higher BMI [41,42], our results indicate that the lower vitamin D intake by these individuals should also be considered.

Our results showed no relationship between cholesterol and vitamin D intake, however, when considering serum 25(OH)D levels, cholesterol levels increased with vitamin D decrease. Although the relationship between vitamin D intake and cardiovascular outcomes is less studied, these results are in accordance with previous studies, in which the relationship between vitamin D intake and cardiometabolic risk factors was less robust than comparing to serum 25(OH)D levels [24]. The mechanism that can explain the relationship between vitamin D and cholesterol is not yet clarified, nevertheless, it has been suggested that through an increased calcium level, vitamin D may reduce hepatic triglyceride formation and/or secretion, and, also, that it can influence lipid metabolism through the effect on both insulin secretion and insulin sensitivity [43].

Studies have suggested that vitamin D may be a negative endocrine regulator of the renin-angiotensin system, through the inhibition of the renin gene expression by the activated metabolite of 25(OH)D, 1,25-dihydroxyvitamin D (1,25[OH]2D) [44]. However, in the present study no association was found between low vitamin D levels (intake and serum) and high blood pressure. A systematic review conducted in 2010, which evaluated 13 observational studies and 18 randomized trials verified that while in a meta-analysis of 3 cohorts, lower 25(OH)D concentration was associated with incident hypertension, in another meta-analysis of 10 trials, the supplementation with vitamin D did not significantly reduce blood pressure, concluding that the association between vitamin D deficiency and hypertension is not firmly established [45].

No differences were also observed between quartiles of vitamin D and glucose, insulin and, consequently, HOMA, possibly due to the lower prevalence of these features which may prevent the effect to be estimated.

In general, no association was found regarding vitamin D intake, which could be explained by measurement errors. The fact that the FFQ was not specifically designed for assessing vitamin D intake might be considered a restraint of the study, however, the main sources of vitamin D intake (e.g. fatty fish, milk, eggs) were specifically asked. Additionally, the FFQ was designed for the Portuguese population [27] and foods or food groups eaten more frequently by adolescents were included in the questionnaire. Also, adolescents were encouraged to list foods eaten at least once weekly not enumerated in the FFQ, in an open section. Vitamin D supplementation was not considered to the estimative of vitamin D intake, which may have contributed to the low vitamin D intake observed. However, since vitamin supplementation was significantly higher in participants with higher vitamin D intake, it indicates that the association between intake and MetS features would not have been affected.

Besides the low vitamin D intake observed, serum 25(OH)D concentrations were also very low. This is consistent with the high prevalence

Table 4
Adjusted odds ratios (95% CI) of selected cardiometabolic risk factors according to vitamin D levels (intake and serum).

Variable	Quartiles of Vitamin D intake, μg			
	I (<2.88)	II (2.88–4.10)	III (4.11–5.60)	IV (>5.60)
BMI ≥ 95th percentile[a]				
Prevalence, %	15.2	8.9	8.1	8.5
Adjusted OR[b]	1.87 (1.04–3.35)	1.08 (0.58–2.04)	0.99 (0.52–1.90)	1.00 (reference)
Waist circumference ≥ 75th percentile				
Prevalence, %	30.0	27.5	25.5	21.2
Adjusted OR[c]	1.02 (0.50–2.06)	1.30 (0.65–2.57)	1.32 (0.67–2.60)	1.00 (reference)
High blood pressure[d]				
Prevalence, %	35.0	32.9	32.8	33.6
Adjusted OR[c]	0.96 (0.64–1.42)	0.93 (0.62–1.38)	1.00 (0.67–1.48)	1.00 (reference)
Fasting glucose ≥ 110 mg/dL[†]				
Prevalence, %	0.8	0.4	0.8	0.4
HDL cholesterol < 50 mg/dL				
Prevalence, %	59.1	55.0	47.9	51.4
Adjusted OR[c]	1.22 (0.84–1.78)	1.07 (0.74–1.55)	0.84 (0.58–1.22)	1.00 (reference)
Triglycerides ≥ 110 mg/dL				
Prevalence, %	12.1	8.9	10.4	14.7
Adjusted OR[c]	0.76 (0.44–1.32)	0.49 (0.27–0.89)	0.75 (0.43–1.32)	1.00 (reference)
Metabolic syndrome[e]				
Prevalence, %	16.0	12.4	10.8	13.5
Adjusted OR[c]	0.82 (0.43–1.54)	0.66 (0.34–1.28)	0.66 (0.33–1.32)	1.00 (reference)
	Quartiles of serum vitamin D, ng/mL			
	I (<13.0)	II (13.0–16.0)	III (17.0–20.0)	IV (>20.0)
BMI ≥ 95th percentile[a]				
Prevalence, %	8.6	9.3	9.6	10.3
Adjusted OR[b]	0.64 (0.26–1.54)	0.84 (0.32–2.14)	0.82 (0.32–2.10)	1.00 (reference)
Waist circumference ≥ 75th percentile				
Prevalence, %	29.9	25.0	27.8	24.8
Adjusted OR[c]	1.26 (0.48–3.29)	1.03 (0.36–2.89)	1.64 (0.58–4.50)	1.00 (reference)
High blood pressure[d]				
Prevalence, %	33.3	36.1	32.2	29.9
Adjusted OR[c]	1.08 (0.62–1.90)	1.29 (0.70–2.36)	1.06 (0.58–1.96)	1.00 (reference)
Fasting glucose ≥ 110 mg/dL[†]				
Prevalence, %	1.1	0	0	0.9
HDL cholesterol < 50 mg/dL				
Prevalence, %	49.4	53.7	51.3	56.4
Adjusted OR[c]	0.84 (0.50–1.42)	0.96 (0.54–1.70)	0.90 (0.51–1.59)	1.00 (reference)
Triglycerides ≥ 110 mg/dL				
Prevalence, %	9.2	19.4	7.0	6.0
Adjusted OR[c]	1.74 (0.63–4.75)	4.91 (1.84–13.10)	1.17 (0.37–3.68)	1.00 (reference)
Metabolic syndrome[e]				
Prevalence, %	10.9	15.7	10.4	10.3
Adjusted OR[c]	1.13 (0.44–2.93)	1.97 (0.74–5.22)	0.88 (0.30–2.53)	1.00 (reference)

[a] BMI classified according to age and sex [33].
[b] Adjusted for sex, parental education, physical activity and season.
[c] Adjusted for sex, parental education, physical activity, BMI and season.
[d] Defined as systolic or diastolic blood pressure ≥ the 90th percentile for age, sex, and height [31].
[e] Insufficient cases to measure the association.
[†] Defined according to the definition of de Ferranti et al. [33].

of vitamin D deficiency described in other countries. A possible explanation for the low serum levels observed could be the time of storage, since all blood samples were stored after collection and analyzed ≈10 years after. However, participants evaluated during the winter season (November–February) presented lower levels of vitamin D. In fact, in the northern hemisphere at latitudes >40°N (latitude of Porto where the study was conducted) sunlight appears not to be strong enough to trigger cutaneous synthesis from November to February [38,46]. This supports that the loss in storage is non differential, therefore, the association between serum 25(OH)D levels and the cardiometabolic risk factors is also not expected to be affected.

Notwithstanding the above limitations, this study is, to the best of our knowledge, the first examining the association between vitamin D levels, both intake and serum 25(OH)D, with cardiometabolic risk factors in adolescents. Moreover, the dataset employed was from a population-based sample, which may enhance generalizability. However, because data are cross-sectional, the causative nature of associations cannot be determined, yet, it is not expected that participants have altered their behaviour in consequence of the features evaluated. Therefore, we can assume that the relationship verified is not the result of reverse causality.

5. Conclusion

Although, in general, no significant associations were found, a trend for increasing levels of cardiometabolic risk factors was verified with decreasing vitamin D levels. The effect was stronger for serum than for intake levels, possibly because serum 25(OH)D levels reflect vitamin D from both intake and cutaneous synthesis. The low levels observed for both vitamin D intake and serum 25(OH)D levels, and the reduced variability of exposure may have limited the ability to detect a possible association with the cardiometabolic risk factors.

Conflict of interest

There are no conflicts of interest to disclose.

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