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1,25-dihydroxyvitamin D and cardiometabolic risk in healthy sedentary adults: The FIT-AGEING study

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ABSTRACT

Background: A growing body of scientific works investigating the physio-pathological mechanisms behind cardiovascular disease has suggested that vitamin D deficiency could play a key role on its development. However, it remains unclear whether its active form (1,25-dihydroxyvitamin D [$1,25(OH)_2D$] is associated with cardiometabolic risk factors in healthy individuals. The aim of the present study was to investigate the relationships of $1,25(OH)_2D$ plasma levels with cardiometabolic risk factors in a sample of healthy sedentary adults.

Methods: A total of 73 adults (~53% women; 54 ± 5 years old) were included in the current cross-sectional study. A sex-specific cardiometabolic risk score (MetScore) was calculated for each subject based on clinical parameters (*i.e.,* waist circumference, systolic and diastolic blood pressure, plasma glucose, high-density lipoprotein cholesterol, and triglycerides) according to the International Diabetes Federation's clinical criteria. Plasma levels of $1,25(OH)_2D$ were measured using a DiaSorin Liaison® immunochemiluminometric analyzer.

Results: No significant association was detected between $1,25(OH)_2D$ and MetScore ($\beta = 0.037$, $R^2 = 0.001$, $p = 0.77$), independently of age, sex and fat body mass index. A significant inverse association were observed between $1,25(OH)_2D$ and waist circumference ($\beta = -0{,}303$, $R^2 = 0.092$, $p = 0.01$). These results were consistent after controlling by potential confounders.

Conclusion: In summary, the present results suggest that $1,25(OH)_2D$ plasma levels are not associated with either cardiometabolic risk factors or insulin resistance in healthy sedentary adults. However, an inverse association of $1,25(OH)_2D$ plasma levels with central adiposity was observed in our study sample.

1. Introduction

The incidence of chronic cardiometabolic disorders has dramatically increased during the last decades representing the leading cause of morbidity and mortality in the developed world [9,44,63]. Several cardiometabolic diseases (*e.g.* cardiovascular diseases/CVD or type II Diabetes Mellitus/T2DM) are usually initiated by the presence of metabolic syndrome (MetS), which is defined as a clustering of abnormal physiological conditions (*i.e.*, hypertension, central obesity, elevated triglycerides, glycaemic dysregulations, dyslipidaemia, and high concentrations of pro-inflammatory biomarkers) [13,16,18,27]. In this context, the identification of potential biomarkers capable of detect the risk and progression of cardiometabolic disease is a major goal of clinical medicine for promoting general health [23,25].

Vitamin D is a fat-soluble steroid pro-hormone endogenously synthesized as vitamin D_3 (cholecalciferol) in the skin upon exposure to ultraviolet B radiation from sunlight and/or obtained from the diet or vitamin D supplements as vitamin D_2 (ergocalciferol) or vitamin D_3 [30]. These pro-hormones are transported to the liver and subsequently hydroxylated producing the biologically inactive 25-hydroxyvitamin D [25(OH)D] [40,46]. 25(OH)D requires to be converted in 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) by the 1-α-hydroxylase in the kidney to be biologically active [40,46]. $1,25(OH)_2D$, also known as calcitriol, is, therefore, the main responsible of vitamin D biological functions [40,46].

Vitamin D deficiency is highly prevalent in different populations

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[1] These authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation

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Table 1
Characteristics of participants at baseline.

	N	All		*N*	Men		*N*	Women	
Age (years)	73	54	(5)	34	55	(5)	39	53	(5)
$1,25(OH)_2D$ (pg/ml)	73	40.3	(14.1)	34	38	(13)	39	42	(15)
Anthropometric and body composition									
Weight (kg)	73	75.5	(15.0)	34	87.3	(11.1)	39	65.3	(9.3)*
Body mass index (kg/m^2)	73	26.7	(3.8)	34	28.3	(3.7)	39	25.3	(3.3)*
Waist circumference (cm)	73	95.0	(11.8)	34	102.8	(8.9)	39	88.2	(9.7)*
Fat mass (%)	73	40.1	(9.0)	34	35.0	(8.0)	39	44.5	(7.4)*
Fat mass (kg)	73	30.1	(8.5)	34	31.0	(9.8)	39	29.2	(7.1)
Lean mass (kg)	73	43.2	(11.5)	34	53.6	(6.4)	39	34.1	(5.8)*
Blood Pressure									
Systolic blood pressure (mm Hg)	66	126.9	(15.8)	30	134.0	(14.0)	36	120.9	(14.8)*
Diastolic blood pressure (mm Hg)	66	81.1	(11.8)	30	85.1	(11.1)	36	77.6	(11.4)*
Mean blood pressure (mm Hg)	66	104.0	(13.2)	30	109.6	(11.9)	36	99.3	(12.5)*
Glucose Metabolism									
Plasma glucose (mg/dL)	70	93.6	(11.4)	33	95.0	(13.6)	37	92.3	(8.9)
Plasma insulin (UI/mL)	70	8.1	(5.7)	33	8.9	(6.7)	37	7.3	(4.5)
Insulin glucose ratio	70	12.6	(7.6)	33	13.4	(8.1)	37	11.9	(7.1)
QUICKI	70	0.362	(0.036)	33	0.357	(0.039)	37	0.365	(0.033)
HOMA-IR	70	1.79	(1.19)	33	1.91	(1,26)	37	1.69	(1.12)
Lipid Metabolism									
Total cholesterol (mg/dL)	70	206.4	(31.9)	33	200.7	(32.3)	37	211.5	(31.0)
HDL-C (mg/dL)	70	134.2	(68.2)	33	55.3	(12.9)	37	61.7	(11.1)*
LDL-C (mg/dL)	70	58.7	(12.3)	33	125.1	(27.9)	37	127.3	(26.6)
Triglycerides (mg/dL)	70	126.2	(27.1)	33	144.8	(83.7)	37	124.8	(49.9)
LDL-C/HDL-C	70	2.31	(0.90)	33	2.45	(0.96)	37	2.18	(0.84)
Triglycerides/ HDL-C	70	2.57	(1.92)	33	3.02	(2.39)	37	2.16	(1.25)
MetScore	66	−0.0002	(0.3414)	30	0.0187	(0.3836)	36	−0.0160	(0.3065)
Dietary Intake									
Total Energy (kcal/day)	72	2094.9	(478.8)	34	2302.8	(466.6)	38	1909.0	(413.0)*
Fat (g/day)	72	87.5	(25.0)	34	97.6	(24.4)	38	78.4	(22.2)*
Carbohydrate (g/day)	72	218.3	(70.2)	34	238.3	(75.0)	38	200.4	(61.1)*
Protein (g/day)	72	89.0	(34.5)	34	92.4	(30.8)	38	86.1	(37.6)
Ethanol (g/day)	72	11.2	(13.2)	34	16.2	(16.3)	38	6.6	(7.2)
Vitamin D (μg/day)	72	5.0	(6.0)	34	3.8	(3.3)	38	6.1	(7.6)
Calcium (mg/day)	72	763.4	(340.5)	34	867.3	(396.9)	38	670.5	(251.4)*
Phosphorus (mg/day)	72	1324.7	(558.9)	34	1507.6	(689.6)	38	1161.0	(342.2)*
Physical activity levels									
Sedentary time (min/day)	70	745.9	(84.8)	33	770.7	(81.4)	37	723.7	(82.6)*
Total physical activity (min/day)	70	269.5	(75.1)	33	265.2	(79.3)	37	273.3	(72.0)

Data are shown as means (standard deviation). Abbreviations: QUICKI, quantitative insulin sensitivity check index; HOMA-IR, homeostasis model assessment for insulin resistance index; HDL—C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; MetScore, cardiometabolic risk score; PA, physical activity.

across the world [4, 22,29,34,54], which is mainly due to a decreased capacity to synthesize vitamin D from sunlight as well as increased body adiposity or low physical activity levels [22,24,57]. Vitamin D status has been also linked with a range of extra-skeletal properties (*e.g.* muscle function, cardiovascular homeostasis, nervous function, and immune response) beyond its key role on calcium/phosphate homeostasis [36,64]. In this sense, a growing body of scientific works investigating the physio-pathological mechanisms behind cardiometabolic disorders has suggested that vitamin D deficiency could play a key role on its development [39,45,50,61].

Previous studies have examined whether vitamin D deficiency -routinely measured as 25(OH)D– is associated with a higher risk of suffering cardiometabolic disease, with controversial findings [12,17,31,45,48,55]. However, considerably less attention has been paid to the relationship between the biologically active form of vitamin D (*i.e.,* $1,25(OH)_2D$) and cardiometabolic risk factors. Concretely, low $1,25(OH)_2D$ levels have been linked with glycaemic and lipid alterations in patients with psoriasis [49] and acute coronary syndrome [11]. It remains unclear, however, whether $1,25(OH)_2D$ levels are associated with cardiometabolic risk factors in healthy individuals [66]. Given that identifying new potential biomarkers to detect cardiometabolic alterations in still healthy subjects potentially allows the application of preventive strategies which are likely preferable to the treatment of cardiometabolic diseases already established. Therefore, since it seems of scientific importance to determine whether $1,25(OH)_2D$ levels are associated with cardiometabolic risk factors in individuals free of chronic diseases [14,15]. The present study is aimed to investigate the relationships of $1,25(OH)_2D$ plasma levels with cardiometabolic risk factors in a sample of healthy sedentary adults.

2. Materials and methods

2.1. Study design and participants

The present study analyzed data from a sample of healthy sedentary adults ($n = 73$ [~ 50% women]). The subjects included in this cross-sectional study were recruited from the FIT-AGEING study, a randomized controlled trial (clinicaltrial.gov: ID: NCT03334357), *via* social networks, electronic media, and leaflets. Data from the baseline assessment were collected during September–October 2016/17 at the Sport and Health University Research Institute (iMUDS, Granada, Spain) and at the "Campus de la Salud" Hospital (Granada, Spain) and, subsequently used for the current study. Details concerning to the study design, procedures, and inclusion/exclusion criteria have been described in detail elsewhere [2]. Briefly, the inclusion criteria were: (i) age between 45 and 65 years old, (ii) physically inactive (*i.e.,* <20 min on 3 days/week), (iii) stable body weight (*i.e.,* body weight changes <3 kg) during the previous 3 months, (iv) non-smoker, (v) non-pregnant, (vi) no long-term medications, and (vi) no cardiometabolic diseases. The FIT-AGEING study was approved by the Ethics Committee on

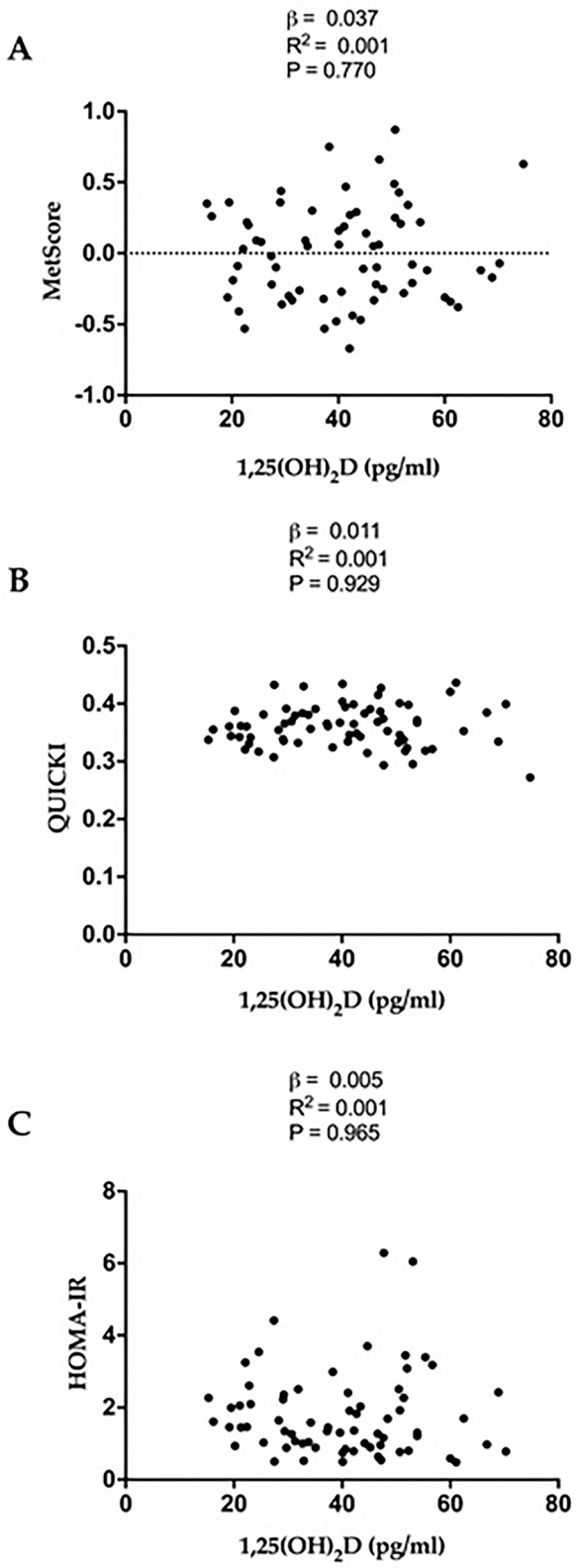

Fig. 1. Association of between $1,25(OH)_2D$ with cardiometabolic risk *Z*-score (MetScore), QUICKI and HOMA-IR index. β (standardized regression coefficient), R^2 and *P* values are for simple linear regression analyses.

Human Research at the University of Granada and the Regional Government of Andalucía [0838-N-2017]. The study protocols and experimental design were applied following the last revised ethical guidelines of the Declaration of Helsinki (last revision guidelines, 2013), with all participants providing written informed consent.

2.2. Anthropometric parameters and body composition

Body weight and height were measured using a Seca model 799 scale and stadiometer (Seca, Hamburg, Germany) to the nearest 0.1 kg and 0.1 cm, respectively, with participants wearing lightweight clothes and barefoot. Body mass index (BMI) was then calculated as weight (kg)/height $(m)^2$. Waist circumference (WC) was registered according to the standard procedures of the International Society for the Advancement of Kinanthropometry (ISAK) [42], and assessed in a standing position from the mid-point between the bottom of the rib cage and the iliac crest at the end of a normal expiration. Body composition analysis was performed using a dual-energy X-ray absorptiometer scanner (Discovery Wi, Hologic, Inc., Bedford, MA, USA), obtaining lean and fat body mass in kg following the manufacture's recommendations. From these measurements, fat BMI (FMI) and lean BMI (LMI) were calculated by the following equations:

$$\text{FMI} = \text{fat body mass [kg]}/\text{height}^2\ \text{[m]})$$

$$\text{LMI} = \text{lean body mass [kg]}/\text{height}^2\ \text{[m]})$$

2.3. Blood pressure (BP)

Systolic and diastolic BP (SBP; DBP) were measured with an Omrom® HEM 705 CP device (Omrom Health-care Co, Kyoto, Japan), an automated oscillometric sphygmomanometer that uses an upper arm cuff. The measurements were taken from the right arm with participants sitting and rested, following the most updated recommendations of the European Heart Society [62]. Readings were taken twice and the mean was subsequently calculated and used for further analysis. Mean BP was calculated using the following formula: (SBP – (DBP/3)) [62].

2.4. Blood samples

The blood samples were taken from the antecubital vein in the morning ($8{:}30_{\text{AM}} - 10_{\text{AM}}$) after overnight fasting and collected using the Vacutainer SST system (Becton Dickinson, Plymouth, UK) in ethylenediamine tetra-acetic acid-containing tubes. All samples were centrifuged at 4000 rpm for 7 min at 4 °C, aliquoted, and stored at −80 °C until further analyses. $1,25(OH)_2D$ plasma levels were measured using a DiaSorin Liaison® immunochemiluminometric analyzer (DiaSorin Ltd., Wokingham, Berkshire, UK) and expressed in pg/mL. Plasma glucose, triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL-C), alanine transaminase (ALT), and γ-glutamyl transferase (γ-GT) were determined using an AU5800 absorption spectrophotometer (Beckman Coulter, Brea, CA, USA). Plasma insulin was assessed by chemiluminescence immunoassay using a UniCel DxI 800 paramagnetic particles (Beckman Coulter, Brea, CA, USA). Low-density lipoprotein cholesterol (LDL-C) was calculated according to the following equation: LDL-C = (total cholesterol) – (HDL-C) – 0.45 x (triglycerides). Additionally, insulin/glucose, LDL-C/HDL-C, and triglycerides/HDL-C ratios were also calculated.

All blood samples were measured in the same laboratory located within the "Campus de la Salud" Hospital (Granada, Spain). All participants were requested to abstain from drugs and/or caffeine 24 h before blood extraction, to refrain from any physical activity (PA) at moderate intensity (24 h before) and/or vigorous intensity (48 h before), and to eat a standardized dinner (*i.e.,* egg omelette, boiled rice, and tomato sauce).

Table 2
Hierarchical regression between 1,25(OH)$_2$D levels with MetScore, QUICKI, and HOMA-IR.

	MetScore				QUICKI				HOMA-IR			
	β	R^2 change	Sig. F change	p	β	R^2 change	Sig. F change	p	β	R^2 change	Sig. F change	p
1,25(OH)$_2$D (pg/ml)												
Age (years)	0.183	0.151	**0.002**	0.17	−0.315	0.112	**0.006**	**0.02**	0.174	0.094	**0.012**	0.11
Sex	−0.447	0.001	0.784	**0.04**	0.252	0.001	0.871	0.24	−0.473	0.001	0.856	0.31
Body mass index (kg/m^2)	−2.302	0.037	0.108	0.10	0.269	0.055	**0.045**	0.86	−0.438	0.005	0.565	0.89
Fat mass index (kg/m^2)	2.295	0.144	**0.001**	**0.045**	−0.417	0.017	0.254	0.73	0.545	0.013	0.339	0.86
Lean mass index (kg/m^2)	1.404	0.027	0.129	0.18	−0.017	0.001	0.751	0.99	0.047	0.006	0.536	0.93
Total energy intake (kcal/day)	−0.200	0.017	0.227	0.12	0.160	0.037	0.095	0.22	−0.268	0.012	0.374	0.48
Vitamin D intake (μg/day)	0.165	0.016	0.239	0.23	0.228	0.037	0.092	0.09	−0.173	0.021	0.235	0.19
Total physical activity time (min/day)	−0.064	0.004	0.557	0.56	−0.180	0.031	0.115	0.12	0.067	0.016	0.303	0.23
Total sedentary time (min/day)	0.017	0.004	0.841	0.95	−0.052	0.032	0.286	0.85	0.120	0.026	0.413	0.40

Abbreviations: MetScore, cardiometabolic risk score; QUICKI, Quantitative insulin sensitivity check index; HOMA-IR, homoeostasis model assessment for insulin resistance index. β: Standardized regression coefficient; R^2; and p value were obtained from the hierarchical multiple linear regression analyses.

2.5. Cardiometabolic risk score

A sex-specific cardiometabolic risk score (MetScore) was calculated for each participant based on the clinical guidelines proposed by the International Diabetes Federation according to the following factors: WC, mean BP, plasma glucose, HDL-C, and TGs [6]. Standardized values were calculated for each variable as follows: Standardized values = (value – mean/standard deviation). The standardized HDL-C values were multiplied by −1 to indicate greater risk with higher values. MetScore was determined as the sum of these 5 standardized values divided by 5, to account for the number of variables included. This approach results in a continuous MetScore with a mean of 0 and a standard deviation of 1 by definition, considering lower values as a representation of a better cardiometabolic risk profile.

Quantitative insulin sensitivity check index (QUICKI) [26] was calculated from plasma insulin and glucose levels as:

$$\text{QUICKI} = 1/[log(\text{plasma insulin (UI/mL)}) + log(\text{plasma glucose (mg/dL)})]$$

The homeostasis model assessment for insulin resistance index (HOMA-IR) [35] was calculated as:

$$\text{HOMA} - \text{IR} = \text{plasma insulin (UI/mL)} \text{ x plasma glucose (nmol/L)}/22.5$$

2.6. Dietary intake

Dietary intake was collected *via* three 24-h recalls on non-consecutive days (*i.e.,* 2 days during the week and 1 day on the weekend) by qualified and trained dietitians through face-to-face interviews. The interviews were meal sequence-based where the subjects were asked to describe the different portion sizes of each food item they consumed using a colored photograph guide [32]. Energy, macronutrient, and micronutrient intake derived from food consumption were calculated using the EvalFINUT® software (FINUT, Granada, Spain), which is based on the USDA (United States Department of Agriculture) and BEDCA ("Base de Datos Española de Composición de Alimentos") databases.

2.7. Sedentary behaviour (SB) and PA

Objectively measured SB and PA were assessed with a wrist-worn accelerometer (ActiGraph GT3X+, Pensacola, FL, United States) for seven consecutive days (24 h/day) [2]. Participants were requested to wear the accelerometers constantly, except during bathing or aquatic activities such as swimming. The ActiGraph sampling frequency was initialized to store raw acceleration information at a rate of 100 Hz [37]. The accelerometry data collection were exported and processed using the ActiLife v.6.13.3 software (ActiGraph, Pensacola, FL, United States) and the GGIR package (v.1.5-12, https://cran.r-project.org/web/packages/GGIR/) in R software (v.3.1.2, https://www.cran.r-project.org/) [19,20]. Time spent at various levels of movement intensity (*i.e.,* moderate-vigorous) was determined according to age-specific cut-points for Euclidean Norm Minus One [19]. Data from participants with at least 16 h of daily accelerometer wear time for 4 days (including 1 weekend day) were included in the analyses.

2.8. Statistical analyses

The Shapiro-Wilk test, visual check of histograms, Q-Q, and box plots were used to verify the distribution of all variables. The descriptive parameters are reported as mean and standard deviation. Sex differences for each variable were performed using an unpaired sample *t*-test. There were no significant sex interactions between 1,25(OH)$_2$D plasma levels and all cardiometabolic risk factors (all $p > 0.05$). The analyses were thus performed including both men and women together.

We conducted simple linear regression models to examine the association between 1,25(OH)$_2$D plasma levels and MetScore, QUICKI, and HOMA-IR. Hierarchical regression analyses were subsequently performed in order to check whether 1,25(OH)$_2$D plasma levels predict the above-mentioned variables independently of potential confounders based on theoretical and statistical considerations. The entry order of potential confounder in the hierarchical analysis were as follows: age, sex, BMI, FMI, LMI, total energy intake, vitamin D intake, total PA and SB. Multiple linear regression analyses were built using the derived confounders from the hierarchical regression analyses. Similar analyses were conducted to study the association between 1,25(OH)$_2$D plasma levels and the remaining cardiometabolic risk factors.

All analyses were performed using the Statistical Package for Social Sciences (SPSS, v. 22.0, IBM SPSS Statistics, IBM Corporation, Armonk, NY, USA). Graphical plots were generated using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). Statistical significance was defined as p values <0.05 for all analyses.

3. Results

The baseline characteristics of the participants are shown in Table 1. No significant differences were observed in 1,25(OH)$_2$D plasma levels between men and women ($p = 0.58$).

Simple linear regression analysis revealed no associations of 1,25(OH)$_2$D plasma levels with MetScore ($\beta = 0.037$, $R^2 = 0.001$, $p = 0.77$; Fig. 1A), QUICKI ($\beta = 0.011$, $R^2 = 0.001$, $p = 0.93$; Fig. 1B) and HOMA-IR ($\beta = 0.005$, $R^2 = -0.015$, $p = 0.97$; Fig. 1C).

Based on this hierarchical regression, we discarded LMI, total energy

Table 3
Association between $1,25(OH)_2D$ and cardiometabolic risk factors.

	All (n = 73)		
	β	R^2	p
Weight (kg)			
Model 0	−0.217	0.047	0.07
Model 1	−0.054	0.050	0.08
Model 2	−0.088	0.593	0.29
Model 3	−0.030	0.800	0.59
Waist circumference (cm)			
Model 0	**−0.303**	**0.092**	**0.01**
Model 1	**−0.296**	**0.093**	**0.01**
Model 2	**−0.197**	**0.457**	**0.03**
Model 3	**−0.125**	**0.777**	**0.04**
Systolic blood pressure (mm Hg)			
Model 0	0.166	0.028	0.18
Model 1	0.076	0.369	0.45
Model 2	0.107	0.467	0.26
Model 3	0.100	0.468	0.31
Diastolic blood pressure (mm Hg)			
Model 0	**0.262**	**0.069**	**0.03**
Model 1	0.185	0.324	0.08
Model 2	**0.208**	**0.380**	**0.045**
Model 3	**0.212**	**0.380**	**0.048**
Mean blood pressure (mm Hg)			
Model 0	0.217	0.047	0.08
Model 1	0.128	0.378	0.21
Model 2	0.157	0.464	0.10
Model 3	0.155	0.464	0.12
Glucose (mg/dL)			
Model 0	−0.008	<0.001	0.95
Model 1	−0.006	<0.001	0.96
Model 2	0.010	0.016	0.93
Model 3	0.036	0.036	0.78
Insulin (UI/mL)			
Model 0	0.185	0.034	0.13
Model 1	0.133	0.134	0.25
Model 2	0.147	0.145	0.21
Model 3	0.193	0.207	0.10
Insulin glucose ratio			
Model 0	0.147	0.022	0.23
Model 1	0.085	0.165	0.46
Model 2	0.091	0.167	0.43
Model 3	0.135	0.225	0.24
Total cholesterol (mg/dL)			
Model 0	**0.267**	**0.071**	**0.03**
Model 1	0.203	0.224	0.07
Model 2	0.173	0.273	0.11
Model 3	0.203	0.301	0.06
Triglycerides (mg/dL)			
Model 0	0.080	0.006	0.51
Model 1	0.017	0.155	0.88
Model 2	0.028	0.163	0.81
Model 3	0.052	0.179	0.66
HDL-C (mg/dL)			
Model 0	−0.182	0.033	0.13
Model 1	−0.103	0.265	0.34
Model 2	−0.129	0.303	0.22
Model 3	−0.138	0.305	0.20
LDL-C (mg/dL)			
Model 0	0.124	0.0.015	0.31
Model 1	0.038	0.296	0.72
Model 2	0.020	0.313	0.85
Model 3	0.034	0.319	0.74
LDL-C/HDL-C			
Model 0	0.172	0.030	0.15
Model 1	0.081	0.342	0.43
Model 2	0.089	0.346	0.38
Model 3	0.110	0.359	0.29
Tryglycerides/HDL-C			
Model 0	0.102	0.010	0.40
Model 1	0.030	0.200	0.78
Model 2	0.052	0.226	0.64
Model 3	0.073	0.239	0.52

Linear regression analyses were performed, unadjusted (Model 0), adjusting for age (Model 1), age and sex (Model 2), age, sex and FMI (Model 3). Standardized β regression coefficient, adjusted R^2, and *p* value of multiple-regression analysis are provided. Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

intake, vitamin D intake, total PA and SB as confounders variables (all $p > 0.05$ and all Sig. F change >0.05), only including age, sex, BMI, and FMI as potential confounders (Table 2). The results persisted when the analyzes were adjusted for age, sex, BMI, and/or FMI (Table S1; all $p \geq 0.59$).

Table 3 shows the associations between $1,25(OH)_2D$ and cardiometabolic risk factors. A significant slightly negative association was observed between $1,25(OH)_2D$ and waist circumference ($\beta = -0,303$, $R^2 = 0.092$, $p = 0.01$), which remained statistically significant after adjusting for age, sex, and FMI (Table 3; all $p \leq 0.04$). There was a significant slightly positive association between $1,25(OH)_2D$ and DBP ($p = 0.03$), which was partially attenuated after adjusting for potential confounders (all $p \leq 0.09$; Table 3). Similarly, we found a significant positive association between $1,25(OH)_2D$ and total cholesterol ($\beta = 267$, $R^2 = 0.071$, $p = 0.03$), which was attenuated once age, sex and FMI were included in the model (all $p \leq 0.11$). No significant association was found between $1,25(OH)_2D$ and others cardiometabolic risk factors (Table 3; all $p > 0.05$).

4. Discussion

The current study sought to elucidate whether $1,25(OH)_2D$ plasma levels are related to cardiometabolic risk factors in sedentary adults free of chronic cardiometabolic diseases. Our results show that $1,25(OH)_2D$ plasma levels are associated with neither the MetScore nor insulin resistance in healthy sedentary adults. However, we observed that higher $1,25(OH)_2D$ plasma levels were consistently associated with low central adiposity/WC in our study sample. These findings support the idea that although $1,25(OH)_2D$ has been proposed as a key factor affecting cardiometabolic health in patients with chronic diseases [11,49], it seems that $1,25(OH)_2D$ plasma levels are not related to cardiometabolic risk factors in healthy individuals with adequate values of these physiological parameters.

$1,25(OH)_2D$ plays a crucial role in mineral homeostasis and skeletal health being its deficiency classically related to rickets in children and osteomalacia in adults (M. F. [21]). Although its main function on the skeletal system is to modulate calcium and phosphorus metabolism through bone resorption, renal retention or intestinal absorption, vitamin D metabolites also exert important physiological functions in other tissues (P. E. [41]). Indeed, previous studies have reported its implication on several chronic pathologies (*e.g.* skin and autoimmune disorders, cancer, T2DM, hypertension, or CVD) [51].

Vitamin D deficiency is currently considered as a serious global problem [38] being the lower skin synthesis (as a consequence of the ageing process) and others environmental (*e.g.* sunlight exposure, season, diet or geographical localization) factors its main cause ([7]; M. F. [21]). It has been reported that the prevalence of vitamin D deficiency depends on age, gender, geographical latitude or ethnicity (M. F. [21,60]). Concretely, an increased incidence of vitamin D deficiency has been described in elderly individuals with CVD [28]. However, excessive levels of vitamin D have been also associated with CVD-related problems [8] including hypercalcemia, hypercalciuria, and kidney stones, among others [28,33,53].

Several molecular and physiological pathways have been described as an explanation of the mechanistic basis of the influence of $1,25(OH)_2D$ on cardiovascular function (P. E. [41]). Experimental studies have demonstrated the important role of $1,25(OH)_2D$ on the immune and inflammatory system during the pathogenesis of CVD, such as atherosclerosis, aneurysm development, and other inflammatory vascular diseases [10,52,58]. Specifically, Beilfuss et al. showed that vitamin D supplementation produced a significant reduction of IL-6 plasma levels in overweight individuals [5]. Moreover, Amer and

Qayyum reported a negative association between 25(OH)D circulating levels and C-reactive protein concentrations in apparently healthy adults suggesting its important influence on T cell regulation [3]. On the other hand, it has been reported that $1,25(OH)_2D$ exerts a direct effect on lipid profile (*i.e.*, *via* reducing triglyceride levels or ApoA1 expression) or indirectly by defeating lipolysis through decreasing parathyroid hormone release [47,59,65,66]. Furthermore, $1,25(OH)_2D$ also inhibits foam cell formation increasing cholesterol efflux [43].

Playford et al. demonstrated that circulating $1,25(OH)_2D$ levels were inversely associated with markers of visceral adiposity, vascular uptake of F-fluorodeoxyglucose (FDG), and coronary plaque burden independently of cardiometabolic risk factors in patients with psoriasis [49], which partially concur with our current findings. However, while we showed a positive association between $1,25(OH)_2D$ plasma levels and low central adiposity in sedentary but healthy individuals, no significant relationships were obtained between $1,25(OH)_2D$ plasma levels and neither the MetScore nor insulin resistance in our study sample. The presently observed lack of associations might be explained because our study subjects were healthy individuals with $1,25(OH)_2D$ and cardiometabolic risk-related factors within normal ranges and the relatively low duration of the intervention [1]. The $1,25(OH)_2D$ normal values obtained in our study sample could be a consequence of their higher sun exposure - blood samples at the baseline were collected in September in the south of Spain- compared with those obtained by other people living in countries far from the equator [56]. Therefore, a potential explanation of why $1,25(OH)_2D$ levels were related to waist circumference but not to MetScore may be that an increased waist circumference seems to be the prelude to the development of further cardiometabolic risk factors and insulin resistance.

4.1. Limitations

The present study, however, suffers from several limitations. Firstly, the cross-sectional design precluded us from making causal conclusions about the association of $1,25(OH)_2D$ plasma levels with cardiometabolic risk factors. Secondly, based on the inclusion criteria of the present study, our findings only apply for healthy sedentary adults (45–65 years old); hence, they may not be generalizable to other populations, such as older, younger, trained, and/or diseased individuals. Thirdly, we have no data on 25-hydroxyvitamin D plasma levels, which would be desirable to well-understand our study findings. Finally, since the relatively small sample size of the present study, the data should be interpreted with caution.

5. Conclusions

In summary, the present results suggest that $1,25(OH)_2D$ plasma levels are not associated with neither cardiometabolic risk factors nor insulin resistance in healthy sedentary adults, independently of several confounders. However, an inverse association of $1,25(OH)_2D$ plasma levels with central adiposity was observed in our study sample. These results have important clinical implications since they suggest that $1,25(OH)_2D$ seems to be related to central adiposity in healthy individuals with normal values of these physiological parameters but do not to others key cardiometabolic risk factors. Our study therefore highlights the importance of including the measurement of $1,25(OH)_2D$ when investigating the effects of sunlight exposure of vitamin D supplementation on the prevention and/or treatment of CVD.

Author contributions

AOP, LJF, MJC, AGS and FJAG conceived and designed the study; AOP, LJF, MJC, AGS and FJAG acquired data; AOP and FJAG, elaborated the statically section; AOP and FJAG, drafted, and CJL, MJC, AGS and FJAG revised the manuscript; all authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2021.10.015.

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