Vitamin D status and circulating biomarkers of endothelial dysfunction and inflammation in non-diabetic obese individuals: a pilot study

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Abstract

Introduction: Obesity and inadequate vitamin D status are associated with endothelial dysfunction and cardiovascular disease. We evaluated the associations between vitamin D status (i.e. serum levels of 25-hydroxyvitamin D (25(OH)D)), biomarkers of endothelial dysfunction (i.e. serum concentrations of soluble intercellular adhesion molecule 1 (sICAM-1) and soluble E-selectin (sE-selectin)), inflammatory markers (i.e. high-sensitivity C-reactive protein (hsCRP) and fibrinogen) and cardiometabolic risk factors.

Material and methods: Fifty obese (body mass index (BMI) ≥ 30 kg/m^2) non-diabetic adults (mean age: 36.2 ±5.4 years) without pre-existing cardiovascular abnormalities and 25 clinically healthy, normal weight and age-matched individuals were included. Anthropometric parameters, markers of glucose and lipid metabolism, and serum levels of inflammatory and endothelial dysfunction biomarkers were assessed in all subjects.

Results: The mean serum 25(OH)D level was significantly lower in the obese group than in controls (33.5 ±15.2 vs. 60.1 ±23.1 nmol/l; $p < 0.001$). In the obese group, sE-selectin (36.4 (32.1–47.2) vs. 32.4 (24.6–35.5) ng/ml, $p < 0.05$) and hsCRP (6.0 ±3.4 vs. 3.5 ±1.0 mg/l, $p < 0.05$) were significantly higher in individuals with lower than median vitamin D levels (i.e. 31 nmol/l) compared with those with higher vitamin D levels. In multivariable linear regression analysis, hsCRP ($\beta = -0.43$; $p < 0.001$) and sE-selectin ($\beta = -0.30$; $p = 0.03$) were independently and significantly associated with serum 25(OH)D levels in the obese group.

Conclusions: Vitamin D levels may be related to increased levels of biomarkers of endothelial dysfunction and inflammation in obese non-diabetic individuals.

Key words: vitamin D, endothelial dysfunction, inflammation, obesity, cardiometabolic risk factors.

Introduction

Excessive food intake, genetic predisposition and lack of physical activity are the main causes of obesity [1]. Visceral depots contribute to a low-grade, chronic inflammatory status, involved in the pathogenesis of insulin resistance and obesity-related diseases such as type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) [2, 3]. This chronic inflammation includes phenotypic conversion of endothelial cells (ECs) into an active form with consequent interactions with circulating leukocytes [4]. Leukocyte-endothelium interactions (rolling, adherence and extravasation) are mediated by cell adhesion molecules expressed on the endothelial cell surface such as the selectin family of adhesion molecules including E-selectin and the immunoglobulin family of adhesion molecules including intercellular adhesion molecule 1 (ICAM-1) [1, 4]. The prolonged exposure of vascular endothelium to cardiometabolic risk factors may lead to continuous endothelial activation and dysfunction [5–10]. In this context, the clinical manifestation of the endothelial phenotype depends on the balance between exposure to risk factors and endothelial repair [6].

Vitamin D has a potential protective effect on the vascular endothelium [11]. Endothelial cells express Vitamin D receptors and 1α-hydroxylase activity, thus allowing autocrine production of 1,25-dihydroxyvitamin D (1,25(OH)D_2), the biologically active form of vitamin D [12, 13]. Apart from the capacity to modulate the effects of proinflammatory cytokines on the vascular endothelium and to decrease the expression of endothelial adhesion molecules, vitamin D may also exert antioxidant properties, and it may be involved in repairing damaged ECs [14]. In clinical studies, it has been proposed that vitamin D deficiency and/or insufficiency is associated with impaired endothelial function [6]. Furthermore, several studies have shown an inverse correlation between serum 25-hydroxyvitamin D (25(OH)D) levels and biomarkers of endothelial dysfunction in patients with hypertension, T2DM, coronary artery disease and stroke [15–17]. Vitamin D insufficiency is more prevalent in obese individuals [18–20]. Moreover, cross-sectional studies have reported inverse associations between low serum 25(OH)D levels and cardiometabolic risk factors (e.g. abdominal obesity, hypertension, hyperglycemia) [21, 22], which may also promote the development and progression of endothelial dysfunction in obese patients.

The main objective of this study was to determine the associations between vitamin D status (represented by serum 25(OH)D levels) and circulating biomarkers of endothelial dysfunction (serum concentration of soluble forms of endothelium-derived adhesion molecules, i.e. sICAM-1 and sE-selectin), inflammation (high-sensitivity C-reactive protein (hsCRP) and fibrinogen) and cardiometabolic risk factors (parameters of glucose and lipid metabolism) in obese non-diabetic middle-aged adults in the absence of pre-existing CVD.

Material and methods

Subjects

This cross-sectional pilot study was performed during 6 months (May to October 2012), at the Department of Endocrinology, Diabetes and Metabolic Disorders, Clinical Center of Vojvodina (CCV). We evaluated 50 (34 female) obese patients (body mass index (BMI) > 30 kg/m^2) with no pre-existing CVD, hypertension (systolic blood pressure (SBP) ≤ 130 mm Hg, diastolic blood pressure (DBP) ≤ 80 mm Hg) or T2DM (2 repeated measurements of fasting glucose levels < 7 mmol/l and glycated hemoglobin A_{1c} (HbA_{1c}) < 6.5%). Patients with hepatic, psychiatric or malignant disorders were excluded from the study. The control group consisted of 25 participants (20 female) with normal weight (BMI < 25 kg/m^2), clinically healthy and matched for age with the obese group. We also excluded smokers, subjects in acute stress situations or with infections (hsCRP > 10 mg/l), as well as those with recent (i.e. 3 months) weight changes, calcium level disturbances or treated with calcium or Vitamin D supplements 3 months prior to the study.

Obese patients (n = 50) were classified into 2 subgroups according to the median serum 25(OH)D levels for this group (i.e. 31 nmol/l).

The study was conducted according to the Declaration of Helsinki and approved by the Ethical Committees of CCV. Informed consent was obtained from every participant.

Study protocol

All participants attended the CCV outpatient clinic for anthropometric measurements and clinical examination; 24 h before blood sampling, participants were asked to refrain from strenuous physical activity and consuming alcoholic beverages or caffeine. Venous blood was drawn from an antecubital vein after a 12 h overnight fast. Analyses were performed immediately after sampling except for serum sICAM-1 and sE-selectin. They were centrifuged 15 min at 1000×g, and stored at –70°C for 1 month. Blood glucose and insulin levels were measured before (fasting plasma glucose (FPG) and fasting plasma insulin (FPI)) and 2 h after the ingestion of a 75 g oral glucose load (2 h plasma glucose (2hPG) and 2 h plasma insulin (2hPI)).

Anthropometric and clinical measurements

Body weight (BW, kg), fat adipose tissue percentage (FAT%) and trunk fat mass percentage

(FAT trunk %) were determined by the bioelectrical impedance method (Tanita TBF-310 Body Composition Analyzer; Tanita Corporation, Tokyo, Japan). Body height (BH, m) was measured using a Harpenden anthropometer (Holtain Ltd, Croswell, UK). Body mass index was calculated as BW/BH^2 (kg/m^2). WC was measured using a tape to 0.1 cm. Blood pressure was measured according to a standard protocol (Riva Rocci method, mercury sphygmomanometer), in the sitting position after a 10 min rest.

Analytical procedures

Standard methods were used to assess blood glucose levels (hexokinase method), insulin levels (chemiluminescent immunoassay, Siemens Advia Centaur XP), HbA_{1c} (immuno-inhibitory test), hsCRP levels (Olympus AU 400, Beckman-Coulter, Ireland), fibrinogen (ACL system, Instrumentation Laboratory, Italy) and lipids (Olympus AU 400, Beckman-Coulter, Ireland). Serum 25(OH)D concentrations were measured by the electrochemiluminescence immunoassay using an automated clinical chemistry analyzer (Elecsys 2010, Roche Diagnostics); sICAM-1 and sE-selectin levels were measured by the Quantikine Human sICAM-1 immunoassay and Quantikine Human sE-Selectin immunoassay, ELISA, respectively (R&D Systems, Minneapolis, USA), according to the manufacturers' instructions.

Insulin resistance was defined as HOMA-IR ≥ 2.5 [23]. Hypertriglyceridemia (i.e. triglycerides (TG) ≥ 1.7 mmol/l), low high-density lipoprotein cholesterol (HDL-C) (i.e. HDL-C ≤ 1.0 mmol/l in men and ≤ 1.3 mmol/l in women) and central obesity (i.e. WC ≥ 102 cm in men and ≥ 88 cm in women) were recorded for each patient [24].

Statistical analysis

Normal distribution of continuous variables was assessed with the Shapiro-Wilk test. Data were presented as mean ± standard deviation for normally distributed continuous variables and median (interquartile range) for non-parametric continuous variables, while categorical data were presented as percentages. Parametric (*t*-test) and non-parametric (Mann-Whitney) statistical tests were used. The χ^2 test was used for categorical variables. Correlations between 25(OH)D levels and other variables were evaluated by Pearson or Spearman coefficients. All correlations were weighted by BMI. Further, we performed a multivariate regression analysis to assess the associations between serum 25(OH)D levels, biomarkers of inflammation and endothelial dysfunction. Regression analysis was adjusted for BMI by adding it to the regression model. Statistical analysis was performed using MedCalc 12.1.4.0 statistical software (MedCalc Software, Mariakerke, Belgium). Differences were considered significant if p (2-tailed) < 0.05.

Results

Comparisons of anthropometric characteristics, biochemical measurements and biomarkers of inflammation and endothelial dysfunction between groups are shown in Table I. Significant differences between the obese and control group were observed in all studied variables except for DBP, FPG and 2hPG. The mean serum 25(OH)D level was significantly lower in the obese group than in controls (33.5 ±15.2 vs. 60.1 ±23.1 nmol/l; $p < 0.001$).

Regarding HOMA-IR, the prevalence of insulin resistance among obese subjects was 78%. The prevalence of hypertriglyceridemia (4% vs. 36%, $p = 0.03$) and low HDL-C levels (8% vs. 41%, $p = 0.04$) was significantly lower in controls compared with the obese group.

In the obese group (Table II), BMI, 2hPG, hsCRP and sE-selectin levels were significantly higher in those patients with 25(OH)D < 31 nmol/l compared with those with 25(OH)D ≥ 31 nmol/l ($p < 0.05$ for all comparisons). The prevalence of insulin resistance (72% vs. 60%, $p = 0.85$), hypertriglyceridemia (32% vs. 40%, $p = 0.89$) and low HDL-C levels (56% vs. 41%, $p = 0.93$) did not differ significantly between the 2 subgroups of obese patients.

Correlation analysis showed significant negative correlations between serum 25(OH)D levels and BMI ($r = –0.33$, $p = 0.02$), WC ($r = –0.25$, $p = 0.03$), FAT trunk % ($r = –0.19$, $p = 0.03$), sE-selectin ($r = –0.41$, $p = 0.01$), sICAM-1 ($r = –0.30$, $p = 0.02$), hsCRP ($r = –0.46$, $p = 0.01$) and HOMA-IR ($r = –0.29$, $p = 0.03$) in all (obese and control subjects) patients. There were no significant correlations between serum 25(OH)D levels, fibrinogen and lipid parameters.

In linear regression analysis serum 25(OH)D levels were significantly associated with hsCRP ($\beta = –0.49$; $p = 0.00$), sE-selectin ($\beta = –0.32$; $p = 0.03$), sICAM ($\beta = –0.12$; $p = 0.04$) and BMI ($\beta = –0.25$; $p = 0.02$) in the obese group. In contrast, the association between serum 25(OH) level and HOMA-IR was insignificant among obese patients. In obese patients, the association between serum 25(OH)D levels and biomarkers, hsCRP ($\beta = –0.43$; $p = 0.00$) and sE-selectin ($\beta = –0.30$; $p = 0.03$) was confirmed in a multivariable (stepwise) linear regression analysis ($R^2 = 0.40$, R^2 adjusted = 0.38) after including BMI ($p = 0.02$) in the analysis. Accordingly, per unit increase in serum 25(OH)D (nmol/l) levels we observed a corresponding significant reduction of hsCRP (0.43 mg/l) and sE-selectin (0.30 ng/ml).

Table I. Study population characteristics

Parameter	Obese (n = 50)	Control (n = 25)	P-value
Age [years]	36.2 ±5.4	34.3 ±3.6	0.09
Gender (m/f)	16/34	13/12	0.15
BMI [kg/m^2]	40.8 ±8.5	22.8 ±2.3	< 0.001
WC [cm]	127.6 ±19.2	82.0 ±9.3	< 0.001
FAT trunk (%)	38.6 ±7.6	18.9 ±6.2	< 0.001
SBP [mm Hg]	123 ±9	114 ±9	< 0.001
DBP [mm Hg]	81 ±5	79 ±4	0.79
FPG [mmol/l]	5.0 ±1.2	4.7 ±0.4	0.41
2hPG [mmol/l]	5.7 ±1.9	5.0 ±1.1	0.21
FPI [mU/ml]	18.3 ±11.8	6.8 ±4.4	< 0.001
2hPI [mU/ml]	44.7 ±34.1	20.8 ±21.4	< 0.001
HOMA-IR	4.2 ±2.9	1.5 ±1.0	< 0.001
HbA_{1c} [mmol/l]	35.3 ±5.6	31.2 ±1.9	< 0.001
LDL-C [mmol/l]	3.7 ±0.9	2.7 ±0.4	0.0001
HDL-C [mmol/l]	1.1 ±0.3	1.4 ±0.3	< 0.001
TG [mmol/l]	1.37 (1.08–1.77)	0.82 (0.59–1.04)	0.0005
hsCRP [mg/l]	5.1 (2.92–6.27)	0.2 (0.1–0.8)	< 0.001
Fibrinogen [g/l]	3.6 ±0.8	2.5 ±0.5	< 0.001
sE-selectin [ng/ml]	36 (29–44)	26 (23–31)	< 0.001
sICAM-1 [ng/ml]	263 (229–332)	185 (166–208)	< 0.001
25(OH)D [nmol/l]	33.5 ±15.2	60.1 ±23.1	< 0.001

Continuous variables are expressed as means ± SD or median (interquartile range) and categorical data as percentages (%). 25(OH)D – 25-hydroxyvitamin D, BMI – body mass index, WC – waist circumference, FAT trunk % – trunk fat mass percentage, SBP – systolic blood pressure, DBP – diastolic blood pressure, FPG – fasting plasma glucose, 2hPG – 2 h plasma glucose, FPI – fasting plasma insulin, 2hPI – 2h plasma insulin, HOMA-IR – homeostasis model assessment of insulin resistance, HbA_{1c} – glycated hemoglobin, LDL-C – low-density lipoprotein cholesterol, HDL-C – high-density lipoprotein cholesterol, TG – triglycerides, hsCRP – high-sensitivity C reactive protein, sE-selectin – soluble E-selectin, sICAM-1 – soluble intercellular adhesion molecule-1.

Discussion

The vascular endothelium is an important source of circulating soluble adhesion molecules [25, 26]. Elevated levels of these markers reflect endothelial dysfunction and can be used as surrogates for increased CVD risk [27]. Previous studies suggest that vitamin D deficiency is associated with a proinflammatory state in obesity [28, 29], but there are still confounding data with regard to the associations of vitamin D levels and certain biomarkers of endothelial dysfunction in obese non-diabetic patients without pre-existing CVD. Vitamin D insufficiency may also be associated with the metabolic syndrome [30].

In the present study, obese non-diabetic individuals had significantly lower serum 25(OH)D levels compared with normal weight controls as well as significantly increased sICAM-1, sE-selectin, hsCRP and fibrinogen levels. Furthermore, BMI, 2hPG, hsCRP and sE-selectin levels were significantly higher in the obese patients with 25(OH)D < 31 nmol/l than in those with 25(OH)D ≥ 31 nmol/l. Insulin resistance was frequent in the obese group (i.e. 78%). The prevalence of hypertriglyceridemia and low HDL-C levels was significantly higher in obese patients compared with controls. In contrast, the frequency of these metabolic disorders did not significantly differ between the 2 obese subgroups according to 25(OH)D levels (i.e. < or ≥ 31 nmol/l).

Obesity markers are important independent predictors of CV risk [3]. We found that BMI, but not WC, was significantly higher in obese patients with 25(OH)D levels < 31 nmol/l than in those obese with 25(OH)D ≥ 31 nmol/l. Although BMI, WC and FAT trunk % had a significant and inverse correlation with vitamin D levels in all studied sub-

Table II. Characteristics of the obese subjects according to median serum 25(OH)D levels (i.e. 31 nmol/l)

Parameter	Obese with 25(OH)D < 31 nmol/l (*n* = 25)	Obese with 25(OH)D ≥ 31 nmol/l (*n* = 25)	*P*-value
Age [years]	37.2 ±4.9	36.7 ±4.8	0.54
Gender (m/f)	18/7	13/12	0.24
BMI [kg/m^2]	43.2 ±10.2	37.5 ±4.9	0.03
WC [cm]	129.1 ±21.5	126.8 ±12.4	0.12
FAT trunk (%)	41.9 ±6.2	40.4 ±4.9	0.19
SBP [mm Hg]	126 ±10	125 ±7	0.26
DBP [mm Hg]	80 ±2	78 ±5	0.68
FPG [mmol/l]	5.0 ±1.0	4.7 ±0.6	0.16
2hPG [mmol/l]	5.5 ±2.0	5.0 ±1.3	0.02
FPI [mU/ml]	14.6 (11.2–23.4)	17.4 (13.2–24.6)	0.24
2hPI [mU/ml]	30.2 (22.8–68.4)	25.4 (20.8–65.9)	0.19
HOMA-IR	4.3 ±3.0	4.0 ±2.2	0.21
Prevalence of insulin resistance (%)	72	60	0.85
HbA_{1c} (%)	5.7 ±0.8	5.5 ±0.4	0.74
LDL-C [mmol/l]	3.6 ±0.9	3.5 ±0.7	0.36
HDL-C [mmol/l]	1.0 ±0.2	1.2 ±0.4	0.14
Prevalence of hypo-HDL cholesterol (%)	56	48	0.93
TG [mmol/l]	1.3 (0.7–1.9)	1.4 (1.0–1.9)	0.51
Prevalence of hypertriglyceridemia (%)	32	40	0.89
hsCRP [mg/l]	6.0 ±3.4	3.5 ±1.0	0.02
Fibrinogen [g/l]	3.9 ±0.7	3.3 ±1.0	0.62
sE-selectin [ng/ml]	36 (32–47)	32 (25–35)	0.04
sICAM-1 [ng/ml]	297 (239–340)	259 (223–326)	0.71
25(OH)D [nmol/l]	20.2 ±6.5	50.2 ±14.3	0.001

Continuous variables are expressed as means ± SD or median (interquartile range) and categorical data as percentages (%). 25(OH)D – 25-hydroxyvitamin D, BMI – body mass index, WC – waist circumference, FAT trunk % – trunk fat mass percentage, SBP – systolic blood pressure, DBP – diastolic blood pressure, FPG – fasting plasma glucose, 2hPG – 2h plasma glucose, FPI – fasting plasma insulin, 2hPI – 2h plasma insulin, HOMA-IR – homeostasis model assessment of insulin resistance, HbA_{1c} – glycated hemoglobin, LDL-C – low-density lipoprotein cholesterol, HDL-C – high-density lipoprotein cholesterol, TG – triglycerides, hsCRP – high-sensitivity C reactive protein, sE-selectin – soluble E selectin, sICAM-1 – soluble intercellular adhesion molecule-1. Insulin resistance was defined as HOMA IR ≥ 2.5. Hypertriglyceridemia was defined as TG ≥ 1.7 mmol/l. Hypo-HDL cholesterol was defined as HDL-C ≤ 1.0 mmol/l in men and ≤ 1.3 mmol/l in women.

jects, only BMI was significantly associated with 25(OH)D levels in obese patients. This finding is consistent with previous studies suggesting that an increase in BMI may decrease vitamin D levels in obese patients, and different mechanisms have been proposed to explain the relationship between obesity and low vitamin D status [18, 19, 21].

As previously shown, obese patients have elevated levels of soluble adhesion molecules in the presence of CV risk factors (e.g. insulin resistance, atherogenic dyslipidemia, hypertension, inflammation) [5, 9, 10, 26]. Considering the role of adhesions molecules in vascular inflammation and atherosclerosis, it has been suggested that levels of their soluble form may be a predictor of atherosclerotic CVD in apparently healthy men and women [31]. Additionally, a recent study suggested that an increase in serum level of the soluble form of endothelial cell-derived chemokines (CXCL16) could be associated with the presence of atherosclerotic cerebrovascular disease (ischemic stroke) [32].

In the present study, obese subjects with a lower 25(OH)D level (< 31 mmol/l) had significantly higher sE-selectin levels. Although recent studies suggest that serum 25(OH)D levels are associated with vascular endothelial dysfunction (proinflammatory endothelial activation and vasodilator function) even in asymptomatic lean individuals [26, 27], BW can be a confounding factor for interpreting the impact of low vitamin D status on endothelial dysfunction in obesity. In previous studies with obese patients, BW and abnormal glucose metabolism were the main determinants of increased soluble adhesion molecule levels [9, 25, 28]. In our study, additionally to sE-selectin levels, only BMI and 2hPG were significantly higher in obese patients with lower vitamin D levels. Moreover, sE-selectin was inversely associated with 25(OH)D levels in obese patients, suggesting that low vitamin D status may promote endothelial dysfunction (proinflammatory endothelial activation/dysfunction) in obesity. Also, some studies have shown an inverse association between vitamin D level and parameters of glucose metabolism [20, 22], but our study did not demonstrate such a relationship.

Vitamin D insufficiency has been associated with higher endothelial activation and dysfunction [17]. According to some authors, vitamin D inadequacy in an adult, i.e. true insufficiency, exists when the level is < 37.5 nmol/l [33]. Precisely defining vitamin D insufficiency in obese subjects on the basis of serum 25(OH)D levels is still a matter of debate. Due to the lack of standardized cut-off points for 25(OH)D insufficiency in our country, and considering that the majority of obese subjects in general as in our study had a lower than optimal vitamin D serum level, we selected our analysis according to the median serum 25(OH)D nmol/l in the obese study group (i.e. 31 nmol/l). Additionally, according to the results of the first meta-analysis that assessed the association between obesity (i.e. different levels of BMI) and vitamin D deficiency, the cut-offs used for vitamin D deficiency were ≤ 25, ≤ 35 or ≤ 50 nmol/l [34].

We observed a negative association between sICAM-1 and vitamin D levels in obese individuals, but this association was not significant in multivariate analysis. The confounding effect of obesity could be a reason for this finding, since in the Framingham Offspring Study for every 5 kg/m^2 increase in BMI, sICAM-1 levels were raised by 5 ng/ml [35]. Some studies have reported that sICAM-1 levels may reflect its production not only by ECs outside adipose tissue, but also by adipocytes and macrophages within adipose tissue, thus creating a proinflammatory state in obesity [36]. Experimental data indicate that treatment with vitamin D may regulate the expression of adhesion molecules by decreasing ICAM-1 levels [37], though results from interventional studies are discordant regarding the potential effect of vitamin D supplementation in reducing the levels of endothelial dysfunction markers in obese individuals [38, 39].

In addition to increased levels of endothelial dysfunction biomarkers, obese patients in our study had significantly higher hsCRP levels compared with controls. Adipocytes, predominantly in the visceral compartment, may express and secrete proinflammatory cytokines, which induce a systemic acute-phase response via the production of acute-phase reactants in hepatocytes and ECs. Thus, adipose tissue mass may be an important mediator in the interaction between obesity and inflammation [40]. Also in ECs, acute-phase reactants activate nuclear factor κB (NF-κB), a proinflammatory transcription factor, which stimulates the production of proinflammatory cytokines and the expression of adhesion molecules such as ICAM-1 and E-selectin [17]. *In vitro* studies suggest that vitamin D may exert immunomodulatory effects and decrease CRP levels [41]. In the present study, we found significantly lower hsCRP levels in obese patients with ≥ 31 nmol/l than those with 25(OH)D < 31 nmol/l as well as an inverse association between 25(OH)D and hsCRP levels in the obese group. Furthermore, multivariate regression analysis suggesting an inverse association between hsCRP and sE-selectin with serum 25 (OH) D level could highlight the role of vitamin D bioavailability in the systemic and vascular endothelial inflammatory response in obese individuals. hsCRP is regarded as a better marker of CVD than soluble adhesion molecules, cytokines and other acute-phase reactants [42]. It is also important to consider that vitamin D insufficiency may affect adherence to statin therapy [43].

This study has several limitations. It is a cross-sectional pilot study with a small number of patients, and we did not have any specific information about sunlight exposure and dietary vitamin D. Comparisons with previous studies are limited by differences in population characteristics, vitamin D insufficiency cut-off points and assay methods.

In conclusion, adipose tissue and the endothelium maintain vascular homeostasis. Vitamin D insufficiency associated with obesity may further promote the inflammatory status and endothelial dysfunction, significantly increasing CV risk. Both obesity and vitamin D insufficiency/deficiency are modifiable factors with high prevalence in several countries [18, 44]. Further trials are needed to establish the clinical implications of preventing, or at least adequately treating, these chronic cardiometabolic abnormalities.

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Conflict of interest

The authors BI, ES, ZS, NEK and EI declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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References

1. Gregor MF, Hotamisligil GS. Inflammatory mechanisms in obesity. Annu Rev Immunol 2011; 29: 415-45.
2. Hotamisligil GS. Inflammation and metabolic disorders. Nature 2006; 444: 860-7.
3. Tchernof A, Despres JP. Pathophysiology of human visceral obesity: an update. Physiol Rev 2013; 93: 359-404.
4. Sun S, Ji Y, Kersten S, Qi L. Mechanisms of inflammatory responses in obese adipose tissue. Annu Rev Nutr 2012; 32: 261-6.
5. Parikh NI, Keyes MJ, Larson MG, et al. Visceral and subcutaneous adiposity and brachial artery vasodilator function. Obesity (Silver Spring) 2009; 17: 2054-9.
6. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. Circulation 2007; 115: 1285-95.
7. Bellien J, Thuillez C, Joannides R. Contribution of endothelium-derived hyperpolarizing factors to the regulation of vascular tone in humans. Fundam Clin Pharmacol 2008; 22: 363-7.
8. Goch A, Banach M, Mikhailidis DP, Rysz J, Goch JH. Endothelial dysfunction in patients with noncomplicated and complicated hypertension. Clin Exp Hypertens 2009; 31: 20-30.
9. de Jongh RT, Serne EH, Ijzerman RG, de Vries G, Stehouwer CD. Impaired microvascular function in obesity: implications for obesity-associated microangiopathy, hypertension, and insulin resistance. Circulation 2004; 109: 2529-35.
10. Jonk AM, Houben AJ, Schaper NC, et al. Obesity is associated with impaired endothelial function in the postprandial state. Microvasc Res 2011; 82: 423-9.
11. Molinari C, Uberti F, Grossini E, et al. 1alpha, 25-dihydroxycholecalciferol induces nitric oxide production in cultured endothelial cells. Cell Physiol Biochem 2011; 27: 661-8.
12. Polidoro L, Properzi G, Marampon F, et al. Vitamin D protects human endothelial cells from H2O2 oxidant injury through theErk/Erk-Sirt-1axis activation. J Cardiovasc Transl Res 2013; 6: 221-31.
13. Jablonski KL, Chonchol M, Pierce GL, Walker AE, Seals DR. 25-hydroxyvitamin D deficiency is associated with inflammation-linked vascular endothelial dysfunction in middle-aged and older adults. Hypertension 2011; 57: 63-9.
14. Uberti F, Lattuada D, Morsanuto V, et al. Vitamin D protects human endothelial cells from oxidative stress through autophagic and survival pathways. J Clin Endocrinol Metab 2014; 99: 1367-74.
15. Tarcin O, Yavuz DG, Ozben B, et al. Effect of vitamin D deficiency and replacement on endothelial function in asymptomatic subjects. J Clin Endocrinol Metab 2009; 94: 4023-30.
16. Al Mheid I, Patel R, Murrow J, et al. Vitamin D status is associated with arterial stiffness and vascular dysfunction in healthy humans. J Am Coll Cardiol 2011; 58: 186-92.
17. Oz F, Cizgici AY, Oflaz H et al. Impact of vitamin D insufficiency on the epicardial coronary flow velocity and endothelial function. Coron Artery Dis 2013; 24: 392-7.
18. Vanlint S. Vitamin D and obesity. Nutrients 2013; 5: 949-56.
19. Drincic AT, Armas LA, Van Diest EE, Heaney RP. Volumetric dilution, rather than sequestration best explains the low vitamin D status of obesity. Obesity (Silver Spring) 2012; 20: 1444-8.
20. Liu E, Meigs JB, Pittas AG, et al. Predicted 25-hydroxyvitamin D score and incident type 2 diabetes in the Framingham Offspring study. Am J Clin Nutr 2010; 91: 1627-33.
21. Stokić E, Kupusinac A, Tomić-Naglić D, et al. Obesity and vitamin D deficiency: trends to promote a more proatherogenic cardiometabolic risk profile. Angiology 2015; 66: 237-43.
22. Cheng S, Massaro JM, Fox CS, et al. Adiposity, cardiometabolic risk, and vitamin D status: the Framingham Heart Study. Diabetes 2010; 59: 242-8.
23. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28: 412-9.
24. Grundy SM, Cleeman JI, Merz CNB, et al. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. Circulation 2004; 110: 227-39.
25. Scalia R. The microcirculation in adipose tissue inflammation. Rev Endocr Metab Disord 2013; 14: 69-76.
26. Pontiroli AE, Pizzocri P, Koprivec D, et al. Body weight and glucose metabolism have a different effect on circulating levels of ICAM-1, E-selectin, and endothelin-1 in humans. Eur J Endocrinol 2004; 150: 195-200.
27. Ertek S, Akgül E, Cicero AF, et al. 25-Hydroxy vitamin D levels and endothelial vasodilator function in normotensive women. Arch Med Sci 2012; 8: 47-52.
28. Ngo DT, Sverdlov AL, McNeil JJ, Horowitz D. Does vitamin D modulate asymmetric dimethylarginine and C-reactive protein concentrations? Am J Med 2010; 123: 335-41.
29. Devaraj S, Kumaresan PR, Jialal I. C-reactive protein induces release of both endothelial microparticles and circulating endothelial cells in vitro and in vivo: further evidence of endothelial dysfunction. Clin Chem 2011; 57: 1757-61.
30. Katsiki N, Athyros VG, Karagiannis A, Mikhailidis DP. Characteristics other than the diagnostic criteria associated with metabolic syndrome: an overview. Curr Vasc Pharmacol 2014; 12: 627-41.
31. Mulvihill NT, Foley JB, Crean P, Walsh M. Prediction of cardiovascular risk using soluble cell adhesion molecules. Eur Heart J 2002; 23: 1569-74.
32. Ma A, Pan X, Xing Y, Wu M, Wang Y, Ma C. Elevation of serum CXCL16 level correlates well with atherosclerotic ischemic stroke. Arch Med Sci 2014; 10: 47-52.

33. Naumović N. Vitamin D: physiological importance. Med Pregl 2010; 63: 301-4.
34. Pereira-Santos M, Costa PR, Assis AM, Santos CA, Santos DB. Obesity and vitamin D deficiency: a systematic review and meta-analysis. Obes Rev 2015; 16: 341-9.
35. Pou KM, Massaro JM, Hoffmann U, et al. Visceral and subcutaneous adipose tissue volumes are cross-sectionally related to markers of inflammation and oxidative stress: the Framingham Heart Study. Circulation 2007; 116: 1234-41.
36. Bosanská L, Michalský D, Lacinová Z, et al. The influence of obesity and different fat depots on adipose tissue gene expression and protein levels of cell adhesion molecules. Physiol Res 2010; 59: 79-88.
37. Martinesi M, Treves C, Albasio G, Bagnoli S, Bonanomi AG, Stio M. Vitamin D derivatives induce apoptosis and downregulate ICAM-1 levels in peripheral blood mononuclear cells of inflammatory bowel disease patients. Inflamm Bowel Dis 2008; 14: 597-604.
38. Keogh JB, Brinkworth GD, Noakes M, Belobrajdic DP, Buckley JD, Clifton PM. Effects of weight loss from a very low-carbohydrate diet on endothelial function and markers of cardiovascular disease risk in subjects with abdominal obesity. Am J Clin Nutr 2008; 87: 567-76.
39. Wamberg L, Kampmann U, Stødkilde-Jørgensen H, Rejnmark L, Pedersen SB, Richelsen B. Effects of vitamin D supplementation on body fat accumulation, inflammation, and metabolic risk factors in obese adults with low vitamin D levels-Results from a randomized trial. Eur J Int Med 2013; 24: 644-9.
40. Rodríguez-Hernández H, Simental-Mendía LE, Rodríguez-Ramírez G, ReyesRomero MA. Obesity and inflammation: epidemiology, risk factors, and markers of inflammation. Int J Endocrinol 2013; 2013: 678159.
41. Chen N, Wan Z, Han SF, Li BY, Zhang ZL, Qin LQ. Effect of vitamin D supplementation on the level of circulating high-sensitivity C-reactive protein: a meta-analysis of randomized controlled trials. Nutrients 2014; 6: 2206-16.
42. Wilson PW, Pencina M, Jacques P, Selhub J, D'Agostino R Jr, O'Donnell CJ. C-reactive protein and reclassification of cardiovascular risk in the Framingham Heart Study. Circ Cardiovasc Qual Outcomes 2008; 1: 92-7.
43. Michalska-Kasiczak M, Sahebkar A, Mikhailidis DP, et al.; Lipid and Blood Pressure Meta-analysis Collaboration (LBPMC) Group. Analysis of vitamin D levels in patients with and without statin-associated myalgia – a systematic review and meta-analysis of 7 studies with 2420 patients. Int J Cardiol 2015; 178: 111-6.
44. Spiro A, Buttriss JL. Vitamin D: an overview of vitamin D status and intake in Europe. Nutr Bull 2014; 39: 322-50.