Endocrine CONNECTIONS

Cardiometabolic healthy and unhealthy obesity: does vitamin D play a role?

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Abstract

Objective: The aim of this observational study was to clarify the link between vitamin D status and metabolic syndrome (MetS) in people with visceral obesity.
Design and methods: One hundred ninety-six consecutive patients (152 women; mean age 51 ± 13 years) with visceral obesity (mean body weight 103 ± 20 kg, mean waist circumference (WC) 119 ± 13 cm) were enrolled at the Obesity Outpatient Clinic of the University of Insubria in Varese. Anthropometric measurements were recorded. Laboratory tests, including vitamin D (25(OH)D)), fasting blood glucose (FBG), lipid profile, liver and kidney function tests were assessed. Vitamin D status was defined according to the European Society of Endocrinology guidelines, MetS to the 2009 harmonized definition.
Results: An inverse association emerged among 25(OH)D, body mass index (BMI) ($P = 0.001$) and WC (all $P = 0.003$). Serum 25(OH)D levels were inversely related to FBG and systolic blood pressure (SBP) (respectively, $P = 0.01$ and 0.02). Median serum 25(OH) D levels were 13.3 ng/mL (CI 95% 12; 15) in MetS and 16 ng/mL (CI 95% 14; 18) ($P = 0.01$) in non-MetS patients. Among patients with MetS, lower 25(OH)D concentrations were related to higher risk of hypertension (HT) (odds ratio (OR) 1.7, CI 95%, 0.7;4) and hyperglycemia (IFG)/type 2 diabetes (OR 5.5, CI 95% 2; 14).
Conclusion: Vitamin D status and MetS are inversely correlated in visceral obesity, particularly with regard to glucose homeostasis and BP. More extensive studies are required to investigate the potential for causality.

Key Words
- vitamin D
- metabolic syndrome
- diabetes
- obesity

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Introduction

Dermal synthesis (90%) and intestinal absorption (10%) are the two main sources of vitamin D (1, 2). In the classical pathway, serum vitamin D is metabolized to calcidiol (25(OH)D) in the liver and converted to its active form $1,25(OH)_2D$ (or calcitriol) in the kidney (3).

Decrease in serum calcium/vitamin D concentrations directly induces the secretion of PTH that influences 25(OH)D activation. $1,25(OH)_2D$ promotes intestinal and renal absorption of calcium and acts with PTH in activating osteoclasts, promoting bone resorption and freeing calcium (1, 2, 3, 4, 5). Evidence suggests that the extrarenal conversion of 25(OH)D to $1,25(OH)_2D$ in numerous tissues contributes to the biological actions of vitamin D (autocrine and paracrine pathways) (2, 3, 4, 5).

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More recently, vitamin D has been implied in reducing inflammation, keeping normal resting levels of intracellular calcium and reactive oxygen radicals and preventing gene hypermethylation (6, 7, 8, 9). These mechanisms may be protective for type 2 diabetes (T2DM) development, but are defective in vitamin D deficiency (6, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27). The interplay between $1,25(OH)_2D$ and PTH with the renin-angiotensin-aldosterone system activity (RAA) is related to myocardic hypertrophy and HT (20). Recent experiments found that $1,25(OH)_2D$ induces a dose-related increase in intracellular calcium along with an increase in adipocyte fatty acid synthase and glycerol-3-phosphate dehydrogenase activities and lipolysis inhibition (28).

Metabolic syndrome (MetS) is a multifactorial condition, having central obesity as a causative factor, though the exact mechanisms remain to be elucidated (29, 30, 31, 32). Figure 1 illustrates the possible additional role of vitamin D in the development of MetS (12, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29). Polymorphisms of vitamin D receptors provide additional explanations (12, 33).

Obesity and vitamin D status are known to be associated, but the nature and direction of this association are controversial (12, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27). Indeed, both obesity and the obesity-related disorders may be linked to 25(OH)D (17, 18). Further, two trials reported no effect of vitamin D supplementation on weight loss, while a small case–control trial found a beneficial significant role on fat mass reduction (6, 7, 8).

We performed this study to observe the magnitude of correlations between 25(OH)D status and MetS in adults at higher risk for metabolic disorders.

Materials and methods

Subjects

Consecutive Caucasian obese adults with visceral obesity attending the Obesity Outpatient Clinic of the Endocrine Unite at the University of Insubria were enrolled during the years 2013–2015. All patients were Caucasian obese adults with visceral obesity (31, 32) and gave their informed consent to participate in the study that has been approved by the Local Ethics Committee based in Ospedale di Circolo, ASST dei Sette Laghi, Viale Borri 57, 21100, Varese, Italy.

Inclusion criteria include no concomitant supplementation with vitamin D, no evidence of psychiatric diseases, active cancer, malabsorption, type 1 diabetes, kidney failure, previous non-reversible bariatric surgery, poverty and pregnancy. Disability and cardiovascular diseases (CVD) were the main reasons for hospital admission. Patients were referred to our Clinic by Primary Care Practitioners and Specialists. Each subject was administered a questionnaire about personal habits (smoking, physical activity), comorbidities (chronic obstructive pulmonary disease, arthropathy, ischemic heart disease) and medical treatments. A second questionnaire was administered about previous attempts for weight loss (32).

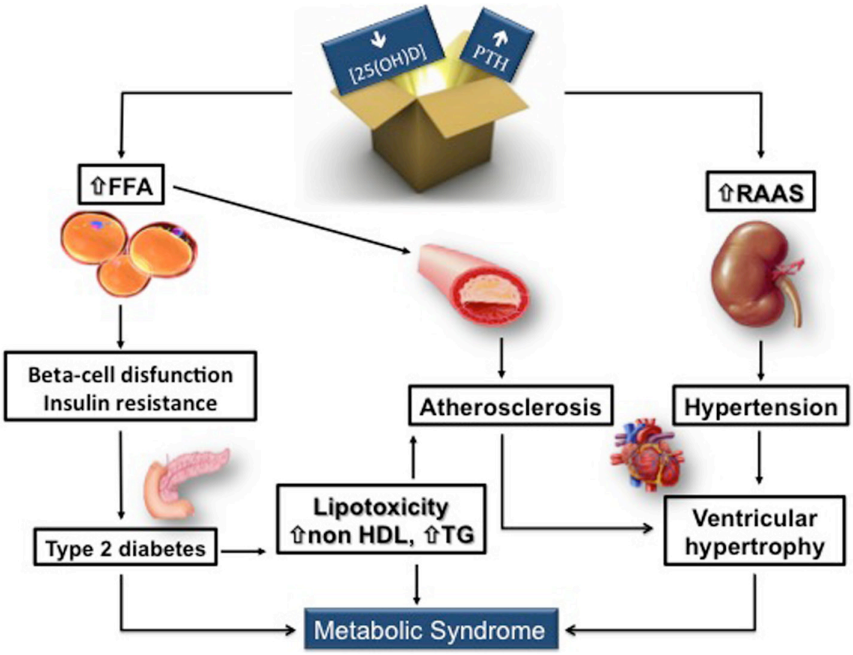

Figure 1
Possible role of vitamin D in the development of MetS.

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Physical examination

Body weight (100 g approximation) and height (0.5 cm approximation) were measured with subjects in light clothing without shoes. The body mass index (BMI) was calculated by dividing weight (kg) by height (square meters). Obesity was classified as follows: grade I obesity for BMI 30–34.99 kg/m^2, grade II obesity 35–39.99 kg/m^2 and grade III obesity BMI ≥40 kg/m^2. To identify abdominal distribution of fat, we referred to ethnic group definition for waist circumference (WC) (male ≥94 cm; female ≥80 cm) (31, 32).

Blood pressure (BP), approximated at 5 mmHg, was measured using calibrated sphygmomanometer with appropriate-size cuff. Heart rate was measured.

Definition of MetS

According to the 2009 harmonized definition (31), MetS was defined as the coexistence of at least 3 out of 5 risk factors, including increased WC, atherosclerotic dyslipidemia (triglycerides ≥150 mg/dL and/or HDL cholesterol <40 mg/dL in men, <50 mg/dL in women); elevated BP (SBP ≥130 mmHg and/or DBP ≥85 mmHg); impaired fasting glucose (IFG) (≥100 mg/dL) or on-going treatment for any of the above conditions.

Blood tests and vitamin D status

Fasting venous blood samples were collected in 2 consecutive days, after an overnight fast, appropriate abstinence from physical activity, from smoking, alcohol and coffee. Intact parathyroid hormone (PTH): serum kept at 2–8°C, Kit 'h-PTH 120 min-IRMA', 2-step immunoradiometric assay based on coated-tube separation, sensitivity 1 pg/mL, nv 6–30 pg/mL (Scantibodies Laboratory, Santee, CA, USA). Radioimmunoassays: Insulin (Kit 'BI-INS-IRMA', based on 2 monoclonal antibodies, one radiolabelled with ^{125}iodine and the other adsorbed onto the inner walls of the tube, sensitivity 0.1 µU/mL, nv<30 µU/mL, Cisbio Assay, Codolet, France); TSH (Serum Separatur Tube, nv 0.31–4.5 U/L, DiaSorin S.p.A, Saluggia, Italy). Chemiluminescent immunoassay: quantitative determination of 25(OH)D, sensitivity 1.5 ng/mL, precision interval 7–11%, Liaison 25-Vitamin D Total Assay, DiaSorin. Assay performance is unaffected by triglycerides (up to 549 mg/dL) and cholesterolemia (up to 259 mg/dL) (33, 34, 35).

Vitamin D status was categorized as 'severe deficiency' (S-DEF$_D$) <10 ng/mL 25(OH)D, 'deficiency' (DEF$_D$) 10–19.9 ng/mL, 'insufficiency' (INSUF$_D$) 20–29.9 ng/mL and 'sufficiency' (SUF$_D$) ≥30 ng/mL (3, 33). Normal values for chemistry and hematology determinations were as follows: AST and ALT (9–36 U/L), calcium (8–10 mg/dL), total cholesterol (<200 mg/dL), HDL (>45 mg/dL), triglycerides (50–175 mg/dL) and hemoglobin A1c (HbA$_{1c}$, nv 4.3–6.1% = 23–43 mmol/mol). Creatinine clearance (mL per min) was directly measured on 24-h diuresis or calculated according to the Cockcroft–Gault equation (36). The Friedewald formula was applied to calculate LDL cholesterol (37). FPG and insulin levels were used to calculate homeostatis model analysis of insulin resistance (HOMA-IR, normal value <2.5) and beta-cell function (HOMA-B, normal value <81.7). HOMA-IR was calculated according to the formula (insulin (µU/mL) × glycemia (mg/dL)/405 (38, 39). HOMA-B = 20 × fasting insulin (µU/mL)/(fasting plasma glucose (mg/dL) − 63) (39). The Nugent test excluded Cushing's syndrome (cortisol radioimmunoassay; normal cortisol values after 1 mg dexamethasone suppression <18 ng/mL) (40).

Statistical analysis

Main demographic, clinical and laboratory parameters have been summarized as mean and standard deviation (S.D.) for continuous values, as frequency distribution for categorical features. Descriptive statistics have been estimated for all patients and categorized by 25(OH) D classes. Since only 9 obese patients were in the SUF class (5%), a 3-category study variable (<10, 10–19.9 and ≥20 ng/mL) was taken into consideration for 25(OH) D values. To test the null hypothesis of no association between patients' characteristics and 25(OH)D, we reported the *P* values from *F*-test or chi-square test, for continuous and categorical variables, respectively. Comparable analyses were performed for obesity parameters, including BMI and WC. In addition, the distribution of 25(OH)D across the range of BMI values is presented as box-plot (Fig. 2).

To investigate the association between MetS and 25(OH)D, the OR with 95% confidence intervals of MetS were estimated for patients with <10 and 10–20 ng/mL of 25(OH)D, using the ≥20 ng/mL as reference class, from a logistic model that included BMI as a covariate. Statistical analysis was expanded by using logistic regression models to estimate the association between 25(OH)D classes and each MetS component, including IR. For the statistical analyses, we used the software SAS 9.4 release (SAS Institute Inc., Cary, NC, USA).

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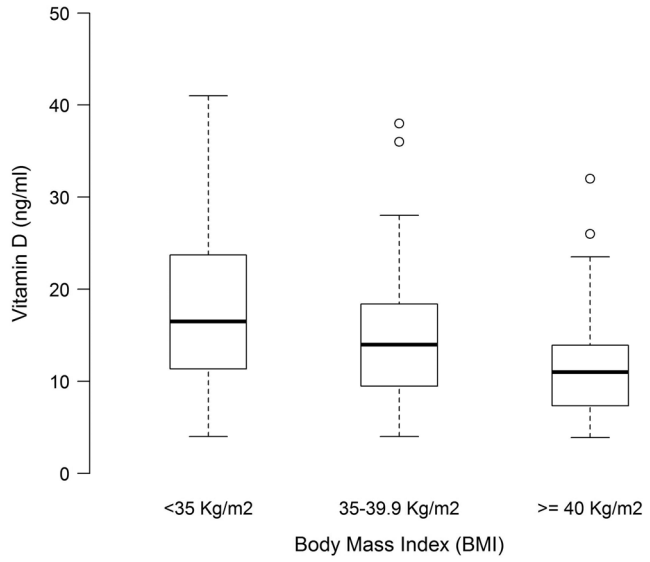

Figure 2
Distribution of serum 25(OH)D across BMI groups. Data represented as box-plot.

Results

In the study period, a cohort of 196 obese adults (152 women, 51 ± 13 years) fulfilled the inclusion criteria. Fifty-eight percent of patients had never participated in a lifestyle intervention program, 31% reported a long history of weight loss attempts (either lifestyle interventions or medications), 5% had undergone reversible bariatric surgery, 6% had recently undertaken an effective hypocaloric diet. Sedentary lifestyle was common (82%). According to the WHO criteria (32), 26% of patients had grade I obesity, 35% grade II obesity and 39% were morbidly obese. Table 1 illustrates demographic features of the study group. No associations emerged between 25(OH)D, demographic features, personal habits, previous weight loss attempts or season of the study visit ($P=0.23$). Over half of patients with BMI >40 kg/m^2 had S-DEF$_D$.

Table 2 depicts the linear negative correlation between 25(OH)D and anthropometric parameters ($P=0.0001$) that was confirmed stratifying the population by gender and by menopausal status (data not shown). The box-plot illustrates the negative correlation with BMI.

S-DEF$_D$ and DEF$_D$ had markedly higher FBG (respectively, 113 ± 39 mg/dL and 109 ± 26 mg/dL) than patients with 25(OH)D ≥20 mg/dL (95 ± 13 mg/dL; *F*-test *P*-value for association 0.01) (Table 3). Likewise, the mean HOMA-IR was significantly higher in S-DEF$_D$ (3.6 ± 3) and DEF$_D$ (3.8 ± 3) than in the group with 25(OH)D ≥20 ng/mL (2.2 ± 1, $P=0.02$). In a preliminary analysis, the relationship between PTH and HOMA-IR failed to reach statistical significance (data not shown). Mean HOMA-B was 131 ± 87 (data not shown). Correlation with HOMA index was $r=0.3988$, with fasting glycemia $r=-0.362$ and with fasting insulin $r=0.64$ (data not shown). The mean HOMA-B had a similar correlation with vitamin D levels compared to HOMA index (data not shown). Mean SBP inversely correlated to 25(OH)D ($P=0.02$), although without a linear trend. Among lipid parameters, triglycerides levels were slightly higher in S-DEF$_D$ than in the other vitamin D classes ($P>0.005$) (Table 3).

Association between 25(OH)D and urate, GOT, GPT and creatinine clearance failed to reach statistical significance (data not shown). Among the complications of obesity, arthropathy occurred more frequently in patients with S-DEF$_D$ than in other groups ($P=0.01$) (Table 3). Patients presenting the cluster of MetS (120/196, 61%) showed lower 25(OH)D than those not fulfilling diagnostic criteria for MetS (13.3 ng/mL, CI 95% 12; 15; vs 16 ng/mL, CI 95% 14; 18 ng/mL, $P=0.01$).

Table 1 Association between clinical features (demographic characteristics, personal habits, weight loss history) and vitamin D levels, in the study population of obese patients ($n=196$).

Vitamin D (ng/mL)	**<10**	**10–20**	**≥20**	***P***
N	60	96	40	–
Age (s.d.)	46 (14)	51 (13)	50 (14)	0.7
Women (%)	46 (77)	76 (79)	30 (75)	0.6
Weight loss treatments				
None (%)	36 (60)	59 (63)	19 (48)	0.6
Previous lifestyle/pharmacological intervention (%)	18 (30)	25 (27)	17 (43)	
Previous restrictive bariatric surgery (%)	3 (5)	5 (5)	1 (3)	
Current lifestyle intervention (%)	3 (5)	5 (5)	3 (8)	
Current smokers (%)	4 (7)	10 (10)	8 (20)	0.2
Physical activity (%)	8 (13)	20 (21)	8 (20)	0.5

Continuous variables are presented as mean (standard deviation, s.d.); discrete variables presented as *n* (%). *P*: *P* value(s) for overall comparisons across the study groups. 'Physical activity' includes both outdoor and indoor aerobic physical activity, practiced at least for 150 min for week.

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Table 2 Association between vitamin D levels and anthropometric measures.

Vitamin D (ng/mL)	**<10**	**10–20**	**≥20**	***P***	****P***	*****P***
N	60	96	40	–	–	–
Mean weight (S.D.) (kg)	108.1 (21)	102.7 (20)	96.1 (15)	0.01	0.08	0.07
Mean waist circumference (S.D.) (cm)	124.2 (13)	118.2 (12)	114.5 (11)	0.0003	0.003	0.1
Mean BMI (S.D.) (kg/m^2)	41.8 (7)	39.3 (6)	36.3 (4)	0.0001	0.01	0.005
BMI classes (%)						
BMI <35 kg/m^2	9 (15%)	25 (26%)	18 (45%)			
BMI <40 kg/m^2	20 (33%)	33 (34%)	16 (40%)	0.002	0.2	0.01
BMI ≥40 kg/m^2	31 (52%)	38 (40%)	6 (15%)			

Continuous variables are presented as mean (standard deviation, S.D.); discrete variables presented as *n* (%). Waist circumference was approximated at 1 cm, weight at 100 g and height (in BMI formula) at 0.5 cm. *P* value(s) for overall comparisons across the study groups (*F*-test and chi-square test for continuous and discrete variables, respectively) and for testing pairwise comparisons (*vitamin D <10 ng/mL vs 10–20 ng/mL; **10–20 ng/mL vs ≥20 ng/mL) whenever the overall test was statistically significant.

Interestingly, 25(OH)D averaged 21 ng/mL (CI 95% 18; 24 ng/mL) in isolated obesity, decreasing to 13.7 ng/mL (CI 95% 11; 16 ng/mL) when all 5 MetS components were present ($P=0.001$). IFG/T2DM occurred more frequently in deficiency (65%) than when 25(OH)D was ≥20 ng/mL (25%, $P=0.0002$) (Supplementary material, see section on supplementary data given at the end of this article). HT had a similar trend ($P=0.01$), whereas the prevalence of HTG was just slightly different among vitamin D classes, a trend emerged for low HDL dyslipidemia. Compared to the reference category, the OR of IFG/T2DM revealed a significant risk for vitamin D-deficient subjects (S-DEF_D 5.5, CI 95% 2; 14; DEF_D 5.4 CI 95% 2; 13). Figure 3 shows how the risks for HT (S-DEF_D 1.65, CI 95% 0.7;4; DEF_D 3.2, CI 95% 1.5;7) and MetS (S-DEF_D 1.8, CI 95% 0.8;4 and DEF_D 3.3, CI 95% 1.5;7) were higher in 25(OH)D deficiency compared to reference.

Table 3 Association between main laboratory characteristics and obesity-related comorbidities with vitamin D levels, in the study population of obese patients ($n=196$).

Vitamin D (ng/mL)	**<10**	**10–20**	**≥20**	***P***	****P***	*****P***
Glycemic profile						
Mean fasting glycemia (S.D.) (mg/dL)	113 (39)	109 (26)	95 (13)	0.01	0.4	0.01
Mean HOMA-IR°° (S.D.)	3.6 (3)	3.8 (3)	2.2 (1)	0.02	0.7	0.006
Treatment for diabetes (%)	14 (23%)	14 (15%)	3 (8%)	0.09	–	–
Blood pressure						
Mean SBP (S.D.) (mmHg)	131 (14)	135 (12)	129 (10)	0.02	0.04	0.008
Mean DBP (S.D.) (mmHg)	80 (8)	82 (11)	80.6 (5)	0.4	–	–
Antihypertensive treatment (%)	31 (52%)	44 (46%)	15 (38%)	0.4	–	–
Lipid profile						
Mean total cholesterol (mg/dL)	202 (36)	210 (40)	213 (32)	0.3	–	–
Mean HDL (S.D.) (mg/dL)	51 (12)	52 (13)	53 (12)	0.3	–	–
Mean LDL (S.D.) (mg/dL)	123 (32)	131 (36)	131 (31)	0.3	–	–
Mean TG (S.D.) (mg/dL)	142 (74)	134 (59)	134 (59)	0.7	–	–
Lipid lowering treatment (%)	17 (28%)	28 (29%)	9 (23%)	0.7	–	–
Comorbidities and uricemia (%)						
Ischemic cardiopathy	6 (10%)	11 (12%)	3 (8%)	0.8	–	–
Artropathy	28 (48%)	24 (25%)	11 (28%)	0.01	0.005	0.7
COPD	35 (19%)	29 (20%)	6 (16%)	0.6	–	–
Uricemia^^ (mg/dL)	5.6 (1)	5.6 (1)	5.5 (2)	1.0	–	–
Other medications (%)						
Levothyroxine	10 (17.2%)	19 (21.1%)	8 (23.5%)	0.7	–	–
Allopurinol	8 (13.3%)	3 (3.1%)	3 (7.5%)	0.055	–	–
Mean TSH levels (S.D.) (U/L)	2.4 (2)	3 (9)	2.4 (2)	0.7	–	–

Continuous variables are presented as mean (standard deviation, S.D.); discrete variables are presented as *n* (%). *P*: *P*-value(s) for overall comparisons across the study groups (*F*-test and chi-square test for continuous and discrete variables, respectively) and for testing pairwise comparisons (*vitamin D <10 ng/mL vs 10–20 ng/mL; **10–20 ng/mL vs ≥20 ng/mL) whenever the overall test was statistically significant. Data available for °°172 cases and for ^^160 cases.

COPD, chronic obstructive pulmonary disease.

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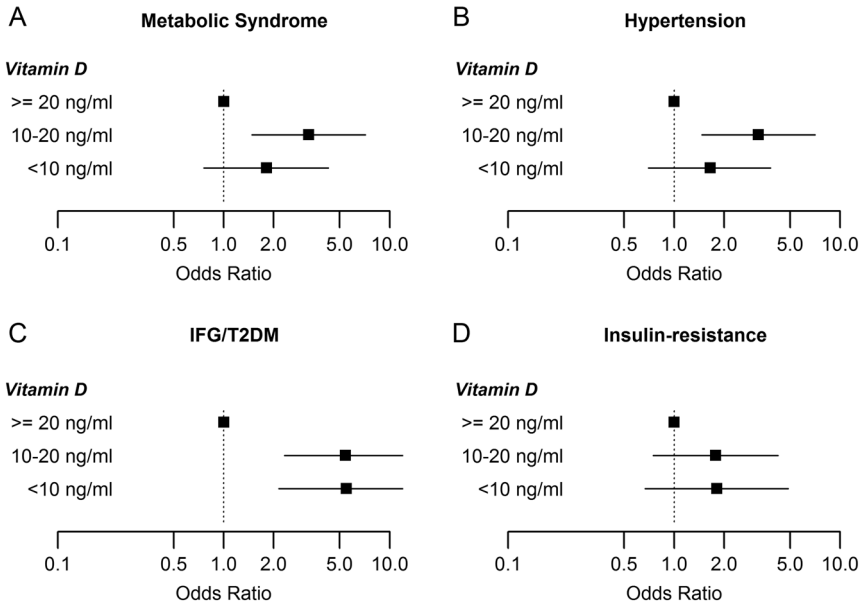

Figure 3
OR (CI 95%) of MetS (A) and its components (B–D) for DEF_D and S- DEF_D, using 25(OH)D ≥20 ng/mL as reference class, from a logistic model, including BMI as covariate.

Discussion

The relationship between 25(OH)D levels and cardiometabolic morbidities in metabolically healthy and unhealthy obesity was studied. Serum 25(OH)D concentration is widely considered the best indicator for vitamin D reserve, reflecting both diet intake and exposure to UV radiation (3, 7). Relatively high variability in 25(OH)D can be problematic when comparing concentrations across studies (19, 20, 21). The Liaison assay has been validated by several groups as an appropriate measurement method (33, 34, 35).

According to recent reports, an increase of 1 kg/m^2 of BMI is associated with 1.15% reduction in serum 25(OH)D (13, 14, 15, 16). In this study, 25(OH)D, BMI and WC were inversely correlated. Cardiometabolic parameters such as IFG, T2DM and HOMA-IR showed the same trend.

The regulating role of vitamin D on insulin secretion is a possible explanation (19, 20, 21, 22, 23, 24, 25, 26, 27, 41). Nevertheless, vitamin D may have a restricted effect in the early phase of beta-cell damage, explaining the failure of intervention studies (43, 44). Additionally, abnormalities of vascular smooth cells mediated both by insulin resistance and by the RAA (8, 9) may contribute to the elevated SBP identified.

It recently appears that people with 25(OH)D <10 ng/mL have higher risk for chronic diseases than those with 25(OH)D ≥20 ng/mL and even more if compared to those with 25(OH)D ≥30 ng/mL (21). The most recent data from randomized clinical trials on CVD state that serum 25(OH)D ≥20 ng/mL are sufficient to meet the requirements of 97.5% of the population (21).

In this study, the OR analysis of MetS, its components and IR demonstrated that patients with 25(OH)D <20 ng/mL had increased risk for IFG/T2DM, HT and MetS. The risk for IFG/T2FM had a slight correlation with the severity of 25(OH)D deficiency, suggesting a central role of insulin and glucose metabolism. The PROspective study involving a non-diabetic population demonstrated an increased risk to develop glycemic disorders over a 10-year time (18, 44). In the current study, the lack of a linear trend for HT prevalence–vitamin D relationship, even by excluding patients being treated for HT, influenced the trend for MetS.

Recently, the concomitant deficiency of magnesium and vitamin D in obesity has been associated with an increased risk of cardiometabolic disease (45, 46).

A multivariate analysis involving analysis of demographic and anthropometric data and personal habits could better clarify this point.

The Australian Diabetes, Obesity and Lifestyle Study reported that, among risk factors, BMI was conditioning MetS occurrence more than vitamin D levels (24). Because our results were confirmed after adjusting for BMI, we suggest an independent relevant role of vitamin D levels. At variance from previous studies (17), a significant correlation between 25(OH)D and lipid profile could not be detected. However, even if not significant, the OR for low HDL was in positive correlation with 25(OH)D. The fact that the data of the literature only report small variations in cholesterol levels in DEF_D may explain the results (8, 47).

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The potential strengths and limitations of this study deserve considerations. First, the cross-sectional design does not allow defining a causality link. Secondly, due to the low prevalence of patients with optimal 25(OH)D, a larger sample size would have been required to adequately assess the parameters of obese patients in the right tail of the 25(OH)D distribution. Although vitamin D classes were well balanced with respect to major demographic and clinical characteristics that may interfere with 25(OH)D and fat distribution, including comorbidities, physical activity and smoking habits, the presence of residual confounders cannot be ruled out. The main strength is the uniformity of the investigated cohort. To our knowledge, this is the first study selecting Caucasian adults at higher risk of metabolic morbidities. This may provide homogeneity in results, given for instance the different bioavailability of vitamin D in blacks compared to whites, as well as in fat storage (48). Previous studies on this topic differ in methodology and population; thus, evidence remains inconclusive (17).

Other strengths were the centralization of all tests and the direct assessment of patients, evaluated by a single trained team. Moreover, the survey considered comorbidities, such as arthropathy, ischemic heart disease and pulmonary diseases possibly responsible for reverse causality effect (severely ill patients could have low vitamin D for shorter exposure to sun rather than the other way around) (14).

In conclusion, the results of this original observational study confirm a significant inverse association of vitamin D status and MetS. For instance, vitamin D deficiency might represent one of the environmental factors in the pathogenesis and progression of T2DM, obesity and MetS. A detailed metabolic phenotyping could support more qualified intervention programs and detect patients at higher risk for metabolic disorders (20, 23, 30, 31, 46, 47, 48, 49).

Vitamin D supplementations proposed to prevent and correct the well-established bone consequences of 25(OH)D deficiency, in addition to lifestyle intervention programs, may be helpful to prevent the progression of metabolic disorders (19). Prospective data on the effect of vitamin D supplementation in these patients will be collected and analyzed. Consistent evidences from large, randomized, placebo-control trials with adequate statistic power and eventually exploring the association of vitamin D and other nutritional elements (46) are warranted to confirm these findings.

Supplementary data
This is linked to the online version of the paper at http://dx.doi.org/10.1530/EC-17-0304.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Compliance with ethical standards
The study has been approved by the Ethics Committee of the Ospedale di Circolo, Varese, Italy. Informed consent has been obtained from all subjects before enrollment.

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