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ORIGINAL ARTICLE

Is plasma 25(OH) D related to adipokines, inflammatory cytokines and insulin resistance in both a healthy and morbidly obese population?

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Abstract To analyse in a cohort of healthy subjects and in a group of morbidly obese patients, we studied the association amongst 25(OH) D and plasma concentrations of adipocytokines, inflammatory cytokines and insulin resistance. We also aimed to determine whether vitamin D-deficient patients showed a greater inflammatory profile. In the observational study that the authors conducted, plasma concentrations of 25(OH) D, leptin, resistin, adiponectin and interleukine-18 were determined in 134 healthy men and 127 women. In the population consisting of 44 patients with morbid obesity, plasma concentrations of 25(OH) D, leptin, resistin, adiponectin, interleukine-18, soluble tumor necrosis factor receptors 1 and 2 and C-reactive protein were analysed. In the healthy population, plasma 25(OH) D showed a negative correlation with body mass index, body fat, waist, hip circumference and with leptin. However, no significant associations were found amongst 25(OH) D and plasma concentrations of resistin, adiponectin or interleukine-18. Patients with vitamin D deficiency showed higher body mass index, fat mass percentage and higher leptin concentrations compared with subjects with normal 25(OH) D concentrations. In the morbidly obese subjects, 25(OH) D did not correlate with leptin, resistin, adiponectin, interleukine-18, soluble tumor necrosis factor receptors 1 and 2 or with C-reactive protein. In patients with morbid obesity, no differences were found in adipokines and inflammatory cytokines concentrations regarding 25(OH) D status. No associations were found either between 25(OH) D and plasma glucose and insulin resistance or with lipid profile. Plasma 25(OH) D concentrations are associated with adiposity markers but not with adipocytokines implicated in inflammation. This lack of association does not support a major role of 25(OH) D in the pro-inflammatory environment observed in morbidly obese subjects. In addition, subjects with vitamin D deficiency are not characterized by a greater inflammatory state.

Keywords Adiponectin · Adipocytokines · Inflammatory cytokines · Insulin resistance · Morbidly obesity · Vitamin D

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Introduction

Vitamin D has a major biological action maintaining mineral homeostasis and regulating bone remodelling [1, 2]. In this sense, active vitamin D is responsible for maintaining calcium homeostasis primarily by increasing the efficiency of intestinal calcium absorption and by stimulating the differentiation of bone-resorbing osteoclasts [3, 4]. Apart from its role in bone metabolism, the existence of vitamin D receptors in activated T lymphocytes, macrophages and thymus tissue and in pancreatic β-cells has suggested that

this vitamin might also function as an immune and insulin secretion modulator [5–9]. To date, a wide range of immune actions have been reported for vitamin D, which suppresses adaptive immunity by inhibiting the maturation of dentritic cells, reducing their capacity to present antigens to CD4 cells and inhibiting the proliferation and differentiation of CD4 cells into T helpers [10, 11]. Vitamin D has also been described to downregulate the production of several cytokines in in vitro studies such as IL-2, IL-6, IL-12, interferon γ, TNF-α and TNF-β [12, 13]. On the other hand, vitamin D deficiency can lead to immune disregulation, and some studies have shown defective macrophage function, such as impaired chemotaxis, phagocytosis, and increased production of proinflammatory cytokines, in vitamin D-insufficiency [14]. However, the number of studies analysing the relationship between vitamin D status and inflammatory cytokines in humans are very scarce and have basically been focussed on IL-10 [15–17], IL-6 [15, 18] and TNF-α [15–17]. C-reactive protein (CRP) is a marker of inflammatory activation produced by hepatocytes, which has been implicated in comorbidities associated with obesity and cardiovascular risk factors. To date, few studies have analysed the relationship of CRP with vitamin D concentrations and the reported results have been contradictory [18].

Regarding the role of vitamin D in insulin secretion, it has been shown in both in vitro and in vivo models that vitamin D is essential for normal insulin release in response to glucose and for maintenance of glucose tolerance [19]. Epidemiological studies assessing vitamin D status and the risk of hyperglycaemia or insulin resistance have been suggestive of inverse associations but are inconclusive [20–22].

The aims of our study were to analyse in a cohort of healthy subjects and in a group of morbidly obese patients the association between 25-hydroxyvitamin D [25(OH) D], an accurate measure of vitamin D status, and plasma concentrations of several adipokines implicated in inflammation (leptin, resistin, adiponectin, etc.), and some inflammatory cytokines (IL-18 and TNF-α-soluble receptors 1 and 2 [sTNFR1 and sTNFR2, respectively]) and the inflammatory marker CRP. We also aimed to determine whether vitamin D-deficient patients showed a greater inflammatory profile. On the other hand, in severely obese subjects, the association between 25(OH) D and hyperglycaemia has not been established; we have, therefore, analysed this relationship in our cohort of morbidly obese patients.

Subjects and methods

Study population

The study was performed in L'Hospitalet de Llobregat, a city adjacent to Barcelona with 280,000 inhabitants. Eight hundred and eighty adult subjects, representative of the whole population of the city in sex and decade distribution, were identified both by the census and self-identification as having lived for at least the last four years in the city. The subjects were randomly selected from the census of the city and were invited to participate in the study, directly by mail and phone call, and finally by verbal information, with the purpose of determining anthropometrics, body composition variables, plasma concentrations of 25(OH) D, leptin, resistin, IL-18 and adiponectin. They were fully informed of the purpose of the study, who gave their written voluntary consent to take part in the investigation. The study was approved by the Research Ethics Boards of the Hospital. The participation rate was 44% of the 880 subjects initially selected, and the age limit ranged from 15 to 70 years. The subjects were explored to determine whether they presented previous diseases, and an appropriate questionnaire was administered to the 387 subjects that consented to participate in the study. The primary exclusion criteria were the presence of diabetes mellitus, cardiovascular disease, chronic hepatic or renal failure, psychiatric problems, past or present thyroid dysfunction or treatment with thyroid hormones and pregnancy. Subjects with blood pressure above 140/90 mmHg or under antihypertensive treatment were considered hypertensive subjects. The subjects were, therefore, recruited in good health with no known diseases, and were not currently following a weight-loss diet or exercise programme. Subjects with past or present thyroid dysfunction, or those treated with thyroid hormones were not included in the sample. In order to exclude the presence of diabetes mellitus, a blood test was performed for the analysis of fasting plasma glucose concentrations. Finally, 261 subjects were included in the study, representative of the whole population of the city in sex and decade distribution, 134 men (mean age 41.6 years) and 127 women (mean age 40.8 years). This sample was also representative of the population from 15- to 70-year-old subjects of the city.

Morbidly obese subjects were consecutively recruited from the Endocrinology Clinic of the Hospital de Bellvitge (Barcelona, Spain). Anthropometric and body composition measurements were made, and blood samples were collected to determine plasma concentrations of 25(OH) D, leptin, sTNFR1 and 2 resistin, IL-18, adiponectin and CRP. Primary exclusion criteria were an acute major cardiovascular event in the previous six months, recent or ongoing infection, a history of cancer disease, or treatment with anti-inflammatory drugs or insulin. Patients with Cushing's syndrome, thyroid dysfunction or other major endocrine disorders were also omitted. Forty-four morbidly obese patients (mean age 45.3 years) were finally included in the study. All the participants had signed a written consent to be included, which was approved by the Research Ethics Board of our Hospital.

Anthropometrical measurements

Height and weight were measured with the patient standing in light clothes and without shoes. Body mass index (BMI) was calculated as body weight divided by height squared (kg/m^2). Waist-to-hip ratio (WHR) was calculated as the ratio of waist and hip circumferences. Body composition was assessed by bioelectrical impedance analysis, using a Holtain BC Analyser (Cambridge, UK) [23]. The precision of this test in determining body fat (BF) is within ±3%. The same physician performed all the examinations. Obesity was classified according to the World Health Organization criteria [24].

Analytical methods

Blood samples were drawn from each subject before breakfast between 8 and 9 a.m., after fasting for at least 12 h and an overnight rest. All the samples were stored at −80°C until analytical measurements were performed, except for glucose, which was determined immediately after blood was drawn.

Serum glucose was measured with a glucose oxidase method using a Hitachi autoanalyser. Serum insulin was measured using a specific immunoradiometric assay (Medgenix Diagnostics, Fleunes, Belgium) in which proinsulin did not cross react. The intra- and inter-assay coefficients of variation (CV) were 6 and 7%, respectively. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated from fasting plasma insulin and glucose levels as (insulin × glucose/22.5), where insulin concentration was reported as mU/l and glucose as mmol concentrations. Hemoglobin A_{1c} was measured by chromatographic method (Glico Hb Quick Column Procedure, Helena Laboratories, Beaumont, TX, USA).

Total serum cholesterol was measured through the reaction of cholesterol esterase/cholesterol oxidase/peroxidase. High-density lipoprotein cholesterol (HDLc) was quantified after precipitation with polyethylene glycol at room temperature. Serum triglycerides were measured through the reaction of glycerol–phosphatase–oxidase and peroxidase. Low-density lipoprotein cholesterol (LDLc) was calculated using the Friedewald formula).

25-hydroxyvitamin D [25(OH) D] concentrations were determined using a radioimmunoassay (DiaSorin, Stillwater, MN, USA). Subjects with plasma circulating levels lower than 38 nmol/l, were considered as having 25(OH) D deficiency [25, 26].

Intact serum parathyroid hormone (PTH) was measured by a two-site immunoradiometric assay (Diagnostic System Laboratories, Webster, TX, USA); intra- and inter-assay CV were 10 and 11%, respectively.

Plasma resistin levels were measured by sandwich enzyme-linked immunosorbent assay (Biovendor Laboratory Medicine, Inc Palackeho, Czech Republic). The sensitivity was 0.2 ng/ml. The intra- and inter-assay CV were 5.8 and 14.7%, respectively.

Plasma adiponectin concentrations were measured using standard radioimmunoassay kits (Linco Research, MS, USA). For human adiponectin the kit has a sensitivity of 1 ng/ml in a 100-µl sample size, and a range of 1–200 ng/ml. All samples were diluted 1/500. The intra- and inter-assay CV were 8 and 12%, respectively. sTNFR1 and 2 were determined by solid phase enzymoimmunoassay with amplified reactivity (Bio Source Europe SA, Fleunes, Belgium). The limit of detection was 0.5 ng/ml for sTNFR1 and 0.1 ng/ml for sTNFR2, and the intra- and inter-assays CV were 7 and 9%, respectively. The sTNFR1 assay does not cross-react with sTNFR2. TNF α does not interfere with the assay.

Plasma IL-18 levels were measured by human IL-18 sandwich enzyme-linked immunosorbent assay (ELISA; Medical & Biological Laboratories Co. Ltd., Japan). Assay sensitivity was 12.5 pg/ml. Intra- and inter-assay CV were 10.8 and 10.7%, respectively.

Serum CRP was measured by immunonephelometry with N High Sensitivity CRP Kit (Dade Bering USA). CRP has a sensitivity of 0.175 mg/l and intra/interassay precision lower than 4.4 and 5.7%, respectively.

Statistical analysis

Descriptive statistics are presented as mean ± standard deviation (SD) for variables with a normal distribution and as median interquartile range (IQR) for non-normally distributed variables. Student *t*-test and ANOVA were used to compare normal distributed variables and Mann–Whitney *U* and Kruskal–Wallis tests were used to compare non-normal distributed variables. Relationships among variables were sought by Spearman's correlation coefficient. Univariate and multivariate linear regression analysis were performed to identify independent factors affecting 25(OH) D. A P value <0.05, was considered statistically significant. All the statistical analyses were performed using the Statistical Package for Social Sciences (SPSS/Windows version 8.0, SPSS Inc., Chicago IL, USA).

Results

The clinical, anthropometric and biochemical characteristics of healthy subjects studied are summarized in Table 1. The 134 men and 127 women studied were 41 ± 15 years old, and had a BMI of 27.1 ± 5.04 kg/m^2 and plasma 25(OH) D concentrations were 52.7 ± 21.3 nmol/l. In the

Table 1 Anthropometric, body composition parameters, and adipokines and inflammatory cytokines in healthy population and correlations with 25(OH) D concentrations

	Healthy population $N = 261$	25(OH) D R	P
Sex (M/F)	134/127	−0.098	0.121
Age (years)	41.1 ± 15.7	−0.251	0.000
BMI (kg/m^2)	27.1 ± 5.04	−0.164	0.009
Waist to hip ratio	0.86 ± 0.10	−0.251	0.000
Fat mass (kg)	18.5 ± 9.8	−0.219	0.000
Fat free mass (kg)	53.3 ± 10.3	0.010	0.878
Body fat (%)	24.9 ± 10.2	−0.207	0.001
25(OH) D (nmol/l)	52.7 ± 21.3	−0.262	0.000
Leptin nmol/lml	8.5 (10.9)	−0.262	0.000
Resistin (ng/ml)	3.8 (2.2)	−0.047	0.475
IL-18 (pg/ml)	158.2 (93.6)	0.045	0.481
Adiponectin (μg/ml)	11.4 (9.3)	−0.030	0.688

Data are expressed as mean ± standard deviation for variables with normal distribution and as median (IQR) for leptin, resistin, IL-18 and adiponectin

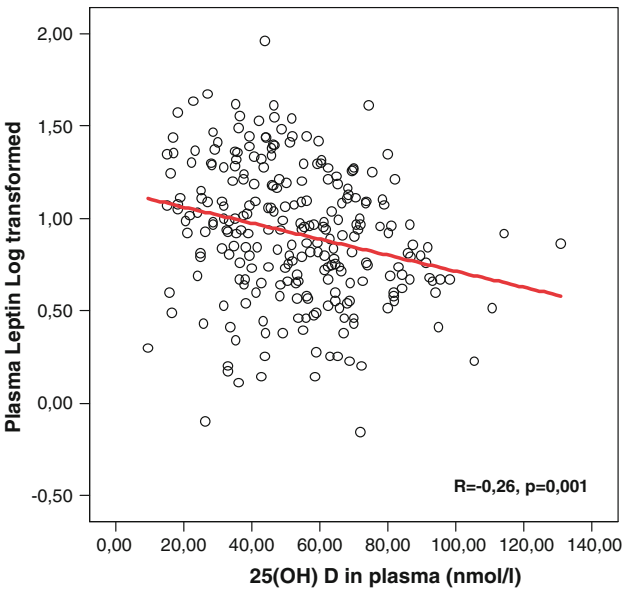

Fig. 1 Correlation between 25(OH) D and leptin plasma concentrations

univariate correlation analysis 25(OH) D showed a negative correlation with BMI, BF and WHR (Table 1); an inverse correlation was found between vitamin D and plasma leptin (Fig. 1). Regarding 25(OH) D association with adipokines and inflammatory cytokines, however, no significant associations were found between 25(OH) D and leptin, resistin, IL-18 or adiponectin. Results were also found to be similar when the analysis was performed according to gender.

Thirty percent of healthy subjects ($n = 73$) had 25(OH) D concentrations below 38 nmol/l and were considered vitamin D deficient. When comparing subjects with and without vitamin D deficiency, higher BMI and a greater fat mass percentage was found in healthy subjects with low vitamin D levels. In the univariate correlation analysis, 25(OH) D showed a negative correlation with BMI, BF and WHR (Table 2). No significant differences were found between groups regarding plasma concentrations of resistin, adiponectin and IL-18. In the multiple regression analysis after adjusting for age, BMI, fat mass and WHR, 25(OH) D remained inversely associated with plasma concentrations of leptin ($\beta = -0.20$, $P = 0.022$, $R^2 = 0.081$) and this correlation remained significant in the multiple regression analysis after adjusting for BMI.

Ninety-six healthy subjects of the studied population (35.8%) were overweight (BMI 25–29.9 kg/m^2), out of whom 25(OH) D also correlated negatively with fat mass ($r = -0.219$, $P = 0.039$); BF ($r = -0.251$, $P = 0.017$), WHR ($r = -0.212$, $P = 0.046$) and with leptin ($r = -0.301$, $P = 0.004$). After the exclusion of overweight subjects the association of 25(OH) D with anthropometric parameters and adipocytokines remained unchanged.

The clinical, anthropometric and biochemical characteristics of the morbidly obese cohort studied are summarized in Table 3. The four men and 40 women studied were 44.6 ± 10 years old, had a BMI of 48.2 ± 8.1 kg/m^2 and plasma 25(OH) D concentrations were 37.8 ± 16.5 nmol/l. Twenty-five percent of patients had type 2 diabetes mellitus. In the univariate correlation analysis, 25(OH) D showed no correlation with anthropometric or body composition variables. No associations were found either between 25(OH) D and plasma glucose, insulin concentrations, HOMA-IR nor with lipid profile (Table 3). Plasma adiponectin and inflammatory cytokines sTNFR 1 and 2, IL-18 and CRP were neither statistically significantly correlated with vitamin D (Table 3). No significant differences were found in the analysis of the associations between 25(OH) D and adipokines and inflammatory cytokines according to the presence of diabetes mellitus. Moreover, 25(OH) D concentrations in diabetic and non-diabetic obese patients were similar (35.2 ± 13 vs. 39.2 ± 18 nmol/l, $P = 0.437$).

Fifty percent of obese subjects ($n = 22$) had 25(OH) D concentrations below 38 nmol/l and were considered vitamin D deficient. When these patients were compared with those without vitamin D deficiency, no significant differences were found in BMI, WHR, BF, or in metabolic variables such as plasma glucose, insulin, HbA_{1c}, HOMA-IR and lipid profile. Also, plasma concentrations of leptin, sTNFR 1 and 2, IL-18, adiponectin and CRP were not found to be different between groups.

Table 2 Anthropometric, body composition parameters, adipokines and inflammatory cytokines in healthy patients with and without 25(OH) D deficiency

	25(OH) D < 38 nmol/l $N = 73$	25(OH) D ≥ 38 nmol/l $N = 188$	*P*
Age (years)	43.1 ± 15.7	40.2 ± 15.6	0.186
BMI (kg/m^2)	29.0 ± 6.2	26.4 ± 4.3	0.001
Waist to hip ratio	0.87 ± 0.09	0.85 ± 0.10	0.331
Fat mass (kg)	21.8 ± 12.4	17.2 ± 8.3	0.001
Fat-free mass (kg)	53.3 ± 9.6	53.3 ± 10.6	0.982
Body fat (%)	27.7 ± 11.5	23.9 ± 9.2	0.005
Leptin (nmol/ml)	10.4 (13.6)	7.2 (8.8)	0.025
Resistin (ng/ml)	3.82 (2.1)	3.95 (2.2)	0.557
IL-18 (pg/ml)	161.5 (90.1)	158.5 (95.5)	0.743
Adiponectin (mcg/ml)	11.1 (8.8)	11.8 (9.4)	0.935

Data are expressed as mean ± standard deviation for variables with normal distribution and as median (IQR) for leptin, resistin, IL-18 and adiponectin

Table 3 Anthropometric, body composition parameters, biochemical characteristics, adipokines and inflammatory cytokines in morbidly obese patients and correlations with 25(OH) D

	Morbidly obese patients $N = 44$	25(OH) D *R*	*P*
Sex (M/F)	4/40		
Age (years)	44.6 ± 10	0.010	0.947
BMI (kg/m^2)	48.2 ± 8.1	−0.250	0.102
WHR	0.89 ± 0.06	−0.161	0.320
Fat mass (kg)	52.6 ± 12.4	−0.139	0.387
Fat free mass (kg)	66.7 ± 10.1	−0.122	0.448
Body fat (%)	43.7 ± 4.9	−0.066	0.684
25(OH) D (nmol/l)	37.8 ± 16.5		
Fasting glucose (mmol/l)	6.1 (2.2)	−0.231	0.131
Fasting insulin (pmol/l)	106.5 (75.1)	−0.191	0.220
HOMA-IR	5.1 (5.2)	−0.229	0.139
HbA1c (%)	5.4 (1.1)	−0.282	0.064
Cholesterol LDL (mmol/l)	2.92 ± 0.86	0.055	0.758
Triglycerides (mmol/l)	1.63 ± 0.85	−0.267	0.100
Leptin nmol/ml	36.96(14.48)	−0.035	0.901
TNFR1 (ng/ml)	2.54 (0.78)	−0.107	0.491
TNFR2 (ng/ml)	5.02 (1.89)	−0.254	0.097
Resistin (ng/ml)	5.93(1.7)	0.171	0.441
IL-18 (pg/ml)	227.2 (165.2)	0.173	0.443
Adiponectin (μg/ml)	9.0 (6.9)	0.035	0.826
CRP (mg/l)	7.0 (6.1)	−0.429	0.059

Data are expressed with mean ± standard deviation for variables with normal distribution and median (IQR) for non-normally distributed data

Discussion

In this study, we have analysed the association of vitamin D with adipokines and inflammatory cytokines both in a healthy population of men and women randomly selected and in morbidly obese patients. We have observed a negative relationship among BMI, anthropometric variables and 25(OH) D circulating concentrations in a healthy population, and these correlations are not present in the obese cohort. However, no association with the adipokines and inflammatory cytokines of the studied profile was observed either in this cohort or in morbidly obese patients, arguing against a significant role of vitamin D in the inflammatory state present in obese subjects [2, 5, 7, 27]. We also have demonstrated that 25(OH) D deficient controls or patients showed a similar inflammatory profile. On the other hand, the study did not show any association between 25(OH) D and glucose metabolism and insulin resistance. Only a negative correlation between 25(OH) D and leptin was found in the healthy population, which remained significant in the multiple regression analysis after adjusting for BMI. A previous article has described that vitamin D_3 presents a direct inhibitory effect on leptin secretion from human adipose tissue culture [27]. However, the exact mechanisms by which vitamin D_3 suppresses leptin secretion, as well as its role in the in vivo regulation of plasma leptin levels, remain to be ascertained.

To our knowledge this is the first study in which the association between 25(OH) D and resistin has been analysed in a healthy and morbidly obese population. Resistin was originally described as an adipocyte-secreted peptide that induced insulin resistance in rodents [28] but increasing evidence indicates its role in the regulation of inflammation and immunity [29]. Resistin, at least in humans, shares several features with proinflammatory cytokines and can trigger a proinflammatory state in vitro as well as in vivo [30]. Therefore, we hypothesized that if resistin was a marker of inflammation, then it could be inversely related to vitamin D concentrations. However,

our result does not support the existence of a relationship between resistin and 25(OH) D.

A direct association between vitamin D and adiponectin was first described in an in vitro study wherein calcium and 1,25-dihydroxyvitamin D3 regulated the expression of adipokines in visceral fat and, in particular, a high calcium diet stimulated the expression of the anti-inflammatory factors IL-15 and adiponectin [31]. Moreover, it has been described that 1,25(OH)2 D can down-regulate the TNF α-gene [32] which is known to affect the adiponectin synthesis. However, the relationship between 25(OH) D and adiponectin in vivo has been scarcely studied. Two studies each performed separately in non-diabetic adults and in a young healthy population found a positive correlation between the two variables, although this association disappeared after adjustment for BMI indicating the weakness of this relationship [33, 34]. We were also unable to find a positive association between adiponectin and 25(OH) D in either a large population of healthy subjects or in a group of morbidly obese patients. Among all the adipokines, adiponectin is the only one with anti-inflammatory properties [35]. Therefore, if vitamin D plays a role on inflammation, then a strong association between them would be expected. Our results argue against the existence of a relationship between plasma vitamin D concentrations and adiponectin.

The relationship between the inflammatory cytokine TNF-α and vitamin D has been previously analysed. In an observational study among healthy women, a significant inverse correlation between serum 25(OH) D and TNF-α concentrations was observed [15]. In a controlled clinical trial, vitamin D supplementation resulted in an improvement of the inflammatory milieu in patients with congestive heart failure reflected by an increase in serum concentrations of IL-10 and a prevention of a rise in TNF-α concentrations [16]. In another study, vitamin D supplementation in healthy overweight subjects also showed a decrease in TNF-α concentrations but no effect on glucose metabolism [36]. Despite these previous observations in intervention studies, we did not observe a relationship between sTNFR1 and 2 and 25(OH) D concentrations in morbidly obese patients. One explanation might be that, in the previous studies, the concentrations of vitamin D achieved with the supplementation were high and double that of observed in our patients. Also our study was performed in patients with morbid obesity, but without heart disease. Moreover, we have determined soluble TNF-α receptor concentrations instead of TNF-α because they are very stable proteins with plasma concentrations that remain elevated for longer periods of time and, therefore, are of more value for monitoring inflammatory responses [37]. However, it has been previously described that, in some situations such as weight loss, they behave differently compared to TNF-α [38–40].

Interleukin-18 is a potent proinflammatory cytokine with potential atherogenic properties through the production of TNF-α [41–44]. High IL-18 mRNA expression has been found in unstable atherosclerotic plaques, and increased levels of circulating IL-18 have been described in patients with acute coronary syndromes, suggesting a role for IL-18 in plaque destabilization [45, 46]. Our study is the first to analyse plasma concentration of this cytokine as a marker of inflammation with 25(OH) D, and, wherein, no association was found.

We were also unable to confirm the hypothesis that hypovitaminosis D might induce a higher inflammatory response. In fact, healthy subjects and morbidly obese patients with low vitamin D levels did not have lower plasma concentrations of the anti-inflammatory adipokine adiponectin. Moreover, plasma concentrations of inflammatory cytokines IL-18 and sTNFR1 and 2 and a marker of inflammation such as CRP protein were not different according to vitamin D status in healthy subjects and in patients with morbid obesity.

CRP is a robust marker of inflammation produced by the liver under regulatory control of cytokines IL-6 and TNF-α. CRP association with indicators of elevated BF and cardiovascular disease risk factors has been well established. However, its relationship with vitamin D has not been settled [47]. In a recent study analysing a healthy population, high-sensitivity CRP inversely correlated with plasma 25(OH) D; however, other authors have not been able to demonstrate this association either in a study performed in overweight subjects [48]. Vitamin D deficiency is associated with significant increases in the incidence of cardiovascular risk factors and mortality. However, the mechanisms underlying this association remain unclear. There are studies that have evaluated the possible relationships among vitamin D status, endothelial dysfunction, and inflammation. Therefore, using high-sensitivity PCR determination as a marker of inflammatory activation, low 25(OH) D concentrations are found to be associated with markers of endothelial dysfunction and inflammatory activation, representing potential mechanisms for incremental coronary risk [49]. Also, epidemiological studies indicate a relation between vitamin D status and autoimmune diseases, and in vitro studies demonstrate an effect of 25(OH) D on immune activation. However, the relation between serum concentrations of 25(OH) D and the effect of vitamin D supplementation on serum concentrations of cytokines are not settled. In one study, interleukin (IL)-2, IL-4, IL-5, IL-10, IL-12, IL-13, IL-17, intercellular adhesion molecule-1, interferon-gamma, monocyte chemotactic protein-1, and high sensitivity CRP, were measured after one-year intervention with vitamin D supplementation, and the authors were not able to demonstrate with certainty any significant relation among serum concentrations of 25(OH)D and a number of cytokines and markers of

inflammation [49]. Contrary to other hypotheses, a study also failed to detect a cross-sectional association between serum 25(OH) D levels and coronary artery calcification, carotid intimal medial thickness, or CRP. Either there is no causal relationship between 25(OH) D and cardiovascular disease risk [18, 49]. Therefore, the role of CRP in the regulation over 25(OH) D is controversial.

Clinical studies have largely but not consistently shown that type 2 diabetes and insulin resistance are associated with poor vitamin D status [20, 50]. The mechanisms underlying the association of low 25(OH) D levels and insulin resistance are not fully understood. Vitamin D may have a beneficial effect on insulin action by stimulating the expression of insulin receptor and thereby enhancing insulin responsiveness for glucose transport [51]. Secondly, vitamin D regulates extracellular and intracellular calcium which is essential for insulin-mediated intracellular processes in insulin-responsive tissues such as skeletal muscle and adipose tissue. Finally, it has been suggested that as vitamin D has a modulating effect on the immune system [7], hypovitaminosis D might induce a higher inflammatory response, which would be associated with insulin resistance [52]. However, results in morbid obesity are confusing. In a study performed in morbidly obese patients, no significant differences on fasting glucose, insulin or insulin resistance were found between patients with and without vitamin D deficiency [53]. In the same sense, another study including morbidly obese patients was unable to support an independent contribution of 25(OH) D in the pathogenesis of the metabolic syndrome and hyperglycaemia in severely obese subjects [54]. These previous reports are in agreement with results of this study, the lack of association between 25(OH) D and plasma insulin or HOMA-IR, irrespective of the presence of diabetes mellitus in our study, suggesting that in morbidly obese patients, vitamin D does not have a main role in glucose homeostasis.

In conclusion, plasma 25(OH) D concentrations in this study are associated with adiposity markers either in controls or in obese patients, but not with adipokines implicated in inflammation such as resistin, sTNFR1 and 2, IL-18 or adiponectin. This lack of association does not support a major role of vitamin D in the pro-inflammatory environment observed in morbidly obese subjects. Moreover, healthy population and morbidly obese patients with vitamin D deficiency are not characterized by a higher inflammatory state. The study also did not show any association between 25(OH) D and glucose metabolism and insulin resistance.

However, because of this cross-sectional study design, we cannot suggest any causal or temporal relationship. Further investigations are needed to improve our understanding of 25(OH) D implication in inflammation and its regulation of adipokines secretion.

Declaration of interest There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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