Nutrition Research 96 (2021) 1–8

Available online at www.sciencedirect.com

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journal homepage: www.elsevier.com/locate/NTR

Original Research

Vitamin D is directly associated with favorable glycemic, lipid, and inflammatory profiles in individuals with at least one component of metabolic syndrome irrespective of total adiposity: Pró-Saúde Study, Brazil ☆

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ARTICLE INFO

Article history:
Received 6 July 2021
Revised 15 October 2021
Accepted 19 October 2021

Keywords:
Vitamin D
Metabolic health
Metabolically healthy obesity
25-hydroxycholecalciferol
Inflammation markers
Adipokines

ABSTRACT

Vitamin D insufficiency has been suggested as a risk factor for several metabolic disorders. The objective of the study was to investigate the association between serum 25 hydroxyvitamin D [25(OH)D] and metabolic health markers of Brazilian individuals with normal-weight, overweight or obesity. We hypothesized that serum 25(OH)D would be inversely associated with glycemic, lipid and inflammatory markers indicative of metabolic abnormality. Data of 511 individuals (33-79 years), recruited from a longitudinal investigation (Pró-Saúde Study), were analyzed cross-sectionally. Anthropometric, biochemical, body composition, socio-demographic and lifestyle data were collected. Based on body mass index (BMI; normal weight, overweight, obesity) and metabolic health (metabolically healthy (MH) and metabolically unhealthy (MU)) categories, the participants were classified into 6 phenotypes. Individuals having zero components of the metabolic syndrome were considered as "MH". MH obesity was frequent in 2.0% of the participants and 56.0% exhibited vitamin D insufficiency (<20 ng/mL). In the subgroups of the same BMI category, there were no significant differences in 25(OH)D concentrations between individuals classified as MH and MU. After

☆ Abbreviations: 25(OH)D, 25 hydroxyvitamin D; BMI, Body mass index; CRP, C-reactive protein; CVD, Cardiovascular disease; DBP, Diastolic blood pressure; HDL-c, High-density lipoprotein cholesterol; HOMA-IR, Homeostatic model; assessment; IL-6, Interleukin-6; IR, Insulin resistance; LDL-c, low-density lipoprotein cholesterol; MH, Metabolically healthy; MU, Metabolically unhealthy; SAT, Subcutaneous adipose tissue; SBP, Systolic blood pressure; TNF-α, Tumor necrosis factor α; UERJ , Rio de Janeiro State University; VAT, Visceral adipose tissue.

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https://doi.org/10.1016/j.nutres.2021.10.002

adjustments (including %body fat and BMI), an inverse association was observed between 25(OH)D and visceral adipose tissue (B = -6.46, 95% confidence interval, CI: -12.87, -0.04), leptin (B = -0.09, 95% confidence interval, CI: -0.14, -0.03), insulin (B = -0.21, 95%CI: -0.34, -0.07), HOMA-IR (B = -0.06, 95%CI: -0.10, -0.02), triglycerides (B = -2.44, 95%CI: -3.66, -1.22), and TNF-α (B = -0.12, 95%CI: -0.24, -0.005) only in MU individuals. Our results indicate that the association of 25(OH)D concentrations with a favorable biochemical profile (glycemic, lipidic and inflammatory) seems to depend on the individual's overall metabolic health, suggesting more benefits from higher serum vitamin D in MU individuals, regardless of their adiposity.

1. Introduction

Obesity is often associated with insulin resistance (IR), type 2 diabetes *mellitus*, systemic hypertension, dyslipidemias and cardiovascular disease (CVD) [1]. Nevertheless, it is estimated that 10% to 44% of individuals with obesity have a favorable metabolic profile and are therefore defined as "metabolically healthy (MH) obese individuals" [2]. Although there is no consensus, most authors define metabolic health on the basis of metabolic syndrome biomarkers, which include blood pressure, plasma concentrations of triglycerides, high-density lipoprotein cholesterol (HDL-c) and glucose [3–6]. Given the pleiotropic action of vitamin D, it has been suggested that it can contribute to developing a favorable metabolic profile in people with obesity. Therefore, serum concentrations of 25-hydroxycholecalciferol [25(OH)D] – the main marker of vitamin D status – has been considered as an alternative biomarker of metabolic health [7–8].

An inverse association between serum 25(OH)D concentrations and obesity has already been demonstrated [9–10]. Furthermore, vitamin D insufficiency [serum 25(OH)D <20 ng/mL] [11] has been pointed as a risk factor for the development of insulin resistance, diabetes, and high blood pressure [12–14]. However, the potential relationship between vitamin D status and metabolic health of individuals with obesity has not been sufficiently elucidated. Higher serum 25(OH)D concentrations were reported for MH compared with metabolically unhealthy (MU) individuals with obesity in some [7,15,16] but not in other studies [17–19]. Part of the controversy can be due to different criteria used to define metabolic health. Also, the association of vitamin D status with metabolic health may be better explored by expanding the scope of its markers, by using inflammation markers (CRP, IL-6 and TNF-α), insulin markers (insulin and HOMA-IR) and adipokines (leptin and adiponectin).

Such relationships have not been sufficiently explored in different phenotypes that arise when BMI classification (normal weight, overweight or obesity) is combined with metabolic health status (healthy or unhealthy) [19]. Also, most studies on obesity and metabolic health have not included a non-obese reference group, which limits comparisons of phenotypes. Furthermore, the adoption of a stricter criterion to evaluate metabolic health, together with the investigation of a wide range of biochemical markers and of body composition may contribute to refining the knowledge about the relationship of this vitamin with metabolic outcomes. We hypothesized that serum 25(OH)D concentrations are higher in MH individuals and inversely associated with glycemic, lipid and inflammatory markers indicative of metabolic disorder. By adopting a criterion that does not admit any abnormality in the biomarkers used for diagnosing the metabolic syndrome [3] to classify individuals as MH, we sought to investigate the association between serum 25(OH)D concentrations and the metabolic health of normal-weight, overweight and obese individuals.

2. Methods and materials

2.1. Study design and participants

This study is part of a prospective cohort study (Pró-Saúde Study) of civil servants of a university in Rio de Janeiro (Brazil), designed to investigate social determinants of health and health-related behaviors [20]. Detailed self-administered questionnaires were answered by cohort members (N = 3253) during four waves of data collection (1999, 2001-2002, 2006-2007, and 2012-2013). Concurrently with wave 4, a subset of 520 participants stratified by sex, age (<50 years and ≥50 years) and educational level (< secondary school and ≥ secondary school), considering the proportions of these strata at the cohort baseline, were randomly selected and invited to perform complementary assessments, including biochemical and body composition analyses [21]. All data collection was conducted by well-trained field personnel; a 10% sample of all questionnaires and body composition measurements were checked for quality by supervisors. The study was approved by the Research Ethics Committee of the Institute of Social Medicine (CAAE: 04452412.0.0000.5260), Rio de Janeiro State University (UERJ) and was carried out after informed consent of the volunteers.

2.2. Collection of sociodemographic data and lifestyle

Information on age, sex, smoking and drinking habits, and practice of physical activity was collected based on self-administered questionnaires. Participants self-classified their race/skin color as white, brown, black, yellow, or indigenous, which are categories used by the Brazilian census [22].

The smoking status was categorized as non-smoker, current smoker or former smoker based on the following question: "Do you currently smoke cigarettes?". Alcohol consumption was assessed via a food frequency questionnaire [23]. Daily average consumption of alcoholic drinks was estimated in daily doses (13g of pure alcohol as standard, equal to one dose). Participants who consumed 0g of alcohol were classified as non-drinkers.

Physical activity was investigated through a dichotomous question: "In the last two weeks, have you practiced any physical activity to improve your health, physical condition or with aesthetic purposes or leisure?" (yes vs no). Information about the use of medication was collected using an open-ended question about use in the previous 7 days. Information on the use of vitamin D supplements was collected from the same open-ended question about medication, combined with another question about current treatment for osteoporosis; based on both responses, the medications/supplements containing vitamin D in their formulations were identified, and the individuals were categorized as users or non-users.

2.3. Anthropometry and classification of BMI

The anthropometric measures were determined according to standardized procedures [24]. Height was measured with a portable stadiometer and total body mass with a digital scale. Body mass index (BMI) was calculated and the participants were classified as normal-weight, overweight or obese according to the parameters of the World Health Organization [25]. The same classification was applied to older individuals because there was a small number of them in the study population, and the same are frequently used of these same cutoff points in studies including elderly volunteers [16,19,20].

2.4. Assessment of body composition

Body composition was determined by dual energy X-ray absorptiometry (DXA, Lunar iDXA, GE Healthcare, Madison, WI, USA). Total fat mass was determined based on whole-body fat analysis using the enCore 2008 software, version 12.20. Body fat percentage was used as an indicator of total adiposity. Visceral adipose tissue (VAT) was estimated on the basis of the android region using the CoreScan VAT software for DXA. Total subcutaneous adipose tissue (SAT) was estimated by subtracting VAT from total fat mass. Details on the method have already been published [21]. All scans were performed by the same professional, who had been well trained to handle the equipment and to position of the participants correctly.

2.5. Blood pressure

Triplicate measurements of blood pressure were performed, using a clinically validated oscillometric device that records brachial venous pressure (OMRON HEM-7113), according to the manufacturer's instructions. The mean values of systolic blood pressure (SBP) and diastolic blood pressure (DBP) of the 3 measurements were calculated and used in the present study.

2.6. Blood collection and biochemical markers

Blood samples were collected after a 12-hour overnight fast by a trained professional, using Vacutainer tubes (Becton, Dickinson & Company, Brazil). Serum samples were collected and stored at -80°C until analyses. The analyses of triglycerides, total cholesterol and fractions, glucose and high-sensitive CRP were carried out using an automatic analyzer (A25 BioSystems, Barcelona, Spain). Insulin concentration was determined by enzyme-linked immunosorbent assay (ELISA), using the automatic analyzer BRIO 2 (RADIM, Pomezia, Italy). HOMA-IR was calculated based on fasting glucose and insulin concentrations [26]. Concentrations of TNFα and IL-6 were determined using a sandwich ELISA, according to the manufacturer's instructions (BD Biosciences, USA). Leptin and adiponectin concentrations were measured using ELISA commercial kits (Millipore, USA), and 25(OH)D was determined by using a semiautomated chemiluminescent enzyme-labeled immunometric assay (Liaison, Diasorin, MN, USA). Intra-assay coefficients of variation for TNFα, IL-6, leptin, adiponectin and 25(OH)D were all $<$6%.

2.7. Definition of metabolic health

This study used the definition proposed by Ortega and colleagues [3], who consider as "metabolically healthy" individuals with zero components of metabolic syndrome. Although based on metabolic syndrome components, it excludes waist circumference owing to its collinearity with BMI. The participants with at least one component of the syndrome were considered as metabolically unhealthy. This definition, which is considered to be stricter, was based on a literature review and opinions of specialists, and it has been largely used to classify participants in recent studies on metabolic health [20,27,28]. Based on this proposal, the individuals of this study were considered as metabolically healthy if they did not fall into any of these situations: triglycerides $\geq$150 mg/dL or using drugs to reduce triglycerides; HDL-c $<$40 mg/dL for men and $<$50 mg/dL for women, or using drugs to increase HDL-c; systolic blood pressure $\geq$130 mmHg and diastolic blood pressure $\geq$85 mmHg, or using antihypertensive drugs, and fasting glucose $\geq$100 mg/dL or using hypoglycemic drugs.

2.8. Phenotypes of body weight and metabolic health

The BMI and metabolic health categories described earlier were combined so as to allow the categorization of participants into six phenotypes: MH normal-weight individuals; MU normal-weight individuals; MH and MU overweight individuals, and MH and MU obese individuals.

2.9. Statistical analyses

For the current analyses, nine individuals were excluded due to underweight (n = 5), missing data required for BMI classification (n = 3), and lack of the data required to determine metabolic health (n = 1), which resulted in a total of 511 participants. The variables were presented as medians, minimums, maximums and frequencies, as appropriate. Since the groups according to the phenotypes resulted in unequal sample sizes, comparisons between the groups were evaluated by Kruskal-Wallis followed by Dunn's multiple comparison test. Associations between serum 25(OH)D concentrations and metabolic health indicators were analyzed separately for the individuals classified as MH and MU, using multiple linear regression models, considering the serum 25(OH)D concentrations as the independent variable (exposure), and each metabolic health marker as dependent variables (outcomes), separately. Two models were built with the following adjustments: model 1 – adjusted by sex, age, race/skin color, smoking and physical activity; model 2 – adjusted by the variables of model 1 plus

Table 1 – Participants' sociodemographic and lifestyle characteristics, according to BMI and metabolic health categories. Pró-Saúde Study, Rio de Janeiro, 2012-13

	Total	Normal weight		Overweight		Obese	
		MH	MU	MH	MU	MH	MU
N	511	43	99	41	167	10	151
Age (years)	51 (33-79)	48 (37-61)[a]	52 (33-73)[b]	50 (36-68)[ab]	52 (37-77)[ab]	47 (39-53)[ab]	51 (36-79)[ab]
Sex							
Female	264 (52)	28 (65)	44 (44)	29 (71)	79 (47)	8 (80)	76 (50.)
BMI (Kg/m^2)	27.5 (19.5-50.4)	22.6 (19.5-24.8)[a]	23.1 (19.7-24.9)[a]	26.5 (25.2-29.7)[b]	27.5 (25.1-29.9)[b]	31.5 (30.4-37.3)[c]	33.0 (30.0-50.4)[c]
Smoking							
Non-smoker	306 (60)	35 (81)	62 (63)	23 (56)	93 (56)	6 (60)	87 (58)
Current smoker	53 (10)	2 (4.7)	10 (10)	3 (7.3)	25 (15)	-	13 (8.6)
Former smoker	150 (29)	6 (14)	26 (26)	15 (37)	49 (29)	3 (30)	51 (34)
Alcohol consumption							
Nondrinker	210 (41)	24 (56)	42 (42)	12 (29)	61 (37)	4 (40)	67 (44)
Up to 1 dose	208 (41)	16 (37)	40 (40)	21 (51)	69 (41)	6 (60.)	56 (37)

MH, metabolically healthy; MU, metabolically unhealthy.
Values presented are median (min-max) or frequencies (%).
Frequencies (%) presented in relation to the total of each phenotype.
1 daily dose corresponds to 13g of alcohol/day. For continuous variables (age and BMI) different superscript letters on the same line indicate a statistically significant difference between groups ($P < .05$) by Kruskal-Wallis with Dunn's posthoc test.

body fat percentage and BMI. Adjustment variables were selected on the basis of evidence in the literature regarding the influence of sex, age, race/skin color, smoking and physical activity on metabolic health biomarkers [29–32]. All tests considered a *P*-value <.05 as statistically significant. Data processing and analysis were performed using the SPSS software, version 22.0 (SPSS, Inc.).

3. Results

Table 1 shows the participants' sociodemographic profile, per phenotype. Median age of the participants was 51 years, 81.6% consumed less than one dose of alcoholic beverage per day, and the majority has never smoked (60.0%). Of the total participants, 2% were classified as MH obese individuals, corresponding to 6.2% of the total number of obese individuals (n =161) (Table 1).

The participants' metabolic profile was characterized according to the six previously defined phenotypes (Table 2). Both MH and MU obese individuals exhibited a higher amount of SAT than normal-weight individuals (18.8 kg, $P < .001$ for MH, and 17.9 kg, $P < .001$ for MU). With respect to VAT, obese individuals (1367g and 1980g for MH and MU, respectively) exhibited higher values ($P < .001$) when compared with normal-weight individuals (389g and 712g for MH and MU, respectively).

There were no statistically significant differences in serum leptin and adiponectin concentrations among MH and MU individuals in the same BMI category ($P > .05$). The median of serum leptin concentration was about three times higher in MH obese than in MH normal-weight individuals (9.7 ng/mL and 3.0 ng/mL, respectively, *P*-value = .038) and also in MU obese individuals when compared with MU normal-weight individuals (6.1 ng/mL and 1.9 ng/mL, respectively, *P*-value < .001). For obese individuals classified as MU the median of serum adiponectin concentrations was 37% lower than that of MH normal-weight individuals (32.0 vs 50.9 ng/mL, *P*-value = .004) (Table 2).

Insulin concentrations and HOMA-IR were not significantly different between MH and MU obese individuals. However, MU obese individuals had higher insulin concentrations (17.0 IU/mL) than MH and MU normal-weight individuals (6.5 IU/mL and 9.0 IU/mL, respectively, $P < .001$ for both). The same pattern of difference between the phenotypes was observed for HOMA-IR. The CRP concentrations were higher in MU obese individuals (0.40 mg/dL) when compared with MH and MU normal-weight individuals (0.13 mg/dL and 0.10 mg/dL, respectively, $P < .001$ for both). There were no significant differences among the 6 phenotypes for IL-6 and TNF-α concentrations (Table 2).

In the subgroups of the same BMI category, there were no differences in 25(OH)D concentrations between MH or MU individuals. The lowest median values of serum 25(OH)D concentrations were observed in MH and MU obese individuals [13.7 (7.9-17.5) ng/mL and 17.8 (4.2-41.0) ng/mL respectively]. Serum 25(OH)D was significantly lower among the entire group (MH+MU) of overweight [18.3 (4.9-56.4), $P = .006$] and obese [16.9 (4.2-41.0), $P < .001$] when compared to the normal-weight [20.8 (4.2-53.9)] participants. Vitamin D insufficiency [25(OH)D <20 ng/mL; IOM, 2011] was observed in 56.0% of the total number of study individuals. Frequency of insufficiency was especially high among overweight (57.9%) and obese (65.6%) individuals. Only 6.8% of all participants reported use of vitamin D supplements, and there was no significant difference in mean serum 25(OH)D concentrations between the individuals who take the supplement and those who do not.

The multiple linear regression showed that, in MU individuals, serum 25(OH)D was inversely associated with leptin (B = -0.14, 95%CI: -0.19; -0.08), insulin (B = -0.29, 95%CI: -0.43; -0.15), HOMA-IR (B = -0.09, 95%CI: -0.13; -0.05), triglycerides (B = -2.98, 95%CI: -4.17; -1.80) and TNF-α (B= -0.13, 95%CI: -0.25; -0.02), after adjustment (model 1) by sex, age, race/skin

Table 2 – Participants' metabolic profile according to BMI and metabolic health categories. Pró-Saúde Study, Rio de Janeiro, 2012-13

	Normal weight		Overweight		Obese	
	MH	MU	MH	MU	MH	MU
Body fat (%)	33.2 (14.4-45.1)^{ab}	29.6 (12.7-43.1)^{a}	39.0 (22.1-48.0)^{cd}	34.7 (22.6-47.9)^{bd}	45.1 (33.7-47.5)^{c}	41.2 (22.5-57.5)^{c}
VAT (g)	389 (43-1242) ^{a}	712 (91-1569)^{ab}	878(243-1906)^{bc}	1203 (159-3497)^{c}	1367 (630-2132)^{cd}	1980 (474-6325)^{d}
SAT (kg)	18.8(7.3-28.0)^{a}	17.9 (7.9-26.6)^{a}	26.4 (15.1-35.0)^{b}	25.4 (15.7-36.8)^{b}	35.2 (31.6-45.0)^{c}	34.7 (16.5-86.5)^{c}
Leptin (ng/mL)	3.0 (0.2-19.1)^{ab}	1.9 (0.1-20.4)^{a}	5.0 (0.3-20.0)^{bcd}	4.3 (0.4-25.7)^{bc}	9.7 (3.4-16.6)^{cd}	6.1 (0.3-29.0)^{d}
Adiponectin (ng/mL)	50.9 (20.8-225.4)^{a}	47.1 (0.6-272.8)^{ac}	46.8 (13.5-268)^{ac}	37.3 (0.5-224.6)^{bc}	45.7 (20.5-88.4)^{ac}	32.0 (0.7-347.8)^{bc}
Glucose (mg/dL)	81 (68-97)^{a}	87 (70-192)^{bc}	86 (59-98)^{ac}	90 (62-330)^{b}	83 (78-98)^{ac}	98 (57-364)^{d}
Insulin (mg/dL)	7 (1-21)^{a}	9 (1-60)^{a}	11 (1-27)^{a}	14 (2-72)^{b}	11 (5-40)^{abc}	17 (3-68)^{c}
HOMA-IR	1.2 (0.1-3.7)^{a}	2.0 (0.1-14.2)^{a}	2.0 (0.1-6.0)^{a}	3.2 (0.4-16.6)^{b}	2.0 (1.0-8.4)^{abc}	4.6 (0.5-36.6)^{c}
TG (mg/dL)	76 (40-146)^{a}	115 (46-538)^{bc}	93 (40-144)^{ab}	138 (41-485)^{cd}	84 (46-123)^{ab}	166 (44-704)^{d}
Total cholesterol (mg/dL)	204 (129-266)	207 (101-381)	204 (154-306)	210 (101-349)	188 (143-242)	203 (126-328)
HDL-c (mg/dL)	56 (42-92)^{a}	52 (25-106)^{a}	57 (40-95)^{a}	46 (25-104)^{bc}	55 (50-86)^{ac}	47 (23-84)^{b}
LDL-c (mg/dL)	128 (62-178)	123 (42-280)	126 (68-218)	124 (43-230)	115 (75-161)	115 (45-217)
CRP (mg/dL)	0.13 (0.01-0.60)^{a}	0.10 (0.01-4.00)^{a}	0.21 (0.02-2.94)^{abc}	0.20 (0.01-1.99)^{b}	0.26 (0.06-1.40)^{abc}	0.40 (0.01-5.95)^{c}
IL-6 (pg/ mL)	0.3 (0.0-19.4)	0.5 (0.0-48.3)	0.0 (0.0-10.6)	0.5 (0.0-101.8)	2.1 (0.0-19.3)	0.5 (0.0-55.9)
TNF-α (pg/mL)	0.3 (0.0-13.5)	0.6 (0.0-21.1)	0.3 (0.0-10.4)	0.8 (0.0-93.4)	2.0 (0.0-49.2)	0.8 (0.0-26.7)
SBP (mmHg)	108 (92-127)^{a}	127 (92-177)^{bc}	114 (95-128)^{a}	125 (91-186)^{c}	110 (94-124)^{ab}	125 (99-225)^{c}
DBP (mmHg)	69 (56-81)^{a}	76 (57-101)^{bc}	70 (57-82)^{a}	79 (58-109)^{bc}	73 (65-82)^{ac}	79 (58-117)^{bc}
25(OH)D (ng/mL)	20.0 (4.9-29.3)^{ab}	22.1 (4.2-53.9)^{b}	18.0 (6.5-48.2)^{ab}	18.9 (4.9-56.4)^{a}	13.7 (7.9-17.5)^{a}	17.8 (4.2-41.0)^{a}

DBP, Diastolic blood pressure; IL, interleukin; MH, metabolically healthy; MU, metabolically unhealthy; SAT, subcutaneous adipose tissue; SBP, systolic blood pressure; TNF, tumor necrosis factor; VAT, visceral adipose tissue.
Values are presented as median (min-max). Different letters on the same line indicate a statistically significant difference by Kruskal-Wallis with Dunn's posthoc test ($P < .05$).

color, smoking and physical activity (Table 3). These associations remained statistically significant after an additional adjustment for body fat percentage and BMI (model 2). Among the MH individuals, there was no association between serum 25(OH)D and the variables tested, with the exception of a direct association with glucose concentrations (B = 0.41, 95% CI: 0.20; 0.63).

4. Discussion

In the present study, we investigated the association between serum 25(OH)D concentrations and metabolic health using a wide range of biochemical and body composition markers in normal-weight, overweight and obese individuals. As expected, serum 25(OH)D concentrations were lower in obese individuals, but there was no difference between those classified as MH and MU. Higher serum 25(OH)D concentrations were associated with more favorable biochemical profiles (glycemic, lipidic and inflammatory) only for individuals classified as MU, regardless of adiposity.

Participants were considered as MH only if they did not exhibit any alteration in the components of metabolic syndrome, which resulted in a very low frequency of MH obese individuals (2%). This frequency was similar in previous studies which reported 3-5% of prevalence of MH obesity [20,33,34], despite the fact that some of them have not used similarly strict criteria for classification of metabolic health [20,34].

Consistent with previous studies [35–37], we observed a high prevalence (65%) of vitamin D insufficiency [25(OH)D <20ng/mL] among obese individuals, irrespective of their metabolic health status. As expected, serum 25(OH)D concentrations were lower in participants with overweight (~10%) and obesity (~20%) compared to normal-weight individuals (regardless of their MH status). In addition, our findings did not suggest differences between serum 25(OH)D concentrations in MH and MU obese individuals, similarly to the observed by some [17–19] but not all [7,15,16] studies.

Median of SAT in individuals with obesity (MH and MU) was twice as much as that observed in MH normal-weight individuals, while the differences of VAT between these groups were much higher, reaching values 3.5 times higher than in MH or MU obese individuals, when compared with MH normal-weight ones. VAT has been recognized as having a distinct secretory profile from that of SAT, presenting hyperlipolytic adipocytes and lower sensitivity to insulin. Also, it is often related to the development of a MU phenotype [38].

As expected, serum leptin concentration was higher in individuals with obesity, although there were no differences between MH and MU. Nevertheless, consistent with the hypothesis of absence of systemic inflammation in a subset of obese individuals, in the present study, for MH obese participants, TNF-α, IL-6, CRP and adiponectin concentrations were similar to those found in MH normal-weight individuals. In addition, mean CRP concentrations were below the cutoff point (>0.3 mg/dL) established as of high risk for CVD [39], which corroborates the hypothesis of absence of significant inflammation in obese individuals, as previously reported [40]. Adiponectin has been recognized for having anti-inflammatory and anti-atherogenic properties [41], and the higher concentrations that have been found in some MH obese individuals suggests that it may contribute to reducing the risk for CVD in those individuals [42].

In the present study, it was also observed that in MU obese and non-obese individuals, serum 25(OH)D was inversely associated with visceral adipose tissue, leptin, insulin,

Table 3 – Associations between serum 25(OH)D concentration and markers of metabolic health. Pró-Saúde Study, Rio de Janeiro, 2012-13

	Metabolically Healthy (n = 94)				Metabolically Unhealthy (n = 417)			
	Model 1		Model 2		Model 1		Model 2	
	B	95% CI	B	95% CI	B	95% CI	B	95% CI
Body fat (%)	-0.09	-0.24; 0.07	-	-	-0.17	-0.24; -0.11[c]	-	-
VAT (g)	-6.63	-20.74; 7.49	2.24	-6.86; 11.34	-24.09	-33.61; -14.57[c]	-6.46	-12.87; -0.04[a]
SAT (kg)	0.09	-0.29; 0.09	0.03	-0.04; 0.09	-0.25	-0.35; - 0.15[c]	-0.01	-0.05; 0.03
Leptin (ng/mL)	0.08	-0.05; 0.21	0.12	-0.003; 0.24	-0.14	-0.19; - 0.08[c]	-0.09	-0.14; -0.03[b]
Adiponectin (ng/mL)	-0.57	-1.90; 0.77	-0.82	-2.20; 0.56	0.15	-0.38; 0.69	0.07	-0.47; 0.61
Glucose (mg/dL)	0.27	0.04; 0.49[a]	0.41	0.20; 0.63[c]	-0.47	-0.89; -0.05[a]	-0.29	-0.72; 0.14
Insulin (UI/mL)	-0.09	-0.29; 0.11	-0.03	-0.23; 0.17	-0.29	-0.43; -0.15[b]	-0.21	-0.34; -0.07[b]
HOMA-IR	-0.01	-0.05; 0.03	0.006	-0.04; 0.05	-0.09	-0.13; -0.05[c]	-0.06	-0.10; -0.02[b]
TG (mg/dL)	0.27	-0.58; 1.13	0.48	-0.42; 1.38	-2.98	-4.17; -1.80[c]	-2.44	-3.66; -1.22[c]
Cholesterol (mg/dL)	0.12	-0.90; 1.14	0.16	-0.84; 1.16	-0.44	-0.92; 0.04	-0.47	-0.97; 0.04
HDL-c (mg/dL)	0.17	-0.15; 0.49	0.09	-0.25; 0.43	-0.003	-0.14; 0.13	-0.10	-0.24; 0.04
LDL-c (mg/dL)	-0.10	-1.05; 0.85	-0.03	-0.96; 0.91	0.15	-0.28; 0.58	0.12	-0.33; 0.57
CRP (mg/dL)	-0.002	-0.01; 0.01	0.00	-0.01; 0.01	-0.003	-0.01; 0.002	0.002	-0.006; 0.009
IL-6	-0.03	-0.16; 0.10	-0.04	-0.18; 0.11	-0.10	-0.24; 0.03	-0.09	-0.23; 0.04
TNF- α	-0.03	-0.25; 0.19	0.01	-0.23; 0.25	-0.13	-0.25; -0.02[a]	-0.12	-0.24; -0.005[a]
SBP (mmHg)	-0.13	-0.40; 0.14	-0.10	-0.39; 0.18	0.07	-0.13; 0.26	0.09	-0.10; 0.29
DBP (mmHg)	-0.13	-0.32; 0.06	-0.14	-0.33; 0.05	0.02	-0.10; 0.13	0.05	-0.07; 0.17

CRP, C-reactive protein; DBP, diastolic blood pressure; HDL-C/LDL-C, high-density/low-density lipoprotein cholesterol; IL, interleukin; SAT, subcutaneous adipose tissue; SBP, systolic blood pressure; TG, triglyceride; TNF, tumor necrosis factor; VAT, visceral adipose tissue.
B and 95% CI values obtained by multiple linear regression.
Model 1 - adjusted for sex, age, race/skin color, smoking and physical activity; Model 2 – additionally adjusted for percent body fat and BMI.
Race/skin color: categories adopted by the Brazilian census.
[a] $P < .05$;
[b] $P < .01$;
[c] $P < .001$

HOMA-IR, triglycerides and TNF-α after adjustment by sex, age, race/color, smoking, physical activity, fat mass percentage and BMI. The literature has shown an inverse association of serum 25(OH)D with several of those biochemical markers [7]. For instance, low serum 25(OH)D concentrations have been associated with an increase in inflammatory cytokines, which, in turn, could contribute to the development of metabolic syndrome, as well as to exacerbate liver inflammation [43,44]. Given that alterations in components of metabolic syndrome are associated with metabolic dysfunction-associated fatty liver disease (MAFLD) [45], it appears that, indirectly, vitamin D insufficiency fosters the risk of MAFLD development [46]. However, our results suggest that the presence of these associations may depend on the individual's metabolic health status. In fact, it has already been found that the effect of vitamin D supplementation on the metabolic profile is different between the phenotypes of obesity and metabolic health, suggesting that interventions with supplemental vitamin D may have the potential to diminish the progression of cardiometabolic complications associated with metabolic diseases in MU obese individuals, but not in MH individuals [47]. In the present study, there was an unexpected direct association between serum 25(OH)D and glucose concentrations in MH individuals. However, it should be noted that these individuals had serum glucose concentrations within normal limits.

The strengths of our study include the presence of reference groups of normal-weight individuals (MH or MU) and a wide range of markers of metabolic health, including the measure of visceral adipose tissue. Furthermore, given the lack of consensus on the definition of "metabolically healthy", we adopted stricter criteria as used in the BioSHaRE-EU international project between European and Canadian institutes [28]. Some limitations of the study should be mentioned. First, the cross-sectional design limits causal inference. Second, the indirect question on the use of vitamin D supplementation may have underestimated this information. In addition, there was limited information regarding physical activity. Finally, the study may have had limited statistical power to detect meaningful differences across some subgroups, as exemplified by the small number of MH obese individuals.

5. Conclusions

In summary, the findings of the present study confirm a higher prevalence of vitamin D inadequacy in individuals with obesity, when compared with normal-weight individuals, but they do not support the existence of differences in serum 25(OH)D concentrations in obese individuals with different metabolic health statuses. The results also indicate that the association of serum 25(OH)D concentrations with a favorable biochemical profile seems to depend on individuals' overall metabolic conditions, suggesting that potential benefits from higher serum 25(OH)D concentrations would be more evident in individuals considered to be metabolically unhealthy. Regardless of the coexistence of obesity, it seems relevant to consider in-

dividuals' metabolic health when evaluating the impact of vitamin D interventions.

6. Author Contributions

Mitsu de Azevedo Oliveira: Conceptualization, methodology, formal analysis, writing - Original Draft, writing - Review & Editing; Eduardo Faerstein: Supervision, project administration, funding acquisition, writing - Review & Editing; Josely Koury: Investigation, writing - Review & Editing; Wania F. Pereira Manfro: Investigation, writing - Review & Editing; Lucimar Gonçalves Milagres: Investigation, writing - Review & Editing; José Firmino N. Neto: Investigation, writing - Review & Editing; Flavia F. Bezerra: Conceptualization, supervision, funding acquisition, writing - Original Draft, writing - Review & Editing.

Acknowledgments

The authors acknowledge the team of the Pro-Saúde research network and the staff of Interdisciplinary Nutrition Assessment Laboratory of the State University of Rio de Janeiro for the valuable support.

Sources of Support

This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Grant 484636/2013/8 for EF), Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ, Grant E-26/110.116/2014 for FFB), Brazil and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, MS scholarship).

Author Declarations

The authors declare no conflicts of interest.

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