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Research Article

Vitamin D Nutritional Status and its Relationship with Body Variables in Individuals with Obesity According to Classification of Obesity Phenotype for Different Criteria

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Abstract

Introduction: There is a subgroup of individuals with obesity, called with metabolically healthy obesity (MHO) phenotype, who remains insulin-sensitive, and they may be more protected from metabolic disorders. Low vitamin D serum concentrations were observed in individuals with obesity, mainly with high abdominal adiposity.

Methods: Descriptive cross-sectional study was conducted with individuals, aged ≥ 20 < 60 years. Body variables were evaluated, and metabolic parameters [blood pressure and blood glucose, insulin, lipid profile, high-sensitivity c- reactive protein (hs-CRP) and (25(OH)D)] were obtained. The cut-off point for vitamin D deficiency (VDD) was ≤20 ng/mL. Individuals were classified as MUHO according to four different definitions.

Results: This study comprised 232 individuals with obesity (BMI ≥ 35 Kg/m^2); 178 female (76.7%). MUHO phenotype was observed in 67.7%, 76.7%, 71,5% and 82,7% according to NCEP/ATPIII, HOMA-IR, Wildman, and Karelis definitions, respectively. Only for the HOMA-IR definition, the mean value of 25(OH)D showed a significant difference between the phenotype groups (p=0.011). Among all criteria to classify obesity phenotype, only in HOMA-IR presented some predictors of MUHO in individuals with VDD.

Conclusion: The results show high prevalence of inadequacy of serum concentrations of 25(OH)D in individuals with MUHO phenotype, mainly classified by HOMA-IR definition.

Keywords: Obesity; Vitamin D; Serum 25(OH)D; Metabolically Healthy Obesity; Metabolically Unhealthy Obesity; Adiposity; Body Variables

Introduction

Obesity is defined as an excess amount of body fat that impacts damage of health [1] and has been strongly associated with chronic low-grade or metabolic inflammation. The prevalence of obesity has been increasing exponentially in recent years and it is the fifth greatest risk factor for mortality [2]. However, obesity does not necessarily translate into an increased risk for metabolic comorbidities highlighted by the fact that a subgroup of individuals with obesity remains insulin-sensitive and they appear to be more protected from metabolic disorder. These individuals have an obesity phenotype called metabolically healthy obesity (MHO). The classification of obesity phenotypes can be done for different criteria, once that we do not have a stabilized one [3].

Low vitamin D serum concentrations and obesity have concomitantly reached epidemic levels worldwide and research linking these two public health issues has grown extensively over the last number of years [4]. Studies have opened a new dimension regarding the relevance of vitamin D in health. This vitamin has been suggested to be a potential factor in the prevention of many diseases [5] and, in obesity itself [4]. Association between vitamin D nutritional status and obesity is known, since vitamin D is lipid-soluble and its serum concentration tends to be reduced in individuals with excess body fat, mainly abdominal adiposity [6].

Body mass index (BMI) is the most used tool to identify overweight or/and obesity in individuals. However, this body compo-

sition variable may be inappropriate since it is not possible to distinguish body fat from lean mass [7]. So, others indirect variables for adiposity measures such as waist circumference (WC) and waist-to-height ratio (WHtR) are used to diagnose abdominal obesity. And, have some indexes to verify adiposity, as body adiposity index (BAI), visceral adiposity index (VAI) and body roundness index (BRI) [8,9,10], and they can be a useful tool to predict body fat in this population.

Therefore, the aim of the study was to evaluate vitamin D serum concentrations (25(OH)D) and its relationship with body variables in individuals with obesity in pre-operative of metabolic surgery classified as MHO and metabolically unhealthy obesity (MUHO) by four different criteria of classification of these obesity phenotypes.

Methods

A cross-sectional study comprising 232 individuals with obesity with BMI ≥ 35 Kg/m^2; aged ≥ 20 and lt; 60 years, in the preoperative phase of metabolic surgery, recruited within the patients of a medical clinic specialized in obesity control, in the municipality of Rio de Janeiro, Brazil, and conducted according to CONSORT guidelines. Exclusion criteria were as follows: pregnancy or lactation, presence of chronic kidney [defined by estimated GFR and lt;60 mL/min/1.73m 2 [11]] or liver diseases (except non-alcoholic fatty liver disease), history of hyperparathyroidism or elevated serum calcium levels, malabsorption bowel syndrome, acute and chronic infections, endocrinopathies (hyperparathyroidism, hypothyroidism and hypercortisolemia), previous restrictive and disabsorptive surgeries and use of anticonvulsant medications or drugs known to interfere with vitamin D metabolism as well as current insulin treatment, consumption of any form of vitamin D supplements or having a vitamin D prescription within 6 months prior to blood work. This study was approved by the Research Ethics Committee of Hospital Universitário Clementino Fraga Filho, Federal University of Rio de Janeiro, Brazil (Research Protocol nº 011/06-CEP).

All patients were informed that participation in the study was voluntary. Written informed consent was obtained before carrying out any study-related procedures from all subjects who participated in the study.

Sample size

The sample size was determined to respond to the main aim of the study, which was to evaluate the nutritional status of VD and its relationship with body variables in individuals classified according to obesity phenotype by four different criteria. The following parameters have been assumed: use of bilateral tests, a level of significance of 5%, a statistical power of 80%, and an expected correlation of - 0.25. According to the sample calculation, 224 individuals were required. The sample size value was inflated by 10%, to anticipate possible losses.

Evaluation of physical activity and body variables

Data related to the habit of engaging in physical exercise, such as type, time (in years and minutes/week) and weekly frequency (days/week) were collected through a questionnaire previously prepared [12] during the first consultation.

BMI calculation (Kg/m^2) was conducted based on the anthropometric measurements of weight (Kg) and height (m) [13]. The measurement of the diameter (cm) of the waist circumference (WC) was performed with the patient standing straight, the abdomen relaxed, the arms beside the body and the feet together. A non-extensible tape was used to involve the subject in the greatest abdominal diameter, being the diameter of the WC evaluated at the completion of the individual normal expiration. Waist/height ratio (WHtR) was calculated using the formula: WC (cm)/height (m). The cutoff point was 0.50, according to Zeng Q., *et al.* [14].

BRI is eccentricity quantifies the degree of circularity of an ellipse, and its values range between 0 to 1, with 0 characterizing a perfect circle, and 1, a vertical line. Was calculated using formulae

$$BRI = 364.2 - 365.5 \times \sqrt{1 - \left(\frac{(WC/(2\pi))^2}{(0.5height)^2}\right)}$$

The VAI is an empirical mathematical model, gender-specific, based on simple anthropometric data (BMI and WC) and biochemical parameters (triglycerides [TG] and high lipoprotein density – cholesterol [HDL-c]) and is indicative of fat distribution and function [10].

$$\text{Females: VAI} = \left(\frac{WC}{36.58 + (1.89 \times BMI)}\right) \times \left(\frac{TG}{0.81}\right) \times \left(\frac{1.52}{HDL}\right),$$

$$\text{Males: VAI} = \left(\frac{WC}{39.68 + (1.88 \times BMI)}\right) \times \left(\frac{TG}{1.03}\right) \times \left(\frac{1.31}{HDL}\right),$$

BAI can be used to reflect the percentage of body fat in adults of both genders of different ethnicities, without numerical correction [8]. The formula used is:

$$BAI = \frac{Hip\ circumference}{height(m)^{1.5}} - 18$$

Evaluation of systemic blood pressure

The blood pressure quantification by indirect measurement method was carried out using OMRON HEM-705CP monitor (OM-

RON Healthcare Europe B.V., Hoofddorp, the Netherlands), with a range of 0-300 mmHg and an accuracy of ± 3 mmHg.

At least two measurements were taken, with an interval of approximately one minute, and the mean was calculated.

Evaluation of circulating biochemical and metabolic parameters For biochemical evaluation, blood was obtained by venipuncture, after an overnight fasting period. Laboratory tests were conducted in the serum to characterize the lipid profile [total cholesterol, low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c) and triglycerides] and to evaluate glucose, insulin and high sensitivity-C reactive Protein (hs-CRP) levels.

Determinations of total cholesterol, HDL-c, triglycerides, and glucose were performed using specific enzymatic colorimetric methods (Labtest Diagnóstica S.A., Minas Gerais, Brazil). LDL-c fraction was calculated in accordance with the Friedewald's formula (Friedewald WT, 1972). Insulin was quantified using reversed-phase HPLC (Labtest Diagnóstica S.A., Minas Gerais, Brazil). hs-CRP was analyzed using the Tina-quant® C-reactive protein latex ultrasensitive assay (Roche Diagnostics, London, United of Kington). PTH measurements were performed by an electrochemiluminescence immunoassay (ECLIA) using Modular E170 (Roche Diagnostics GmbH, Mannheim, Germany).

Homeostatic model assessment - insulin resistance (HOMA-IR) was calculated as described by Matthews., *et al*. [15], formula: fasting insulin (microU/L) x fasting glucose (nmol/L)/22.5.

Vitamin D status

Serum vitamin D analysis was conducted in the form of 25(OH) D using HPLC [16]. The volunteers of this study were organized according to the 25(OH)D cutoff points of the clinical practice guideline of the American Endocrine Society [17] and included in the deficiency (≤ 20 ng/mL), insufficiency (20.1 ng/mL – 29.9 ng/mL) and sufficiency (≥ 30 ng/mL and and lt; 100 ng/mL) groups.

To complete the evaluation of the nutritional status of vitamin D, an investigation was conducted on the sun exposure of the individuals, as described by Hanwell., *et al*. [18].

Definitions of the metabolically healthy and unhealthy obesity phenotypes

Participants were divided in two obesity phenotype groups, MHO and MUHO, according to the four different definitions described in chart 1.

		Anthropometric, biochemical and metabolic features								Number of features necessary for MUHO classification	Reference
		HOMA-IR	WC	BP (SBP/ DBP)	TG	Glucose	HDL-c	LDL-c	hs-CRP		
Definition	NCEP/ ATPIII	-	> 88 cm (♀) > 102 cm (♂)	> 130/85 mmHg	≥ 150 mg/dL	≥ 100 mg/dL	< 50 mg/ dL (♀) < 40 mg/ dL (♂)	-	-	≥ 3	Grundy, 2005
	HOMA-IR	≥ 2.5	-	-	-	-	-	-	-	1	Durward., *et al.,* 2012; Calori., *et al*., 2011
	WILDMAN	> 8.3 (i.e. >90th percentile)	-	≥ 130/85 mmHg or antihypertensive medication use	≥ 150 mg/dL	≥ 100 mg/dL	< 50 mg/ dL (♀) < 40 mg/ dL (♂)	-	>2.04 mg/L (i.e. >90th percentile)	≥ 2	Wildman, 2008
	KARELIS	> 2.7	-		> 150.5 mg/dL	-	< 50.2 mg/dL	> 100.5 mg/dL	≥ 3 mg/L	≥ 2	Karelis, 2008; Messier and Karelis, 2011

Chart 1: Four distinct definitions of the healthy and unhealthy obesity phenotypes.

BP: Blood Pressure; HS-CRP: High-Sensitive-C-Reactive Protein; DBP: Diastolic Blood Pressure; HDL-c: High-Density Lipoprotein Cholesterol; HOMA-IR: Homoeostasis Model Assessment for Insulin Resistance; LDL-c: Low-Density Lipoprotein Cholesterol; MUHO: Metabolically Unhealthy Obesity; NCEP/ATPIII: National Cholesterol Education Program/Adult Treatment Panel III; SBP: Systolic Blood Pressure; TG: Triglycerides; WC: Waist Circumference.

Statistical analysis

The Kolmogorov–Smirnov test was used to test the normality of data and expressed as means and standard deviations for clinical and biochemical variables. Categorical variables were reported as count and percentage, while numerical variables were described as mean ± standard deviation (SD). Proportion differences between the MHO and MUHO phenotype groups were evaluated using the Chi-square test. Differences between the MHO and MUHO phenotype groups in the continuous variables were assessed using the two-independent sample t-test. Correlation analysis of 25(OH)D levels with the other body variables were estimated using the Pearson's correlation. Comparison of continuous variables between groups of Vitamin D status was performed by ANOVA if variables were normally distributed. The binary logistic regression was performed using obesity phenotype definition was the dependent variable for all six models. Models were fitted using in each model only one body adiposity parameter as independent variable (BMI, WC, WHtR, BRI, BAI and VAI) and vitamin D deficiency (VDD) (≤ 20 ng/mL) were included as selection variable. The covariates for adjustment were weight and age and the predictor was identified by stepwise selection. Statistical analysis was performed using the SPSS software (SPSS version 21.0, Chicago, IL, USA). p-Values ≤ 0.05 were considered statistically significant.

Results

General characteristics

Table 1 shows the body variables of our population analyzed according to the four definitions taken into consideration for the obesity phenotypes classification. The MUHO phenotype was observed in 67.7%, 76.7%, 71,5% and 82,7% of the population for NCEP/ATPIII, HOMA-IR, Wildman, and Karelis definitions, respectively. There was not statistically significant difference between gender in according of phenotype classifications, NCEP/ATPIII, HOMA-IR, Wildman and Karelis (p = 0.139; p = 0.345; p = 0.416; p = 0.173, respectively).

For the NCEP/ATPIII and Wildman definition, a significant difference (p = 0.013 and p = 0.052, respectively) was observed for the age of the individuals, which prove to be higher in the MUHO than in MHO phenotype group. There were no significant differences for BMI and body weight mean values between MHO and MUHO for each of the four definitions used here. However, WC diameter was significantly higher in the MUHO than in the MHO phenotype group only in HOMA-IR definition (p = 0.041).

	NCEP/ATPIII		*p*	HOMA-IR		*p*	Wildman		*p*	Karelis		*p*
	MHO	MUHO		MHO	MUHO		MHO	MUHO		MHO	MUHO	
Number	75	157		54	178		66	166		40	192	
Gender n(%) male	13 (17.3)	41 (26.1)		10 (18.5)	44 (24.7)		13 (19.7)	41 (24.7)		6 (15.0)	48 (25.0)	
Female	62 (82.7)	116 (73.9)	0.139	44 (81.5)	134 (75.3)	0.345	53 (80.3)	125 (75.3)	0.416	34 (85.0)	144 (75.0)	0.173
Age (years)	39.5 ± 11.1	43.2 ± 10.3	0.013	42.5 ± 9.9	41.8 ± 10.9	0.662	39.8 ± 10.8	42.8 ± 10.5	0.052	42.9 ± 11.3	41.8 ± 10.5	0.528
BMI (Kg/m^2)	43.0 ± 4.5	42.3 ± 4.8	0.308	41.8 ± 3.8	42.8 ± 5.0	0.204	42.9 ± 4.4	42.4 ± 4.9	0.491	42.8 ± 4.7	42.3 ± 4.8	0.119
Weight (Kg)	118.7±16.3	117.2 ± 19.9	0.526	116.5 ± 18.1	118.0 ± 19.1	0.593	119.5 ± 16.0	116.9 ± 19.8	0.355	120.8 ± 16.4	117.0 ± 19.2	0.246
WC (cm)	118.1±11.7	120.3 ± 14.0	0.234	116.4 ± 13.1	120.6 ± 13.3	0.041	117.8 ± 11.9	120.4 ± 13.8	0.182	119.2 ± 13.3	119.7 ± 13.4	0.813
WHtR	0.7 ± 0.1	0.7 ± 0.1	0.204	0.7 ± 0.1	0.7 ± 0.1	0.014	0.7 ± 0.1	0.7 ± 0.1	0.093	0.7 ± 0.1	0.7 ± 0.1	0.976
BRI	8.1 ± 1.8	8.5 ± 2.0	0.160	7.9 ± 1.8	8.6 ± 2.0	0.029	8.0 ± 2.0	8.5 ± 2.0	0.032	8.4 ± 2.2	8.4 ± 1.9	0.889
VAI	2.9 ± 1.9	4.0 ± 3.5	0.010	2.8 ± 2.0	3.9 ± 3.4	0.040	2.7 ± 1.9	4.0 ± 3.4	0.004	2.7 ± 1.5	3.8 ± 3.3	0.028
BAI	37.3 ± 5.6	38.4 ± 5.9	0.216	36.2 ± 5.3	38.6 ± 5.9	0.010	36.7 ± 5.2	38.5 ± 6.0	0.035	37.7 ± 6.5	38.1 ± 5.7	0.717
Physical activity, n (%)												
yes	14 (18.7)	35 (22.3)		12 (22.2)	37 (20.8)		12 (18.2)	37 (22.3)		7 (17.5)	42 (21.9)	
no	61 (81.3)	122 (77.7)	0.527	42 (77.8)	141 (79.2)	0.821	54 (81.8)	129 (77.7)	0.489	33 (82.5)	150 (78.1)	0.537

Table 1: Body variables and physical activity of the MHO and MUHO phenotype groups according to four distinct obesity phenotype definitions.

Values are presented as mean ± SD or as count (and percentage). Differences between groups were assessed with two-independent sample t-test or Chi-square test.

BRI: Body Roundness Index; BAI: Body Adiposity Index; BMI: Body Mass Index; HOMA-IR: Homeostatic Model Assessment Insulin Resistance; MHO: Metabolically Healthy Obesity; MUHO: Metabolically Unhealthy Obesity; NCEP/ATPIII: National Cholesterol Education

MHO phenotype group when considering HOMA-IR (p = 0.014). BRI and BAI mean values were higher in the MUHO than in the MHO groups when classified by HOMA-IR (p = 0.029; p = 0.010) and Wildman (p = 0.032; p = 0.035) definitions, respectively. For all four definitions, the mean values of VAI (NCEP/ATPIII: p = 0.010; HOMA-IR: p = 0.040; Wildman: p = 0.004 and Karelis: p = 0.028) were significantly higher in the MUHO than in the MHO phenotype group.

Vitamin D nutritional status

Table 2 presents the 25(OH)D nutritional status of the MHO and MUHO phenotype groups according to the 4 definitions used. Only for the HOMA-IR definition, the mean value of 25(OH)D showed a significant difference between the MHO and MUHO phenotype groups (p = 0.011). Additionally, and in line, lower mean 25(OH)D values were found in the MUHO versus the MHO phenotype group in the deficiency (p = 0.004), and in insufficiency, yet no significant difference (p = 0.077) 25(OH)D groups. Also, was observed lower mean, for the NCEP/ATPIII definition, in the insufficiency group of MUHO (p = 0.033).

No difference was found for the sun exposure time between MHO and MUHO for any of the four definitions used, as showed: 13.4 ± 3.4 and 7.2 ± 3.1/*p* = 0.233; 8.7 ± 3.2 and 9.3 ± 4.5/*p* = 0.365; 10.2 ± 1.4 and 8.4 ± 4.4/*p* = 0.122; 8.9 ± 3.5 and 9.3 ± 4.2/*p* = 0.431, in MHO and MUHO in NCEP/ATPIII, HOMA-IR, Wildman and Karelis, respectively.

	NCEP/ATPIII		*p*	HOMA-IR		*p*	Wildman		*p*	Karelis		*p*
	MHO	MUHO		MHO	MUHO		MHO	MUHO		MHO	MUHO	
	(n = 75)	(n = 157)		(n = 54)	(n = 178)		(n = 66)	(n = 166)		(n = 40)	(n = 192)	
25(OH)D (ng/mL)	22.3 ± 7.7	22.6 ± 8.2	0.818	24.9 ± 8.0	21.8 ± 7.9	0.011	22.3 ± 7.9	22.6 ± 8.1	0.828	23.5 ± 8.8	22.3 ± 7.9	0.365
Deficiency	15.5 ± 3.2	14.8 ± 3.8	0.418	17.1 ± 2.7	14.5 ± 3.6	0.004	15.4 ± 3.4	14.9 ± 3.7	0.095	15.8 ± 3.2	15.5 ± 3.1	0.284
(≤ 20 ng/mL)	29 (38.7%)	64 (40.8%)		19 (35.2%)	74 (41.6%)		26 (39.4%)	67 (40.3%)		16 (40%)	77 (40.1%)	
Insufficiency	24.0 ± 2.8	25.2 ± 2.8	0.033	25.7 ± 2.6	24.5 ± 2.9	0.077	24.2 ± 2.8	25.0 ± 2.8	0.373	25.0 ± 2.5	24.7 ± 2.9	0.151
(21 - 29 ng/mL)	36 (48.0%)	66 (42.0%)		24 (44.4%)	78 (43.8%)		32 (48.5%)	70 (42.2%)		18 (45%)	84 (43.8%)	
Sufficiency	36.3 ± 6.8	34.6 ± 6.2	0.479	36.9 ± 6.2	34.2 ± 6.4	0.257	37.2 ± 7.4	34.4 ± 6.0	0.103	39.7 ± 6.6	34.1 ± 6.0	0.767
(≥ 30 ng/mL)	10 (13.3%)	27 (17.2%)		11 (20.4%)	26 (14.6%)		8 (12.1%)	29 (17.5%)		6 (15%)	31 (16.1%)	

Table 2: Nutritional status of vitamin D [25(OH)D] of the MHO and MUHO phenotype groups according to four distinct obesity phenotype definitions.

Differences between groups were assessed with two-independent sample t-test or Chi-square test. HOMA-IR: Homeostatic Model Assessment - Insulin Resistance; MHO: metabolically healthy obesity; MUHO: metabolically unhealthy obesity; NCEP/ATPIII: National Cholesterol Education Program/Adult Treatment Panel III.

Association between 25(OH)D and body variables according to nutritional status

Table 3 shows the mean values of body variables according to nutritional status of vitamin D in both obesity phenotype groups to four distinct definitions. VAI mean values were higher in MUHO with deficiency status than MHO in NCEP/ATPIII (p = 0.010) and Karelis (p = 0.028) definitions. In Wildman definition, mean results of BRI, VAI and BAI were higher in MUHO with VDD (p = 0.032, p = 0.004, p = 0.035, respectively). Only in HOMA-IR definition, most body parameters (WC: p = 0.041; WHtR: p = 0.014; BRI: p = 0.029; VAI: p = 0.040; BAI: p = 0.010) present highest values in MUHO with deficiency of 25(OH)D when compared with MHO in all nutritional status.

Odds Ratio for body variables related risk of MUHO

To identify among all adiposity parameters which was the best predictor of MUHO in deficiency of vitamin D nutritional status, within each classification for obesity phenotype, a logistic regression analysis was performed using six different models with adjustment for weight and age. The results show when considering NCEP/ATPIII definition only VAI is a predictor of unhealthy phenotype (B-0.675, 0.487-0.937, p=0.019). HOMA-IR classification was unique which presented some predictors of MUHO in individuals with VDD, as WC, WHtR, BRI and VAI (Table 4). Already, in Wildman and Karelis definitions did not have results with statistical significance.

		NCEP/ATPIII		p	HOMA-IR		p	Wildman		p	Karelis		p
Body variables	**Nutritional status of vitamin D [25(OH)]**	**MHO (n = 75)**	**MUHO (n=157)**		**MHO (n = 54)**	**MUHO (n = 178)**		**MHO (n = 66)**	**MUHO (n = 166)**		**MHO (n = 40)**	**MUHO (n = 192)**	
Weight (Kg)	Deficiency.[a] Insufficiency.[b] Sufficiency [c]	117.9 ± 12.5 121.0 ± 20.1 113.1 ± 8.4	121.5 ± 19.5 113.4 ± 17.4 116.1 ± 25.0	0.554	119.4 ± 16.4 122.6 ± 12.8 119.9 ± 28.5	120.6 ± 18.1 117.2 ± 20.1 113.3 ± 18.3	0.593	118.2 ± 11.2 122.4 ± 20.0 111.8 ± 9.0	121.2 ± 19.6 113.2 ± 17.5 116.2 ± 24.1	0.355	118.9 ± 15.0 120.1 ± 14.2 128.0 ± 25.9	120.7 ± 18.2 115.2 ± 19.4 112.8 ± 20.3	0.246
BMI (Kg/m^2)	Def Insuf Suf	42.5 ± 4.2 43.7 ± 4.9 42.1 ± 4.1	43.4 ± 5.3 41.6 ± 4.6 41.6 ± 4.0	0.308	41.5 ± 3.6 42.2 ± 4.0 41.6 ± 4.2	43.5 ± 5.2 42.4 ± 5.0 41.8 ± 3.9	0.204	42.4 ± 4.6 43.8 ± 4.9 40.6 ± 2.9	43.4 ± 5.2 41.7 ± 4.6 42.0 ± 4.2	0.491	42.9 ± 4.3 44.5 ± 4.5 42.9 ± 3.7	43.2 ± 5.1 41.9 ± 4.7 41.6 ± 4.0	0.119
WC (cm)	Def Insuf Suf	116.5 ± 12.0 118.8 ± 12.2 120.4 ± 9.6	121.9 ± 14.1 118.8 ± 12.4 120.4 ± 17.4	0.234	114.7 ± 12.7 114.8 ± 10.4 122.7 ±18.0	121.7 ± 13.6 120.0 ± 12.6 119.4 ± 14.6	0.041	116.7 ± 11.9 118.5 ± 12.7 118.0 ± 9.2	121.6 ± 14.1 118.9 ± 12.1 121.0 ± 16.9	0.182	118.5 ± 14.5 116.6 ± 11.2 128.5 ± 13.9	120.6 ± 13.5 119.2 ± 12.5 118.8 ± 15.5	0.813
WHtR	Def Insuf Suf	0.7 ± 0.1 0.7 ± 0.1 0.7 ± 0.1	0.7 ± 0.1 0.7 ± 0.1 0.7 ± 0.1	0.204	0.7 ± 0.1 0.7 ± 0.1 0.7 ± 0.1	0.7 ± 0.1 0.7 ± 0.1 0.7 ± 0.1	0.014	0.7 ± 0.1 0.7 ± 0.1 0.7 ± 0.1	0.7 ± 0.1 0.7 ± 0.1 0.7 ± 0.1	0.093	0.7 ± 0.1 0.7 ± 0.1 0.7 ± 0.1	0.7 ± 0.1 0.7 ± 0.1 0.7 ± 0.1	0.976
BRI	Def Insuf Suf	7.8 ± 2.0 8.2 ± 1.6 8.9 ± 2.1	8.6 ± 2.1 8.4 ± 2.0 8.5 ± 1.9	0.160	8.0 ± 1.7 8.0 ± 1.8 8.0 ± 1.8	8.7 ± 2.1 8.5 ± 1.9 8.2 ± 2.1	0.029	7.8 ± 2.0 8.1 ± 1.6 8.2 ± 1.6	8.6 ± 2.1 8.5 ± 2.0 8.4 ± 2.0	0.032	8.4 ± 2.6 8.0 ± 2.0 9.2 ± 1.5	8.3 ± 2.0 8.4 ± 1.8 8.5 ± 2.0	0.889
VAI	Def Insuf Suf	2.9 ± 2.0 2.7 ± 1.7 3.6 ± 2.3	4.6 ± 4.6 3.7 ± 2.7 3.5 ± 1.7	0.010	2.9 ± 1.8 3.2 ± 2.5 2.1 ± 0.8	4.3 ± 4.4 3.4 ± 2.4 4.1 ± 1.8	0.040	2.8 ± 2.1 2.6 ± 1.8 2.7 ± 1.5	4.5 ± 4.5 3.7 ± 2.6 3.7 ± 1.9	0.004	3.0 ± 1.9 2.4 ± 1.2 2.6 ± 1.2	4.3 ± 4.3 3.5 ± 2.6 3.7 ± 1.9	0.028
BAI	Def Insuf Suf	36.3 ± 6.2 39.5 ± 6.6 37.4 ± 5.6	38.6 ± 6.6 38.2 ± 5.7 38.1 ± 4.7		34.2 ± 5.5 37.1 ± 5.2 38.0 ± 4.5	38.8 ± 6.5 38.3 ± 5.4 38.7 ± 5.7	0.010	36.2 ± 6.2 37.0 ± 4.4 37.3 ± 5.2	38.5 ± 6.6 38.4 ± 5.7 38.5 ± 6.0	0.035	37.5 ± 8.0 37.5 ± 5.9 39.0 ± 4.6	38.0 ± 6.3 38.1 ± 5.3 38.4 ± 5.5	0.717

Table 3: Mean and standard deviation of body variables according to nutritional status of vitamin D [25(OH)D] in the MHO and MUHO phenotype groups according to four distinct obesity phenotype definitions.

Values are presented as mean ± SD or as count (and percentage). Differences between groups were assessed with two-independent sample t-test or Chi-square test.

BRI: Body Roundness Index; BAI: Body Adiposity Index; BMI: Body Mass Index; HOMA-IR: Homeostatic Model Assessment Insulin Resistance; MHO: Metabolically Healthy Obesity; MUHO: Metabolically Unhealthy Obesity; NCEP/ATPIII: National Cholesterol Education Program/Adult Treatment Panel III; VAI: Visceral Adiposity Index; WC: Waist Circumference; WHtR: Waist-Height Ratio

Deficiency[a] – def (≤ 20 ng/mL); Insufficiency [b] – insuf (21 - 29 ng/mL); Sufficiency [c] – suf (≥ 30 ng/mL).

	OR	95% CI for Exp (B)	*p*
BMI	0.861	0.738 - 1.005	0.058
WC	0.944	0.896 - 0.996	0.034
WHtR	1.025	0.000 - 0.054	0.007
BRI	0.709	0.521 - 0.964	0.028
VAI	0.756	0.533 - 1.072	0.117
BAI	0.880	0.797 - 0.971	0.011

Table 4: Odds ratios for body variables related risk of the unhealthy phenotype in DVD according to HOMA-IR criteria. Model was adjusted for age and weight.

CI: Confidence Interval; OR: Odds Ratio. BAI: body adiposity index; BMI: body mass index; BRI: body roundness index; HOMA-IR: homeostatic model assessment insulin resistance; VAI: visceral adiposity index; WC: waist circumference; WHtR: waist-height ratio.

Discussion

To the best of our knowledge, the present study is the first to examine the nutritional status of vitamin D associated with the body variables in individuals with obesity classified as MHO and MUHO by four different classifications. Our study observed, in accordance of these classifications of obesity phenotype, the prevalence of MUHO was higher than MHO in our population. Probably, the difference in percentage of MUHO's incidence was in relation of criteria used to define the phenotype. The NCEP/ATPIII is the most used, followed by HOMA-IR in studies conducted worldwide [25,26]. However, we found interesting to include two more classifications, that are used in population studies [22,23], to assess the strength and relevance in consideration of other components to define the MUHO phenotype.

Present study showed significant difference for age between both groups, being higher in MUHO when considered NCEP/ATPIII and Wildman definition. Studies display an overall agreement that there are less MHO individuals as age increases [27]. Information about the prevalence of MHO in youth is scarce but is knowing that the prevalence of MHO in earlier ages will be higher than in oldest ones, probably, for presenting less metabolic abnormalities and consequently to be healthier [28,29].

This study presented that when individuals have classified by presence of insulin resistance (IR), the mean values of WC, WHtR, BRI, BAI and VAI had significant difference between the groups, showing that the presence of abdominal adiposity had an important role to become these results worse in MUHO. Greater adipose tissue inflammation is closely associated with the start and development of several metabolic diseases [30,31], while individuals with obesity without greater adipose tissue inflammation exhibit reduced metabolic risk. The enlarged adipocytes release free fatty acids (FFAs), reactive oxygen species (ROS), and pro-inflammatory cytokines. The possible mechanism is that FFAs activate nuclear factor-kappa B (NF-κB) and P38 MAPK signaling through the MyD88 and TRIF-mediated downstream pathways following activation of TLR4 (Toll-like receptor 4) expression in resident adipocytes and macrophages, enhance oxidative stress and produce ROS, and promote the secretion of pro-inflammatory cytokines [32].

Studies suggest that MHO is characterized by a lower inflammatory cytokine environment than MUHO [3,30,33,34]. The MHO phenotype may be caused by several mechanisms, including preserved insulin sensitivity, specific fat distribution with low visceral and ectopic fat accumulation compared with subcutaneous fat depots, normal adipose tissue function defined by lower adipocyte size, less macrophage infiltration into adipose tissue, and normal adipokine secretion [3]. Pro-inflammatory M1 macrophages release cytokines, including Monocyte chemoattractant protein-1(MCP1), interleucin -1β (IL-1β), and IL6, which can recruit more monocytes, depending on the adipocyte size and metabolic conditions [35].

The present study showed, in HOMA-IR classification, that WC, WHtR, BRI and VAI were considered predictors for unhealthy obesity phenotype in individuals with VDD. WC and WHtR play an important role in the risk assessment of cardiovascular events and it was postulated to include these parameters in routine cardiovascular disease (CVD) risk assessment. A systematic review and meta-analysis carried out by Ezzatvar., *et al*., [36] including 138,561 individuals, showed that the waist-height ratio was the best index for screening cardiometabolic risk in both genders and concluded that the WHtR is a better indicator than BMI to assess cardiometabolic risk. A study developed by Cai., *et al*. [37] validated and compared the predictive capacity for the diagnosis of metabolic associated fatty liver disease (MAFLD) by eight anthropometric indicators and demonstrated that the WHtR had the strongest association with MAFLD, regardless of potential confounding factors.

Published data suggested that clinical practice should also include other indexes such as BAI and VAI, once these are characterized by higher sensitivity and specificity than conventional parameters such as WC and BMI and could significantly improve the assessment of risk of metabolic alterations associated with obesity [8,10,38-41].

The present study showed that VAI had higher mean value in MUHO with VDD according to all obesity phenotype classifications.

Reinforcing that lower 25(OH)D is associated with greater regional adiposity, and this is stronger in visceral adipose tissue (VAT) than subcutaneous adipose tissue, significant across the spectrum of body size [42]. Vitamin D may protect against adipose tissue inflammation in obesity by disrupting the deleterious cycle of macrophage recruitment and has been reported to act as an acute phase reactant because of such an inflammatory response occurs in obesity, which can suppress the concentration of 25(OH)D [43]. VAT accumulation is a plausible mechanism for the metabolically unhealthy phenotype in our study. VAT not only acts as a fat-deposit site, but also as a highly secretory organ with a differential production of adipokines capable of regulating energy expenditure, lipid metabolism, insulin sensitivity, and inflammation [44].

Additionally, nutritional status of vitamin D, only in classification of obesity phenotype by HOMA-IR, was significant difference between both of groups (MHO and MUHO), presenting high prevalence of inadequacy and lowest mean in MUHO. These findings can be answered by the fact that low concentrations of 25(OH)D was associated with IR, suggesting the influence of this metabolic alteration on nutritional status of VD. Because potential mechanisms that link VDD to increased metabolic risk have yet to be established but may involve increased inflammation owing to unregulated increase in the activity of the NFkB signaling pathway. In addition, vitamin D could influence the insulin secretion regulated by the opening and closing of calcium channels, and 1,25(OH) 2 D (active form of vitamin D) may also improve insulin sensitivity by stimulating the expression of insulin receptors and activating peroxisome proliferator-activated receptor delta (PPAR-δ) [45].

Although the present study has its strengths, it is important to consider its limitations due to its cross-sectional design. Therefore, we cannot establish a causal relationship between VDD and changes in body composition shown in obesity phenotypes. Despite these limitations, our study is a pioneer in the evaluation of 25(OH)D serum concentrations and their association with body variables in individuals with obesity classified according to four definitions of MUHO, developed in Brazil. Furthermore, the sample population size is relatively large compared to other available studies.

Conclusion

The results found in this study indicate a high prevalence of inadequacy serum concentrations of 25(OH)D in individuals with the MUHO phenotype, especially those classified by the HOMA-IR definition, who also had higher mean values of body variables such as WC, WHtR, BRI and VAI. Furthermore, these variables were identified as predictors of MUHO in individuals with VDD classified by HOMA-IR.

Regular monitoring of vitamin D nutritional status, along with assessment of body composition using these variables, can help reduce the incidence and development of diseases associated with obesity, as well as provide a more accurate assessment of the metabolic health of individuals with obesity.

However, further researches are needed to provide additional information about the mechanisms underlying the MUHO phenotype, especially when associated with VDD.

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Statement of Ethics

- **Study approval statement:** The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of Hospital Universitário Clementino Fraga Filho, Federal University of Rio de Janeiro, Brazil (Research Protocol number 011/06-CEP).
- **Consent To participate statement:** Informed consent was obtained from all subjects involved in the study.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Conceptualization, A.C., B.C. and A.R.; methodology, A.C. and A.R.; validation, A.C. and A.R.; formal analysis, A.C. and A.R.; investigation, A.C., B. C. and A.R.; data curation, A.C., S.E.P., C.J.S. and A.R.; writing, A.C. and A.R.; visualization, A.C., B.C. and A.R.; resources, S.E.P. and C.J.S.; supervision, project, administration, and funding acquisition, A.R. Critical revision of the manuscript for important intellectual content: All the authors have read and approved the final version of the manuscript.

Data Availability Statement

The data used to support the findings of this study are available from the corresponding author upon request.

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