

Body Adiposity and Endothelial Dysfunction Assessed By Laser Speckle Contrast Imaging in Obese and Overweight Individuals With Suboptimal Vitamin D Status: Sex Differences

Adriana de Castro Carvalho Faria,¹ Michelle Rabello Cunha,¹ Caroline Lyra Moreira,¹ Samanta Souza Mattos,¹ Clara Castro Carvalho Oliveira Faria,² Leticia Castro Carvalho Oliveira Faria,² Wille Oigman,¹ Mario Fritsch Neves¹

Universidade do Estado do Rio de Janeiro,¹ Rio de Janeiro, RJ – Brazil

Fundação Técnico Educacional Souza Marques, Escola de Medicina Souza Marques,² Rio de Janeiro, RJ – Brazil

Abstract

Background: Low Vitamin D levels are prevalent in obesity and have been linked to adverse cardiovascular and metabolic outcomes.

Objective: This cross-sectional study aimed to assess anthropometric indices, vascular, autonomic function in obese and overweight individuals with suboptimal vitamin D status.

Methods: Participants of both sexes, 40-69 years, Body Mass Index (BMI) 25-40 kg/m², were divided into two groups above and under the median vitamin D level (22,6 ng/ml), named Suboptimal (SUB-D) and Lower (LOW-D) vitamin D, respectively. Vascular function was assessed using central hemodynamic parameters (Mobil-O-Graph®), endothelial function (microvascular reactivity; Laser Speckle Contrast Image system), and heart rate variability (Polar® monitor). The groups were compared using an unpaired Student's t-test, and p-values < 0,05 were considered statistically significant.

Results: The groups (n = 90) had similar ages and blood pressures. The LOW-D group had a higher body fat percentage (%BF; 29 ± 4 vs. 33 ± 3, p = 0.020) and glycated Hb (5.4 ± 0.4 vs. 5.8 ± 0.4, p = 0.029) in males. Females had higher Visceral Adiposity Index (VAI; 2.33 ± 0.86 vs. 3.32 ± 1.86, p = 0.008) and Lipid Accumulation Products (LAP; 39 ± 23 vs. 63 ± 40, p = 0.005). The LOW-D group showed a lower percentage increase in the area under the curve in post-occlusive reactive hyperemia (%AUC-PORH) (70 ± 30 vs. 55 ± 32%, p = 0.034). Females showed inverse correlation of vitamin D levels and VAI and LAP, and a positive correlation with %AUC-PORH.

Conclusions: In obese and overweight individuals, lower vitamin D content was associated with higher levels of body adiposity in males. Furthermore, impaired metabolic functions and endothelial dysfunction were related to lower vitamin D levels in females, as in the central illustration.

Keywords: Vitamin D; Obesity; Women; Adiposity.

Introduction

According to the American Heart Association (AHA), obesity is directly associated with an increased incidence of cardiovascular risk factors.¹ However, the impact of obesity on health is not uniform, and the localization of excess body fat is more crucial than the overall body weight.² In fact, intra-abdominal (visceral) adipose tissue has been identified as the most strongly fat depot linked to metabolic complications, forming a key correlation of various

atherogenic, prothrombotic, and inflammatory abnormalities collectively referred to as the metabolic syndrome.^{3,4}

Vitamin D, also known as cholecalciferol, is a steroid hormone primarily responsible for regulating calcium homeostasis and skeletal health. Prolonged deficiency of vitamin D leads to conditions like rickets and an increased risk of fractures associated with osteoporosis.⁵ On the other hand, 25-hydroxyvitamin D (25(OH)D) concentrations between 20 ng/ml and 50 ng/ml (50–125 nmol/l) have been considered safe and sufficient for skeletal health in the general population.⁶

In recent years, emerging evidence has revealed additional functional roles of vitamin D, linking its deficiency (25(OH)D < 20 ng/dl) and suboptimal status (25(OH)D ≥ 20 and < 30 ng/dl) to various diseases and unfavorable metabolic factors such as Insulin Resistance (IR), Type 2 Diabetes Mellitus (DM2), and Cardiovascular Disease (CVD). Epidemiological studies have shown that vitamin D deficiency is associated with an increased risk of future cardiovascular events and is

Mailing Address: Adriana de Castro Carvalho Faria •

Universidade do Estado do Rio de Janeiro. Boulevard 28 de setembro, 77.

Postal code: 20550-900. Rio de Janeiro, RJ – Brazil

E-mail: afmenie@gmail.com

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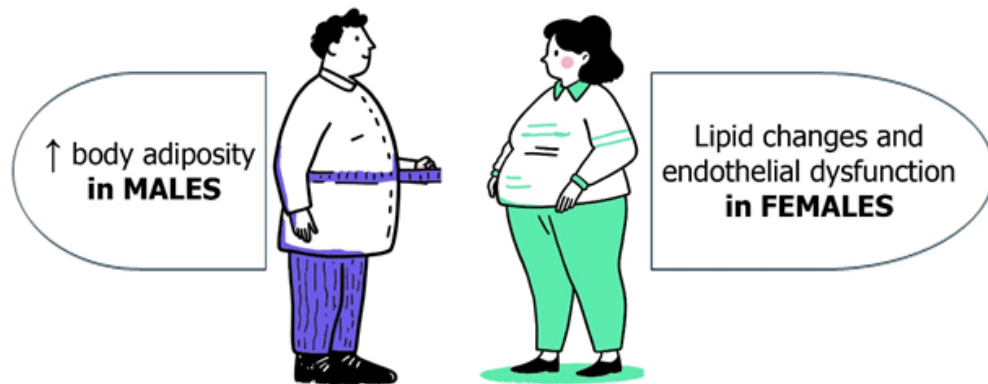
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Central Illustration: Body Adiposity and Endothelial Dysfunction Assessed By Laser Speckle Contrast Imaging in Obese and Overweight Individuals With Suboptimal Vitamin D Status: Sex Differences

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In obese and overweight individuals,
lower vitamin D levels were associated with



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correlated with the presence of endothelial dysfunction and arterial stiffness, particularly in hypertensive individuals.⁷⁻⁹

Obesity and vitamin D deficiency are commonly associated with health hazards. Several potential explanations account for this relationship. One possibility is that the liposoluble nature of vitamin D leads to volumetric dilution in obese individuals.¹⁰ Additionally, the larger amount of adipose tissue in obese individuals can act as a reservoir, sequestering vitamin D and contributing to its reduced availability.^{11,12} Reduced sun exposure in obese populations further limits dermal synthesis of prohormones, resulting in lower serum vitamin D levels.¹³ Furthermore, the response to vitamin D supplementation is less pronounced in obese subjects, suggesting the need for dose adjustments based on body size.¹⁴ The impaired response to vitamin D supplementation in obesity may be attributed to various factors. For instance, patients with Nonalcoholic Fatty Liver Disease (NAFLD) may experience lower hepatic 25-hydroxylation of vitamin D.¹⁵ Moreover, obese individuals may exhibit reduced gene expression of specific enzymes in the cytochrome P450 system responsible for the conversion of vitamin D into its bioactive form in subcutaneous adipose tissue.^{16,17} While adverse dietary habits are common in obesity, they are considered a less likely contributor to low serum vitamin D levels.¹⁸ Overall, the relationship between obesity and vitamin D deficiency involves complex biochemical mechanisms that warrant further investigation for a comprehensive understanding of their health implications.

This study explores the potential interplay between obesity, vitamin D deficiency, and their collective influence on inflammation and vascular dysfunction. Through the modulation of vasoconstriction factors and vasodilator substances, such as nitric oxide, these conditions are believed to contribute significantly to vascular inflammation

and endothelial activation.^{19,20} We aim to investigate the association between body composition, altered biochemical parameters, and vascular function, assessing indices that could be linked to impaired endothelial function, arterial stiffness, and autonomic imbalance in overweight and obese individuals with inadequate vitamin D status.

Methods

Study Population

Potential subjects were recruited from a consecutive convenience sampling of individuals who sought follow-up at our outpatient clinic of arterial hypertension and metabolic associated diseases (CHAMA) at Pedro Ernesto University Hospital, in Brazil. The study population consisted of overweight or obese adults (BMI between 25 Kg/m² and 40 Kg/m²), both sexes, who did not present Vitamin D sufficiency [25(OH)D levels > 30 ng/ml]. Participants were divided into two groups named Suboptimal vitamin D (SUB-D) and Lower vitamin D (LOW-D) for data analysis, according to median 25(OH)D levels among the study participants.

Volunteers were excluded if they had any of the following: diabetes mellitus; coronary heart disease; chronic kidney disease; active malignancy and pregnancy. All subjects provided written informed consent. The protocol was approved by the local Research Ethics Committee (CAAE:61044522.0.0000.5259), and all participants read and signed the participatory and informed consent (PIC).

Biochemical Evaluation

After an 8-h fasting, venous blood samples were collected. Serum glucose, uric acid, creatinine, Total

Cholesterol (TC), High-Density Lipoprotein cholesterol (HDL-c), and Triglycerides (TG) were measured using an Auto Analyzer technique (Technicon DAX96, Miles Inc). Low-density lipoprotein cholesterol (LDL-c) concentrations were calculated using Friedewald's equation. After the results, the Atherogenic Index of Plasma (AIP) that suggests cardiovascular risk, was calculated as $(\log [TG/HDL-C])$ and the Lipid Accumulation Product index (LAP), a simple, non-invasive gender-specific marker for central or abdominal lipid accumulation as: $LAP = (Waist\ Circumference\ [cm] - 65) \times Triglycerides\ [mmol/L]$ for men, and $LAP = (Waist\ Circumference\ [cm] - 58) \times Triglycerides\ [mmol/L]$ for women.²¹ Insulin was measured by radioimmunoassay and Homeostatic Model Assessment-Insulin Resistance (HOMA-IR) index was calculated with the formula $= (Fasting\ Glucose \times Fasting\ Insulin) / 22.5$ and the result estimates insulin resistance based on fasting blood measurements. The determination of the serum concentration of C-reactive Protein (CRP) was performed by the turbidimetry method (High-sensitivity latex). Electrolytes such as magnesium and calcium were measured by the colorimetric method. Parathyroid Hormone (PTH) and 25-OH Vitamin D were analyzed by electrochemiluminescence immunoassay.

Blood Pressure and Anthropometric Evaluation

Measurements of Systolic (SBP) and Diastolic (DBP) blood pressure were obtained using a calibrated electronic device (model HEM-705CP, OMRON Healthcare Inc., Illinois), performed with the patient in a seated position after 5 min of rest.

The nutritional status assessment was obtained by measuring body weight (kg) using electronic scales with a stadiometer (Filizola® SA, São Paulo, SP, Brazil). The Body Mass Index (BMI) was calculated as $body\ weight\ (kg) / height^2\ (m^2)$. Waist Circumference (WC) was determined at the midpoint between the last rib and the iliac crest. Hip Circumference (HC) assessment was performed on the largest diameter of the gluteal region. Waist-to-hip ratio (WHR) and Waist-to-height ratio (WHtR) were obtained by dividing the measures. We also estimated Conicity Index (CI) as $WC / 0.109 \times \text{square root of weight/height}$, Visceral Adiposity index (VAI) as $(WC / (36.58 + (1.89 \times BMI))) \times ((TG / 0.81) \times (1.52 / HDL-c))$ for women and $(WC / (39.68 + (1.88 \times BMI))) \times ((TG / 1.03) \times (1.31 / HDL-c))$ for men (18,19), and LAP as $(WC - 58) \times TG$ for women and $(WC - 65) \times TG$ for men.²²⁻²⁵

Body composition by Bioimpedance analysis (BIA)

Body composition was evaluated by BIA, which estimated body fat percentage (%BF).²⁶ BIA was performed using a Biodynamics 450® analyzer (Biodynamics Corp., Shoreline, WA, USA) in standardized conditions: ambient temperature between 23–25 °C, fast for 8 h, empty bladder, and supine position for 10 min, on a flat, nonconductive bed by using a single frequency tetrapolar technique.

Microvascular Reactivity

Microvascular reactivity was assessed using the Laser Speckle Contrast Image (LSCI) method (Pericam® PSI System,

Perimed, Sweden) in combination with Post Occlusive Reactive Hyperemia (PORH) for continuous analysis of expressed endothelium-dependent microvascular skin perfusion changes in arbitrary perfusion units (APU).²⁷ Through these analyses, we obtained the mean perfusion (baseline perfusion) and the Area Under the Curve (AUC) at 1-min of the baseline period, PORH peak mean, and the area under the curve at 1-min after occlusion of the forearm for 3 minutes at cuff pressure of 50 mmHg over SBP. Cutaneous Vascular Conductance (CVC) of the baseline period and during PORH was obtained by dividing baseline perfusion (or PORH) by Mean Arterial Pressure (MAP). The increased percentage of the AUC from baseline to PORH period (% AUC increase) was calculated as $[(AUC-PORH - AUC\ baseline) / AUC\ baseline \times 100]$. Pimsoft®, Perimed software was used to analyze the images obtained.

Central hemodynamic measurements

Central hemodynamics were assessed using the Mobil-O Graph® device (I.E.M. GmbH, Stolberg, Germany). Parameters were obtained through a non-invasive method, which simultaneously checked the pulse wave of arterial blood flow, allowing the analysis of central SBP (cSBP), central Pulse Pressure (cPP), Pulse Wave Velocity (PWV), Augmentation Pressure (AP), Augmentation index (Aix), and vascular age.²⁸

Heart Rate Variability (HRV)

HRV was used to estimate autonomic function. The individuals were evaluated in a seated position after resting for 5 min, with the initial 1 min of stabilization and after 5 min of recording. All data were processed using the software Kubios® HRV (Kubios Oy, Kuopio, Finland). From the optical heart rate monitor (Polar® Verity Sense, Kempele, Finland) placed snugly on the right forearm, the intervals between each heartbeat (R wave), called R–R intervals (iRR), and other parameters were identified. HRV was analyzed in the time-domain, frequency-domain, and with non-linear parameters.²⁹

Statistical Analysis

The variables were assessed for normality using the Shapiro–Wilk test. The differences between the groups were analyzed using an unpaired Student t-test for normally distributed continuous variables and Mann-Whitney for non-normally distributed ones. Chi-square test was used to compare categorical variables. Results of continuous variables with a normal distribution were presented as mean $\pm$ standard deviation (SD), continuous variables without a normal distribution as median and interquartile ranges (IQR), and categorical variables were presented as absolute (n) and relative (%) frequencies. Pearson and Spearman's coefficients were used to analyze the correlation by gender between normally and non-normally distributed continuous variables. Multiple linear regression analyses were conducted to evaluate independent associations between 25(OH)D as a predictor and the outcomes adjusted for age and sex, with the results expressed

as coefficients (β). The assumptions for multiple linear regression were verified, including linearity, normality, and homoscedasticity of residuals, independence of errors, and absence of multicollinearity. All models satisfied the required assumptions. For all analyses, 95% confidence intervals were adopted and $p < 0,05$ values considered statistically significant. Statistical analysis was performed using the Statistical Package for Social Sciences® (SPSS, Chicago, Illinois, USA) version 25.0.

Results

A total of 90 individuals were selected after having been screened by BMI and Vitamin D levels. The mean age of the study population was 52 years, and the mean BMI was 31 kg/m². The median 25(OH)D was 22.6 [interquartile range: 17.8–26.5] ng/ml. Clinical and anthropometric characteristics of the participants sorted by median serum 25(OH)D levels are shown in Table 1. Although BMI was similar between the groups (SUB-D 30.8 ± 4.0 vs. LOW-D 31.6 ± 3.6 , $p = 0.858$), there were significant gender differences in %BF, which was found to be significantly higher in males than in females in the LOW-D group. On the other hand, females showed higher values of VAI and LAP indices in the same LOW-D group.

Biochemical variables are expressed in Table 2. As expected, the mean Vitamin D levels were significantly lower in the LOW-D group compared to those in the SUB-D group. Regarding the glycidic profile, the LOW-D group presented higher parameter levels, including insulin resistance HOMA-IR > 2.71 ($p = 0.049$), although glycated hemoglobin did not reach statistical significance ($p = 0.058$).

In relation to endothelial function, impaired microvascular reactivity was shown by a smaller % AUC increase in the LOW-D group (Table 3). Concerning the central hemodynamic parameters and HRV, no significant differences were found between the groups, although the LOW-D group patients presented sympathetic hyperactivity with higher LF/HF and SD2/SD1 ratio (Tables 3 and 4).

Concentrations of 25(OH)D showed weak inverse correlation to TG ($r = -0.231$ $p = 0.032$), PTH ($r = -0.259$ $p = 0.015$), and to WHtR, and moderate correlation with Female's VAI and LAP. On the other hand, the increase in area under the curve in post-occlusion reactive hyperemia (AUC-PORH increase, %) was weakly positively correlated to 25(OH)D levels (Figure 1). Considering 25(OH)D concentrations as a dependent variable, those significant parameters were assessed using multiple linear regression and remained statistically significant after adjusting for age and sex (Table 5).

Gender differences were considered in correlations among 25(OH)D concentrations and all continuous variables. In male subjects, some anthropometric indices, such as WC, WHR, WHtR, and CI, were weakly, and the %BF were moderately inversely correlated to 25(OH)D levels. On the other hand, vitamin D in females presented moderated inverse correlations with VAI, LAP, and TG, and weak AIP and TG/HDL. Females also presented a positive weak correlation with the increase in AUC-PORH (Table 6).

Discussion

In this cross-sectional study, the lowest 25(OH)D concentrations were associated with anthropometric, metabolic, and vascular changes in middle-aged and obese adults with suboptimal vitamin D levels.

The connection between reduced vitamin D concentrations and obesity is classic and well-documented in observational studies.³⁰ These sample characteristics, with low vitamin D levels and high BMI, are consistent with previous studies in other populations that have found an inverse relationship between 25(OH) D levels and BMI.³¹

Our results showed significant gender differences in body adiposity content in the LOW-D group. Men with the lower vitamin D serum levels presented a significantly greater content of fat mass than women, as shown by higher adiposity (%BF) in BIA analysis. These findings agree with previously published reports,^{32–35} but do not with an Italian report of 500 subjects, in which females presented higher body adiposity.³⁶ Our females' main characteristics were higher visceral adiposity and the accumulation of lipid products. Visceral depots represent around only 6% of total body fat in women compared to 20% in men.³⁷

Obesity's related cardiovascular risks are more closely related to the location of the excess body fat rather than to an elevated body weight per se.^{24,38,39} VAI and LAP are seen as better indicators of dysfunctional adipose tissue than BMI for recognizing cardiovascular risk in some illness conditions and in the general population.^{25,40,41} Our women's sample does not follow Vague's definitions of the most common phenotype as gynoid obesity.³ So, it seems that our women are at increased health risks as their VAI and LAP indices were significantly higher in the LOW-D group. These findings support that modern dietary habits may overcome phenotypic traits.

The LOW-D group presented biochemical data of insulin resistance and slightly higher HbA1C levels, albeit still within the normal range. Defects of vitamin D and insulin resistance have a superimposable epidemiological distribution that can be considered the direct or indirect consequence of vitamin D deficiency.⁴² Also, this finding aligns with the Saudi study that investigated diabetic and non-diabetic adults, which reported decreased vitamin D levels associated with increased HbA1C in non-diabetic individuals.⁴³ Additionally, participants in the LOW-D group exhibited notably lower levels of vitamin D, as anticipated, and higher levels of PTH, although not significant. These results are likely attributable to secondary reflex hyperparathyroidism.

The LOW-D group exhibited impaired microvascular reactivity in vascular function, as evidenced by a smaller increase in AUC from baseline to the PORH period. Previous studies have demonstrated patterns of endothelial dysfunction in various populations with vitamin D deficiency, including those with ischemic heart disease, as well as in healthy subjects from diverse ethnic backgrounds. In other studies, these patterns are predominantly characterized by lower increments in flow-mediated dilation of the brachial artery^{8,44,45} and, in some cases, by evaluating the fingertip reactive hyperemia index using the Endo-PAT 2000, as described elsewhere.^{46,47}

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Table 1 – Clinical, anthropometric, and bioelectrical impedance analysis variables according to Vitamin D status.

Vitamin D groups			
Variables	SUB-D (n = 45)	LOW-D (n = 45)	P-value
Age, years	52 ± 6	51 ± 8	0.519
Female, n (%)	27 (60)	34 (76)	0.175
Previous hypertension, n (%)	15 (33)	19 (42)	0.515
Hypertension duration, years	9 ± 7	9 ± 12	0.834
Systolic BP, mmHg	123 ± 10	124 ± 17	0.889
Diastolic BP, mmHg	83 ± 6	84 ± 10	0.594
Heart rate, bpm	70 ± 10	70 ± 10	0.900
Body Mass Index, kg/m²			
Male	31.1 ± 3.7	32.5 ± 4.1	0.337
Female	30.6 ± 4.3	31.3 ± 3.4	0.545
Waist circumference, cm			
Male	109 ± 7	114 ± 11	0.130
Female	99 ± 12	100 ± 8	0.652
Waist-to-hip ratio			
Male	0.96 ± 0.04	0.99 ± 0.05	0.099
Female	0.86 ± 0.10	0.88 ± 0.06	0.343
Waist-to-height ratio			
Male	0.63 ± 0.05	0.65 ± 0.07	0.350
Female	0.61 ± 0.07	0.63 ± 0.05	0.266
Conicity Index			
Male	1.36 ± 0.04	1.38 ± 0.05	0.150
Female	1.29 ± 0.10	1.30 ± 0.08	0.531
Visceral adiposity index			
Male	2.67 ± 0.91	2.61 ± 0.94	0.860
Female	2.33 ± 0.86	3.32 ± 1.86	0.008
Lipid accumulation products			
Male	62 ± 34	78 ± 52	0.330
Female	39 ± 23	63 ± 40	0.005
Body fat (%)			
Male	29 ± 4	33 ± 3	0.020
Female	40 ± 3	39 ± 5	0.763

Values are mean ± SD or proportions when appropriate. 1BP, Blood Pressure.

Table 2 – Biochemical variables according to the Vitamin D status

Variables	SUB-D (n = 45)	LOW-D (n = 45)	P-value
Glucose, mg/dl	84 ± 9	87 ± 12	0.179
Insulin, µIU/mL	12 ± 6	16 ± 12	0.085
Glycated hemoglobin, %	5.4 ± 0.4	5.6 ± 0.4	0.058
HOMA-IR ^{1,2}	2.57 ± 1.21	3.53 ± 2.92	0.049
Creatinine, mg/dl	0.99 ± 0.18	0.93 ± 0.2	0.091
Uric acid, mg/dl	5 ± 1.5	4.8 ± 1.5	0.498
Magnesium, mg/dl	2 ± 0.1	2 ± 0.2	0.972
Calcium, mg/dl	9.6 ± 0.5	9.6 ± 0.5	0.533
Total cholesterol, mg/dl	211 ± 34	207 ± 44	0.656
HDL ³ -cholesterol, mg/dl	55 ± 15	52 ± 17	0.490
LDL ⁴ -cholesterol, mg/dl	132 ± 75	125 ± 39	0.328
Triglycerides, mg/dl	108 ± 55	132 ± 75	0.094
AIP ⁵	-0.11 ± 0.3	-0.01 ± 0.31	0.119
C-reactive protein, mg/dl	0.49 ± 0.58	0.4 ± 0.42	0.448
Vitamin D, ng/ml	26.2 ± 1.9	17.7 ± 3.5	< 0.001
Parathyroid hormone, pg/ml	58 ± 18	66 ± 27	0.090

Values are mean ± SD. 1HOMA, Homeostasis model assessment; 2IR, insulin resistance; 3HDL, High density lipoprotein; 4LDL, low-density lipoprotein; 5AIP, Atherogenic index of plasma.

Table 3 – Assessment of endothelial function by microvascular reactivity and central hemodynamic parameters according to the Vitamin D status.

Variables	SUB-D (n = 45)	LOW-D (n = 45)	P-value
Microvascular reactivity			
Baseline perfusion, APU ¹	34 ± 9	35 ± 8	0.579
PORH – perfusion Peak, APU	90 ± 20	88 ± 21	0.515
Perfusion increase, %	171 ± 46	158 ± 74	0.341
Baseline AUC ² , APU	2112 ± 570	2180 ± 514	0.557
PORH ³ – AUC, APU	3480 ± 710	3291 ± 682	0.205
AUC-PORH increase, %	70 ± 30	56 ± 32	0.035
Central hemodynamic parameters			
Central Systolic BP, mmHg	118 ± 9	119 ± 13	0.553
Central PP, mmHg	32 ± 8	31 ± 8	0.552
Augmentation pressure, mmHg	8 ± 5	9 ± 5	0.774
Augmentation index (AIx), %	25 ± 12	28 ± 13	0.424
Pulse wave velocity, m/s	7.5 ± 0.8	7.5 ± 1.1	0.951
Vascular age, years	53 ± 7	52 ± 10	0.827

Values are mean ± SD. 1APU, Arbitrary Perfusion Units; 2AUC, Area Under the curve; 3PORH, Post Occlusive Reactive Hyperemia.

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Table 4 – Heart rate variability according to the Vitamin D status.

Vitamin D groups			
Variables	SUB-D (n = 45)	LOW-D (n = 45)	P-value
iRR ¹ , ms	844 (738-983)	838 (782-964)	0.592
HR ² , bpm	71 (61-82)	72 (62-77)	0.658
SDNN ³ , ms	25 (17-36)	23 (19-37)	0.566
RMSSD ⁴	23 (14-38)	24 (16-35)	0.534
LF ⁵ /HF ⁶ ratio	1.38 (0,80-4,04)	1.21 (0,72-2,32)	0.717
SD2 ⁷ /SD1 ⁸	1.73 (1.32-2.63)	1.77 (1.38-2.37)	0.733
⁹ PNS index	-0.85 (-1.46-0.28)	-0.61 (-1.36-0.03)	0.421
¹⁰ SNS index	1.14 (-0,13-2,74)	1.10 (0.17-2.56)	0.579
Stress Index	15 (11-20)	14 (11-21)	0.586

Values are median $\pm$ IQR. 1iRR, beat-to-beat RR interval series; 2HR, heart rate; 3SDNN, standard deviation of all normal RR intervals; 4RMSSD, root mean square of successive differences between RR intervals; 5LF, low frequency; 6HF, high frequency; 7SD2, standard deviation of overall variability of the Poincare plot; 8SD1, standard deviation of instantaneous beat-to-beat variability of the Poincare plot; 9PNS, parasympathetic nervous system; 10SNS, sympathetic nervous system.

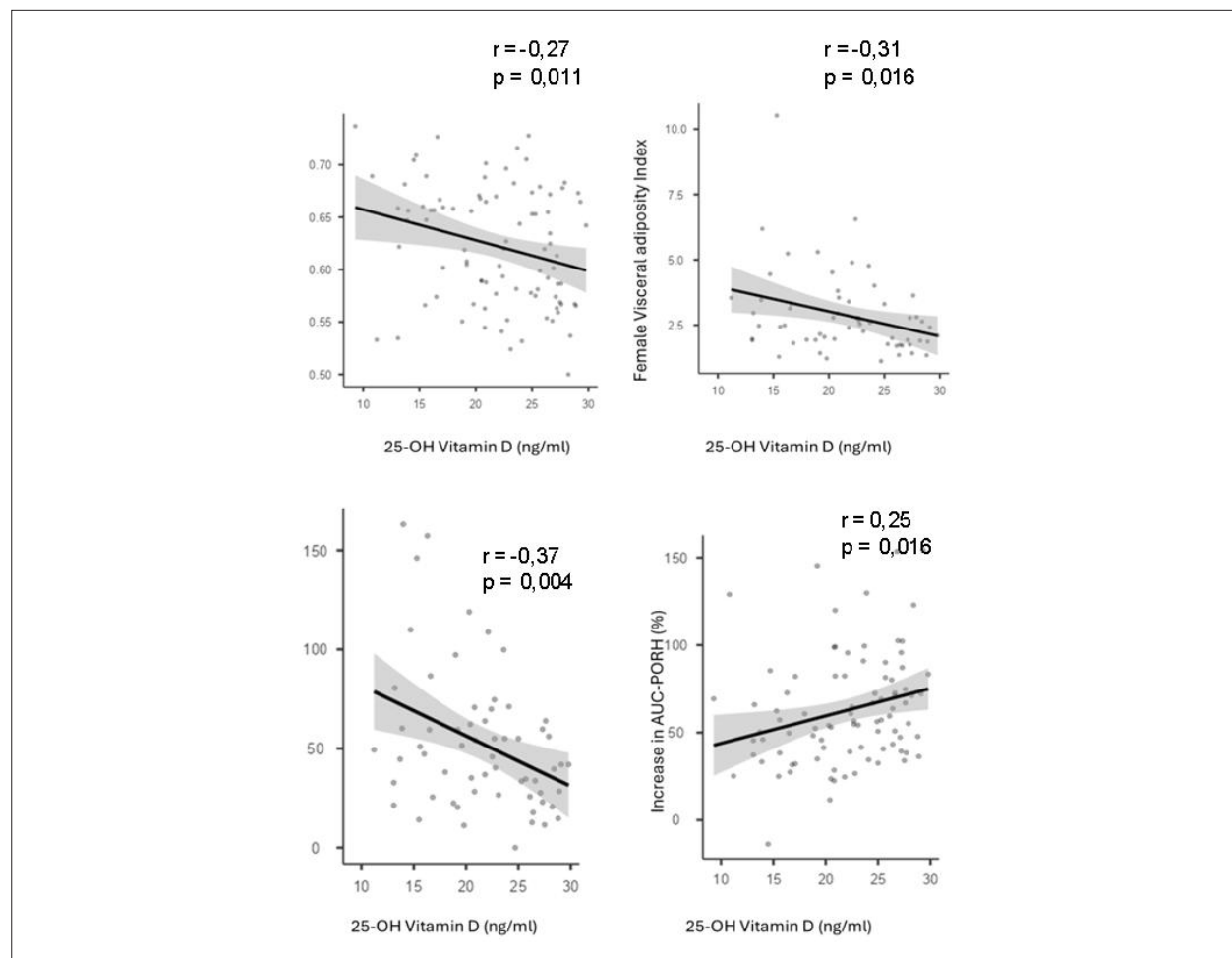


Figure 1 - Vitamin D global correlations

Table 5 – Multiple linear regression considering Vitamin D levels as the predictor variable, adjusted for age and sex.

Dependent variables	Non-standardized B coefficient	CI 95%	P-value
Waist-to-height ratio	-0.003	-0.005 / -0.001	0.016
Female VAI ¹	-0.095	-0.173 / -0.017	0.018
Female LAP ²	-2.421	-4.108/-0.733	0.006
Triglycerides, mg/dl	-3.275	-5.966 / -0.584	0.018
Parathyroid hormone, pg/ml	-1.110	-2.074 / -0.146	0.025
AUC-PORH ³ increase, %	1.476	0.193 / 2.760	0.025

¹VAI, Visceral adiposity index; ²LAP, Lipid accumulation products; ³AUC-PORH, area under the curve in post occlusive reactive hyperemia.

Table 6 – Significant correlations with Vitamin D according to gender.

Variables	MALES		FEMALES	
	Pearson coefficient (r)	P-value	Pearson coefficient (r)	P-value
Waist circumference. cm	-0.414	0.026	-0.127	0.330
Waist-to-hip ratio	-0.492	0.007	-0.130	0.318
Waist-to-height ratio	-0.475	0.009	-0.198	0.126
Conicity Index	-0.410	0.027	-0.108	0.409
Body fat, %	-0.412	0.026	0.047	0.719
GGT ¹	-0.481	0.010	0.065	0.619
VAI ²	-0.042	0.830	-0.307	0.016
LAP ³	-0.254	0.202	-0.365	0.004
Triglycerides, mg/dl	-0.103	0.609	-0.315	0.014
AIP ⁴	-0.047	0.817	-0.291	0.024
TG/HDL ⁵	-0.011	0.958	-0.299	0.020
AUC-PORH ⁶ increase, %	0.197	0.306	0.283	0.029

¹GGT, Gamma glutamyl transferase; ²VAI, Visceral adiposity index; ³LAP, Lipid accumulation products; ⁴AIP, Atherogenic index of plasma; ⁵TG/HDL, Triglycerides/High density lipoprotein ratio; ⁶AUC-PORH, area under the curve in post-occlusive reactive hyperemia.

few publications have evaluated reactive hyperemia, including one of the most recent ones that evaluated overweight post-menopausal women and could only associate lower PORH with age, not with vitamin D levels, although this one has shown a weak inverse correlation with one oxidative stress blood marker (3-nitrotyrosine).⁴⁸ Therefore, to the best of our knowledge, no studies have yet utilized this reactive hyperemia using our LSCI system methodology in populations with our specific characteristics.

Concerning HRV, the LOW-D group patients presented sympathetic hyperactivity with higher LF/HF and SD2/SD1 ratio, although no significant differences were found between the groups. These findings align with a randomized double blind clinical trial where daily vitamin D supplementation resulted in an improvement in autonomic imbalance.⁴⁹

The correlation tests by gender showed an association of lower vitamin D levels with worse anthropometric parameters in males. Our findings align with some results from a large cross-sectional study where the conicity index was negatively associated with vitamin D levels in men. However, there were conflicting findings, as % BF and WHtR were negatively associated with vitamin D levels in both sexes.⁵⁰ In another large cross-sectional Chinese study, mean values of WC and WHtR tended to increase with 25(OH)D insufficiency in both sexes.⁵¹ In contrast, our correlation tests indicate that these parameters were inversely associated with vitamin D levels only in men. It is important to note that WC and WHtR are traditional indicators of abdominal obesity but do not directly reflect metabolic dysfunction. The conicity index, on the other hand, is more closely related to body fat and fat mass,⁵² and

WHR has a better correlation with subcutaneous adipose tissue.⁵³ These findings diverge from the current literature, where not only the amount of fat mass is increased in males, but visceral adiposity as well.³²⁻³⁵

Interestingly, in our female sample, indicators of dysfunctional visceral adiposity were inversely associated with vitamin D levels. To the best of our knowledge, we could not find published data where visceral adiposity was higher in women. Furthermore, in the correlation analysis by sex, we observed a negative association between serum vitamin D levels and TG in women. However, no significant correlation was found between vitamin D status and other lipid markers, as previously reported in non-obese populations.⁵⁴ This inverse association seems to be related only to increased caloric intake in obese individuals.

Lower levels of vitamin D were associated with a smaller percentage increase after post-occlusion reperfusion area, suggesting compromised endothelial function, and we found a positive correlation between vitamin D levels and endothelial dysfunction. Vitamin D plays a crucial role in regulating the synthesis of Nitric Oxide, a potent endothelium-dependent vasodilator, thereby conferring vaso-protective effects. Conversely, vitamin D deficiency represents a risk factor for endothelial dysfunction, which reinforces the significance of our findings.⁵⁵

This study needs to be analyzed for its limitations and strengths. The sample size could be a limitation, but it was sufficient to achieve significant results using different methods of structural and functional assessment. Concerning physical activity, all the participants were considered inactive. Diet and sun exposure were not collected as variables, as in other vitamin D studies. The oscillometric method used to assess PWV is not considered the gold standard, although it has already been validated and used in clinical practice. Assessment of the autonomic nervous system may be influenced by several factors related to the patient's resting environment. However, the HRV measurements provide several parameters related to sympathetic and parasympathetic activities. This cross-sectional design, which impairs causal inference, was elaborated for a specific population of obese individuals and cannot be extrapolated to non-obese individuals or those with sufficient vitamin D levels. Although these findings are crucial to better understand some vascular dysfunctions in this very prevalent condition within the general population, mostly related to sex differences.

Conclusions

In this sample of overweight and obese individuals, endothelial function was impaired in those with the lowest vitamin D levels. Additionally, noteworthy gender differences emerged, with males

showing an excess of fat mass irrespective of body distribution. However, the most intriguing findings were related to the female subjects, who presented a metabolically unfavorable condition characterized by dysfunctional visceral adipose tissue, adverse biochemical markers, and impaired endothelial function.

Author Contributions

Conception and design of the research: Faria ACC, Oigman W, Neves MF; acquisition of data: Faria ACC, Moreira CL, Faria CCCO, LCCO; analysis and interpretation of the data and statistical analysis: Faria ACC, Cunha MR, Mattos SS, Neves MF; obtaining financing: Neves MF; writing of the manuscript: Faria ACC; critical revision of the manuscript for intellectual content: Faria ACC, Cunha MR, Oigman W, Neves MF.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of the Hospital Universitário Pedro Ernesto under the protocol number 5.600.773 (CAAE: 61044522.0.0000.5259. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

Use of Artificial Intelligence

The authors did not use any artificial intelligence tools in the development of this work.

Availability of Research Data

The underlying content of the research text is contained within the manuscript.

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