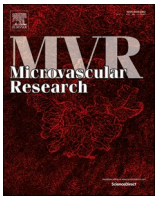
Microvascular Research 148 (2023) 104553

Contents lists available at ScienceDirect

Microvascular Research

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



Evaluation of systemic endothelial-dependent and endothelial-independent microvascular reactivity in metabolically healthy obesity: An observational study

Silas Escobar, Daniela Peçanha, Maíra Duque, Alice Duque, Vinícius Crahim, Andrea De Lorenzo, Eduardo Tibirica [*,1]

National Institute of Cardiology, Rio de Janeiro, Brazil

ARTICLE INFO

Keywords:
Obesity
Metabolically healthy
Laser speckle contrast imaging
Microvascular function

ABSTRACT

Background: Metabolically healthy obesity (MHO), a phenotype of obesity considered to be of lower cardiovascular risk, is still a controversial concept. This study aimed to investigate the presence of subclinical systemic microvascular dysfunction in individuals with MHO.

Methods: This was a cross-sectional study in which 112 volunteers were allocated into three groups: metabolically healthy normal weight (MHNW), MHO, or metabolically unhealthy obesity (MUO). Obesity was defined as a body mass index (BMI) $\geq$ 30 kg/m^2. MHO was defined as the absence of any component of metabolic syndrome, except waist circumference. Microvascular reactivity was evaluated using cutaneous laser speckle contrast imaging.

Results: Mean age was 33.2 $\pm$ 7.66 years. The median BMI in the MHNW, MHO and MUO groups was 23.6, 32.8, and 35.8 kg/m^2, respectively. Baseline microvascular conductance values were lower in the MUO group (0.25 $\pm$ 0.08 APU/mmHg) than in MHO (0.30 $\pm$ 0.10 APU/mmHg) and MHNW groups (0.33 $\pm$ 0.12 APU/mmHg) (P = 0.0008). There were no significant differences regarding endothelial-dependent (acetylcholine stimulation or postocclusive reactive hyperemia) or endothelial-independent (sodium nitroprusside stimulation) microvascular reactivity among the groups.

Conclusions: Individuals with MUO had lower baseline systemic microvascular flow than those with MHNW or MHO, but endothelium-dependent or endothelium-independent microvascular reactivity were not changed in any of the groups. The relatively young age of the study population, the low frequency of class III obesity, or the strict definition of MHO (absence of any metabolic syndrome criteria) might account for the lack of difference of microvascular reactivity among MHNW, MHO or MUO.

1. Introduction

Overweight and obesity are defined by the World Health Organization as abnormal or excessive fat accumulation that presents a risk to health (WHO, 2016). Both may be considered a result of the complex interplay of genetic, behavioral and environmental factors (Danesh et al., 2007). Globally, from 1990 to 2017, the deaths and disability-adjusted life-years (DALYs) attributable to high body mass index (BMI) more than doubled for both females and males, and in 2017, the two main causes of high-BMI-related DALYs were ischemic heart disease and stroke (Dai et al., 2020). Additionally, while formerly considered a problem of high-income societies, the rates of overweight and obesity

Abbreviations: ACh, Acetylcholine; ALT, alanine transaminase; APUs, arbitrary perfusion units; AST, aspartate transaminase; BMI, body mass index; CVC, cutaneous vascular conductance; DALYs, disability-adjusted life-years; FID, flow-induced dilation; HbA1c, Glycated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance; IDF, International Diabetes Federation; IRB, Institutional Review Board; MHNW, metabolically healthy normal weight; MHO, metabolically healthy obesity; MUO, metabolically unhealthy obesity; NAFLD, nonalcoholic fatty liver disease; PORH, postocclusive reactive hyperemia; SAT, subcutaneous visceral tissue; SNP, sodium nitroprusside; T4, thyroxine; TSH, thyroid stimulating hormone; VAT, visceral adipose tissue; WHO, World Health Organization.

* Corresponding author at: Instituto Nacional de Cardiologia, Rua das Laranjeiras, 374, 22240-006 Rio de Janeiro, Brazil.
E-mail address: etibi@uol.com.br (E. Tibirica).

[1] The last two authors contributed equally to this work.

https://doi.org/10.1016/j.mvr.2023.104553
Received 5 January 2023; Received in revised form 18 May 2023; Accepted 19 May 2023
Available online 23 May 2023

have risen in low- and middle-income countries, particularly in urban settings (Dai et al., 2020).

As obesity is currently widespread, it represents an issue that needs continuous attention in every aspect, from the basic, academic understanding of pathophysiological substrates to ultimate clinical management. A topic that has attracted special attention is the existence of a "metabolically healthy" status in individuals with obesity, which would be tied to lower cardiovascular risk (Hamer and Stamatakis, 2012; Ogorodnikova et al., 2012; Appleton et al., 2013; Hosseinpanah et al., 2011; Meigs et al., 2006). However, the definition of "metabolically healthy obesity" (MHO) is not consistent, leading to conflicting prognostic data (Duque et al., 2020; Rey-Lopez et al., 2014; Smith et al., 2019). In addition, there is evidence that MHO is a transitional state between metabolically healthy normal weight (MHNW) and metabolically unhealthy obesity (MUO) (Phillips, 2017; Tsatsoulis and Paschou, 2020; Elias-Lopez et al., 2021); therefore, this concept is still a matter of debate.

In addition to atherosclerosis in the larger arteries, obesity has also been proposed to be involved in the development of functional and structural microvascular alterations in different tissues and organs (Sorop et al., 2017; Bagi et al., 2012). Most studies have been performed using animal models of obesity (Bagi et al., 2012), mainly due to the paucity of noninvasive methods available to evaluate microvascular function in humans. However, using skin video capillaroscopy, Francischetti et al. showed that subjects who fulfill the criteria for metabolic syndrome have structural and functional capillary rarefaction that is proportional to the increase in the degree of global and central obesity (Francischetti et al., 2011).

Laser speckle contrast imaging (LSCI), which is used to assess skin microvascular reactivity, allows for innovative and reproducible noninvasive evaluation of tissue flow with high real-time spatial resolution in patients with cardiometabolic diseases (Cracowski and Roustit, 2016; Hellmann et al., 2015; Roustit and Cracowski, 2012; Tew et al., 2011). Moreover, cutaneous microvascular reactivity has been correlated to microvascular function in different vascular beds, both in intensity and regarding the underlying mechanisms (Holowatz et al., 1985). Our research group has been using LSCI for skin microvascular blood flow evaluation in diverse clinical conditions, including diabetes (Gomes et al., 2008; Matheus et al., 2011), arterial hypertension (Verri et al., 2021; Verri et al., 2018), infectious diseases (Barata Kasal et al., 2021; Sabioni et al., 2023; Sabioni et al., 2021; Barcelos et al., 2018), dietary supplementation (Van Bavel et al., 2019; Moraes et al., 2014) and coronary artery disease (Souza et al., 2014).

The present study sought to evaluate the systemic microcirculatory responses of individuals with MHO, compared to those with MHNW and MUO, using a noninvasive laser-based methodology (LSCI) (Cracowski and Roustit, 2016; Roustit and Cracowski, 2012).

2. Methods

The present study was conducted in accordance with the Helsinki Declaration, revised in 2013, and was approved by the Institutional Review Board (IRB) of the National Institute of Cardiology, Rio de Janeiro, Brazil, under protocol # CAAE 13959019.0.0000.5272 and registered at ClinicalTrials.gov under protocol # NCT03515460 (date of first registration 06/01/2020). Once deemed eligible for participation in this study, all subjects read and signed an informed consent form approved by the IRB.

2.1. Study design

This was a cross-sectional observational study that included one hundred and twelve volunteers aged between 18 and 55 years who were randomly selected and distributed in three groups: individuals with metabolically healthy normal weight (MHNW), metabolically healthy obesity (MHO) and metabolically unhealthy obesity (MUO). The volunteers were allocated to the different groups according to the criteria described below, and the flow chart of the selection of the study population is presented in Fig. 1. The subjects evaluated in this study were recruited between January 2020 and January 2022. Members of the staff of our institution and external volunteers were invited to participate through social media outreach or through direct contact with research participants. All volunteers were evaluated in the morning between 8 and 11 A.M. in a single visit to our institution.

The exclusion criteria were as follows: individuals with overweight (BMI $\geq$ 25 and $<$30 kg/m^2); individuals with low weight (BMI $<$ 18.5 kg/m^2); individuals who were pregnant or lactating; and individuals with endocrine diseases other than diabetes, cardiovascular diseases, autoimmune diseases, malignant neoplasms and use of antihypertensive drugs.

Obesity was defined as body mass index $\geq$30 kg/m^2 and further categorized into classes I, II or III according to World Health Organization criteria (WHO, 2016). In this study, to define a "metabolically healthy" phenotype, the definition used was the absence of any of the components of metabolic syndrome, according to the International Diabetes Federation (IDF) criteria (Alberti et al., 2006), except waist circumference. The IDF defines metabolic syndrome as the mandatory presence of an increased abdominal circumference ($\geq$90 cm for men and $\geq$80 cm for women) plus the presence of two of the following four criteria: an increase in the plasma levels of triglycerides ($\geq$150 mg/dl) or treatment for dyslipidemia; low plasma levels of HDL cholesterol ($<$40 mg/dl for men and $<$50 mg/dl for women) or specific treatment for dyslipidemia; an increase in blood pressure (systolic blood pressure $\geq$ 130 mmHg or diastolic blood pressure $\geq$ 85 mmHg) or treatment for previously diagnosed hypertension; and increased fasting blood glucose levels ($\geq$100 mg/dl or previously diagnosed type 2 diabetes).

The MUO group was defined as the presence of one or more of the components of the metabolic syndrome according to the IDF criteria, excluding waist circumference; therefore, patients in this group might have one, two, three or four of these components. This definition has been employed in other studies, as the one by Lavie et al. (Lavie et al., 2018).

The body composition analysis, including percent body fat, was performed using the electrical bioimpedance method, with a multifrequency tetrapolar device with 8 tactile electrodes (Bioespace, model Inbody 720). A sub-analysis of microvascular function was performed according to the percentage of body fat. Two groups were analyzed: patients with normal body fat (NBF) versus patients with increased body fat (HBF). The latter were defined according to the cutoff suggested by Macek et al. (Macek et al., 2020), of 25.8 % for men and 37.1 % for women.

2.2. Evaluation of microvascular flow and reactivity

Microcirculatory tests were performed in an undisturbed quiet room with a stable temperature (23 $\pm$ 1 °C) after a 20-min resting period in the supine position. Microvascular reactivity was evaluated using a laser speckle contrast imaging system with a laser wavelength of 785 nm (PeriCam PSI system, Perimed, Järfälla, Sweden), and continuous recordings of cutaneous microvascular perfusion changes were measured in arbitrary perfusion units (APUs). The images were analyzed using PIMSoft software (Perimed, Järfälla, Sweden). Two skin sites on the ventral surface of the forearm were randomly chosen for the recordings. Hair, broken skin, areas of skin pigmentation and visible veins were avoided, and two drug-delivery electrodes were installed using adhesive discs (LI 611, Perimed, Järfälla, Sweden). A vacuum cushion (AB Germa, Kristianstad, Sweden) was used to reduce recording artifacts generated by arm movements. Acetylcholine (ACh) or sodium nitroprusside (SNP) 2 % *w/v* (Sigma Chemical CO, St. Louis, USA) were dissolved in deionized water, and iontophoresis was performed using a micropharmacology system (PF 751 PeriIont USB Power Supply, Perimed, Sweden) with increasing anodal currents of 30, 60, 90, 120, 150 and

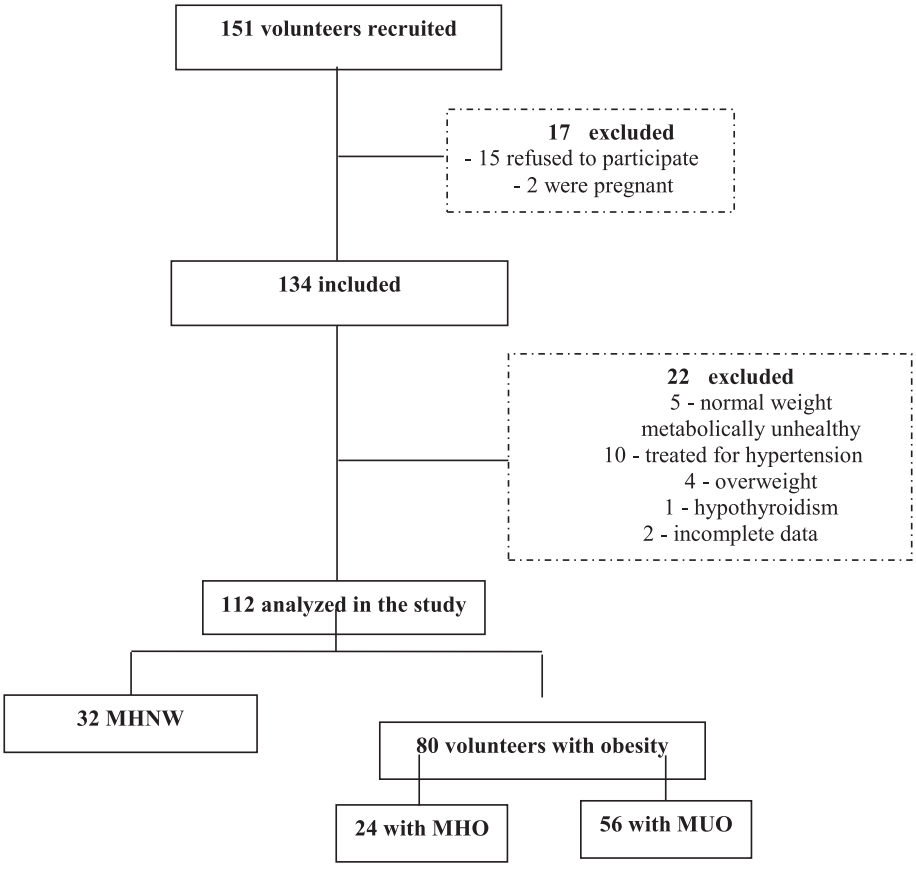


Fig. 1. Flow chart of the selection of the study population.
Abbreviations: metabolically healthy normal weight (MHNW), metabolically healthy obesity (MHO) and metabolically unhealthy obesity (MUO).

180 μA, which were administered for 10-s intervals spaced 1 min apart. The total charges for the above currents were 0.3, 0.6, 0.9, 1.2, 1.5 and 1.8 mC, respectively. After the last current, the microvascular blood flow was recorded for 120 s, resulting in a total recording time of 540 s for the whole ACh and SNP curves. The dispersive electrode was attached approximately 15 cm from the electrophoresis chamber. Skin blood flow measurements in APUs were divided by mean arterial pressure values to yield cutaneous vascular conductance (CVC) values in APU/mmHg. The results of the pharmacological tests were expressed as peak values, representing the maximal vasodilation observed after the highest dose of ACh or SNP and the area under the curve of vasodilation. The use of the area under the blood flow/time curve for assessing skin microvascular reactivity using laser technology is well validated because it represents the global flow response to different physiological and pharmacological stimuli (Roustit and Cracowski, 2013).

During the postocclusive reactive hyperemia (PORH) test, arterial occlusion was performed with suprasystolic pressure, defined as 50 mmHg above the systolic arterial pressure, by applying a sphygmomanometer for 3 min (Yvonne-Tee et al., 2008). Following the release of pressure, the maximum flux was measured. The PORH tests were always performed after the iontophoresis of ACh and SNP to avoid interference with these pharmacological tests, which are limited to local responses in the skin. In contrast, the hyperemic response is a global response that extends to the skin of the whole forearm and thus could interfere with subsequent tests of microvascular reactivity.

2.3. Statistical analysis

The prospective power analysis was based on laser speckle contrast imaging data from a previous study of our group (Verri et al., 2018). This analysis indicated that a sample size of 31 subjects per group would have 80 % power at the 5 % significance level to detect a mean difference of 0.156 APU/mmHg, with a standard deviation of 0.219 APU/mmHg, of increase in cutaneous vascular conductance induced by cutaneous iontophoresis of acetylcholine between the groups (healthy volunteers and patients with dyslipidemia). The calculations were made using classical power calculations with the formula $n = f(\alpha,\beta).\frac{2s^2}{\delta^2}$, where α is the significance level, β is the power of the test, $f(\alpha,\beta)$ is a value calculated from α and β (in this case, 7.9), δ is the difference in the means that we should be able to detect, and s is the standard deviation found in these previous studies.

Variables were tested for normality using the Shapiro-Wilk normality test. The results of normally distributed variables are presented as the mean ± SD. Non-Gaussian variables are presented as medians and interquartile ranges. Statistical analyses of microvascular vasodilation induced by skin iontophoresis were performed using two-way ANOVA followed by multiple comparisons (Tukey's multiple comparisons test), where we compared the interactions between doses of iontophoresis among groups of volunteers. The effects of increasing currents of ACh and SNP iontophoresis are shown as the mean (95 % confidence interval). Categorical variables were analyzed by the chi-square test or Fisher's exact test, when appropriate. Statistical analyses of clinical and microvascular parameters among groups of volunteers were performed using one-way ANOVA followed by multiple comparisons (Tukey's multiple comparisons test). P values < 0.05 were considered significant. Statistical analyses were performed using Prism version 7.0 software (GraphPad Software, La Jolla, CA, USA).

3. Results

The clinical and anthropometric characteristics of the study

volunteers are shown in Table 1. There were no differences in age ($P = 0.26$) or sex ($P = 0.59$) among the three groups of volunteers. As expected, both the MHO and MUO groups had significantly higher values of body weight ($P < 0.0001$), BMI ($P < 0.0001$), waist circumference ($P < 0.0001$) and body fat ($P < 0.0001$) than the control (MHNW) group. Nevertheless, there were no significant differences between the MHO and MUO groups concerning body weight ($P < 0.59$), BMI ($P < 0.15$), waist circumference ($P < 0.07$) and body fat ($P = 1$). Moreover, all volunteers in both MHO and MUO groups had high values of waist circumference (IDF criteria). Volunteers in the MUO group presented higher values of glycated hemoglobin ($P < 0.0001$), LDL cholesterol ($P = 0.007$) and ALT ($P = 0.0008$) than those in both the MHNW and MHO groups. Both the MHO and MUO groups presented higher values of fasting insulin ($P < 0.0001$) and HOMA-IR ($P < 0.0001$) than the MHNW group. The levels of fasting glucose ($P < 0.0001$) and triglycerides ($P < 0.0001$) and the systolic ($P < 0.0001$), diastolic ($P < 0.0001$) and mean ($P < 0.0001$) arterial pressures were higher in the MUO group than in both the MHO and MHNW groups. HDL-cholesterol levels were lower in the MUO group than in both the MHO and MHNW groups ($P < 0.0001$).

Among the 112 participants, only 21 (18.7 %) used diverse medications: oral contraceptives or antidepressant medications (paroxetin, sertraline, vortioxetin, escitalopram, citalopram, bupropion, desvenlafaxin). Five were from the MHNW group, six from the MHO and 10 from the MUO groups.

In the subgroup analysis of microvascular function performed according to the percentage of body fat, we observed that among the 112 study participants, in one case body fat was not assessed, while 78 patients had increased body fat and 33 had normal body fat. From the former, 54 had MUO, 21 MHO, and 3 were MHNW. From the latter, 30 were MHNW, 2 were MHO and 1 had MUO.

Table 1
Clinical characteristics of the volunteers included in the different groups of the study: individuals with metabolically healthy normal weight (MHNW), metabolically healthy obesity (MHO) and metabolically unhealthy obesity (MUO).

	MHNW ($n = 32$)	MHO ($n = 24$)	MUO ($n = 56$)	P values
Age (years)	33 ± 10	34 ± 5	33 ± 7	0.26
Male sex, n (%)	12 (40)	11 (46)	29 (52)	0.59
Body weight (kg)	65 ± 10	102 ± 15****	105 ± 15****	**<0.0001**
Body mass index (kg/m^2)	23.6 (21.5–24.3)	32.8 (31.5–36.1)****	35.8 (33.9–39.3)****	**<0.0001**
Waist circumference (cm)	81 ± 6	115 ± 11	120 ± 10	**<0.0001**
Number of components of metabolic syndrome, n (%)	N/A	N/A		
1			31 (55.4)	
2			19 (33.9)	
3			5 (8.9)	
4			1 (1.8)	
Elevated systolic arterial pressure, n (%)	N/A	N/A	17 (30)	
Elevated diastolic arterial pressure, n (%)	N/A	N/A	16 (28.5)	
Elevated triglycerides, n (%)	N/A	N/A	16 (28.5)	
Elevated blood glucose, n (%)	N/A	N/A	15 (26)	
Low HDL Cholesterol	N/A	N/A	36 (64)	
Smokers, n (%)	0 (0)	2 (8)	7 (12.5)	0.15
Body fat (%)	24.4 ± 8	40.7 ± 8****	42.7 ± 7.5****	**<0.0001**
Grade I obesity, n (%)	N/A	16 (67)	26 (46)	0.14
Grade II obesity, n (%)	N/A	5 (21)	19 (34)	0.29
Grade III obesity, n (%)	N/A	3 (12)	11 (20)	0.53
Fasting glucose (mg/dL)	85 ± 7	88 ± 7	94 ± 11****,†	**<0.0001**
Glycated hemoglobin (%)	5.0 ± 0.3	5.2 ± 0.3	5.5 ± 0.5****	**<0.0001**
Fasting insulin (μUI/mL)	7.0 (5.9–10.5)	16.7 (10.8–26.8)***	22.6 (17.5–31.6)****	**<0.0001**
HOMA-IR	1.6 (1.2–2.3)	3.8 (2.2–5.8)***	5.3 (3.9–7.4)****	**<0.0001**
Total Cholesterol (mg/dL)	178 (167–201)	173 (154–137)	188 (169–205)	0.48
LDL Cholesterol (mg/dL)	101 (91–118)	115 (91–132)	127 (113–149)**	**0.007**
HDL Cholesterol (mg/dL)	67 (54–80)	55 (47–61)**	42 (37–49)****,†††	**<0.0001**
Triglycerides (mg/dL)	65 (49–82)	71 (57–103)	114 (90–166)****,††	**<0.0001**
Creatinine (mg/dL)	0.86 ± 0.16	0.84 ± 0.21	0.85 ± 0.18	0.90
Urea (mg/dL)	28 (23–36)	24 (21–29)	24 (22–28)*	**0.02**
Uric acid (mg/dL)	4.6 (3.7–5.0)	4.6 (3.9–5.5)*	5.7 (4.8–6.7)***,†	**<0.0001**
TSH (μUI/mL)	1.9 ± 0.9	2.1 ± 0.8	2.3 ± 1.2	0.21
Free T4 (ng/dL)	1.2 ± 0.3	1.1 ± 0.2	1.1 ± 0.2	0.21
AST (U/L)	20.1 ± 5.5	19.5 ± 5.2	21.6 ± 8.8	0.50
ALT (U/L)	17.3 ± 6.8	23.6 ± 12.3	28.11 ± 15.3***	**0.0008**
Systolic arterial pressure (mmHg)	112 ± 9	118 ± 8	126 ± 12****,††	**<0.0001**
Diastolic arterial pressure (mmHg)	69 ± 6	74 ± 7*	80 ± 10****,†	**<0.0001**
Mean arterial pressure (mmHg)	83 ± 6	89 ± 7*	96 ± 10****,††	**<0.0001**
Heart rate (bpm)	63 ± 9	67 ± 9	66 ± 8	0.12

The results are presented as the mean ± SD. Values that did not follow a Gaussian distribution are presented as medians (25th–75th percentiles) (Shapiro–Wilk normality test). Statistical analyses were performed using one-way ANOVA followed by Tukey's multiple comparisons test for numerical parameters and with the chi-square test or Fisher's exact test for categorical parameters, when appropriate.
P values in bold denote statistical significance.
Abbreviations: AST: aspartate transaminase; ALT: alanine transaminase; HOMA-IR: homeostasis model assessment of insulin resistance; N/A: not applicable; TSH: thyroid stimulating hormone; T4: thyroxine.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.
**** $P < 0.0001$ compared to MHNW.
† $P < 0.05$.
†† $P < 0.01$.
††† $P < 0.001$ compared to MHO.

3.1. Microvascular reactivity

The skin iontophoresis of both ACh and SNP induced current-dependent increases in cutaneous microvascular conductance values in all groups of volunteers (Fig. 2, A and B). There were no significant differences regarding endothelial-dependent (ACh) or endothelial-independent (SNP) microvascular reactivity among groups (Fig. 2, A and B).

The baseline skin microvascular conductance values, recorded before any pharmacological or physiological stimulation, were lower in the MUO group (0.25 ± 0.08 APU/mmHg) than in the MHNW group (0.33 ± 0.12 APU/mmHg; $P = 0.0007$) and were also lower than in the MHO group, but without statistical significance (0.30 ± 0.10 APU/mmHg; $P = 0.09$) (Fig. 3; 1-way ANOVA between groups). The areas under the curves (AUCs) of microvascular vasodilation induced by ACh iontophoresis were 18,652 ± 5843 APU/s (MHNW), 18,100 ± 5047 APU/s (MHO) and 18,562 ± 6584 APU/s (MUO) (Fig. 4A; $P = 0.73$, 1-way ANOVA between groups). The peak values of microvascular conductance induced by ACh iontophoresis were 0.57 ± 0.22 APU/mmHg (MHNW), 0.53 ± 0.18 (MHO) and 0.55 ± 0.23 APU/mmHg (MUO) (Fig. 4B, P = 0.73, 1-way ANOVA between groups).

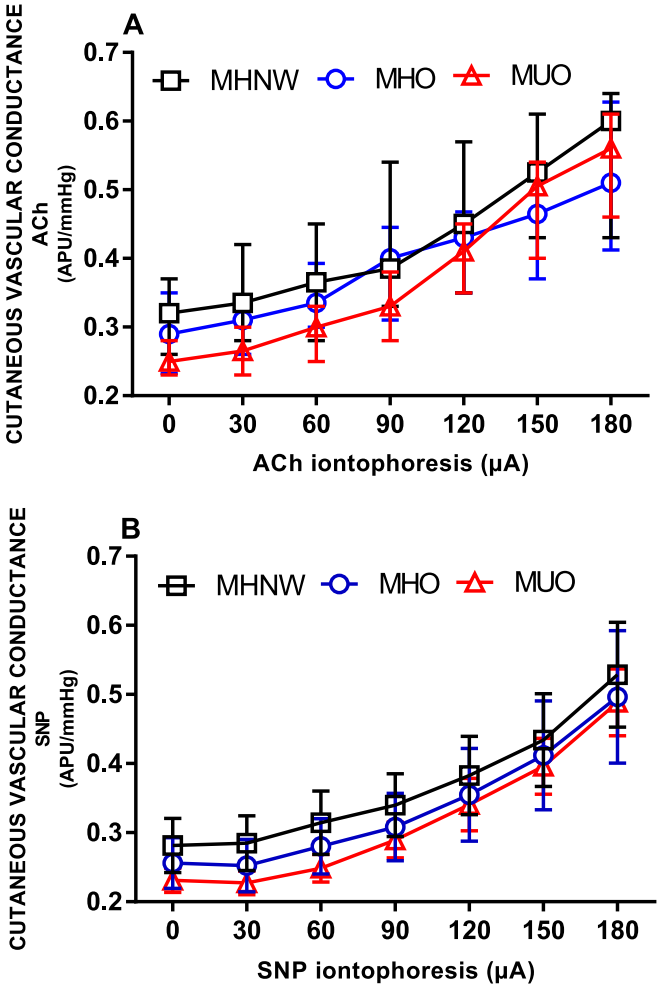


Fig. 2. Effects of skin acetylcholine (A, ACh) or sodium nitroprusside (B, SNP) iontophoresis on the cutaneous microvascular conductance of metabolically healthy normal weight (MHNW) controls, individuals with metabolically healthy obesity (MHO) and individuals with metabolically unhealthy obesity (MUO), expressed in arbitrary perfusion units (APUs) divided by the mean arterial pressure in mmHg, representing endothelium-dependent (ACh) or endothelium-independent (SNP) microvascular vasodilation.
The values are expressed as medians (95 % CIs).

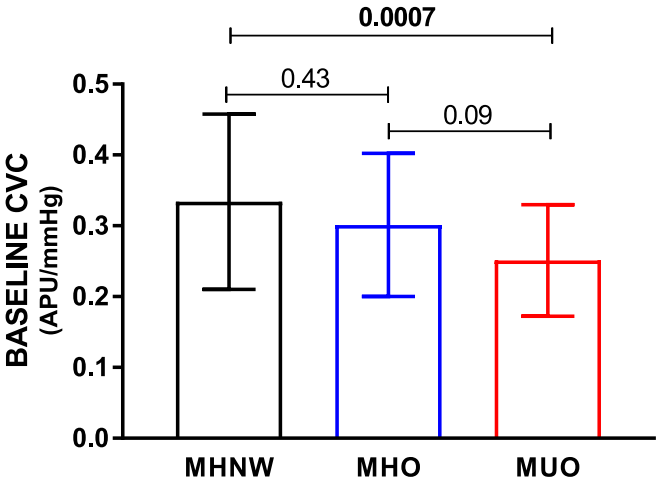


Fig. 3. Baseline cutaneous microvascular conductance (CVC) before skin iontophoresis of metabolically healthy normal weight (MHNW) controls, individuals with metabolically healthy obesity (MHO) and individuals with metabolically unhealthy obesity (MUO), expressed in arbitrary perfusion units (APUs) divided by the mean arterial pressure in mmHg.
The results are shown as the mean ± SD. *P* values in bold denote statistical significance.

The AUCs of microvascular vasodilation induced by SNP iontophoresis were 16,193 ± 5548 APU/s (MHNW), 15,608 ± 5224 APU/s (MHO) and 15,958 ± 4510 APU/s (MUO) (Fig. 4C, $P = 0.90$, 1-way ANOVA between groups). The peak values of microvascular conductance induced by SNP iontophoresis were 0.53 ± 0.21 APU/mmHg (HNOC), 0.50 ± 0.23 (MHO) and 0.49 ± 0.18 APU/mmHg (MUO) (Fig. 4D, $P = 0.66$, 1-way ANOVA between groups).

The peak values of microvascular conductance induced by PORH were 0.82 ± 0.20 APU/mmHg (MHNW), 0.76 ± 0.20 (MHO) and 0.81 ± 0.23 APU/mmHg (MUO) (Fig. 4E, $P = 0.48$, 1-way ANOVA between groups).

3.2. Microvascular reactivity and body composition

This subgroup analyses compared microvascular reactivity in individuals with normal (NBF, $n = 33$) or high (HBF, $n = 78$) body fat indices. The % of body fat was of 25.9 (19.2–31) for NBF and 41.9 (35.3–49.2) for HBF ($P = 0.0001$, Mann-Whitney test).

The areas under the curves (AUCs) of microvascular vasodilation induced by ACh iontophoresis were 17,357 (13,738-23,201) APU/s in NBF individuals and 18,336 (14,292-22,163) APU/s in HBF counterparts ($P = 0.96$, Mann-Whitney test). The peak values of microvascular conductance induced by ACh iontophoresis were 0.55 ± 0.24 APU/mmHg (NBF) and 0.54 ± 0.23 (HBF) ($P = 0.86$, unpaired *t*-test).

The AUCs of microvascular vasodilation induced by SNP iontophoresis were 15,933 (11,351-18,524) APU/s (NBF) and 15,402 (12,487-19,819) APU/s (HBF) ($P = 0.69$, Mann-Whitney test). The peak values of microvascular conductance induced by SNP iontophoresis were 0.48 (0.35–0.65) APU/mmHg (NBF) and 0.48 (0.35–0.63) APU/mmHg (HBF) ($P = 0.67$, Mann-Whitney test).

The peak values of microvascular conductance induced by PORH were 0.76 (0.64–0.97) APU/mmHg (NBF) and 0.82 (0.63–0.96) (HBF) ($P = 0.97$, Mann-Whitney test).

4. Discussion

the main findings of the present study are that (i) individuals with metabolically unhealthy obesity present lower baseline systemic microvascular flow than individuals without obesity; ii) endothelium-dependent and endothelium-independent microvascular reactivity are

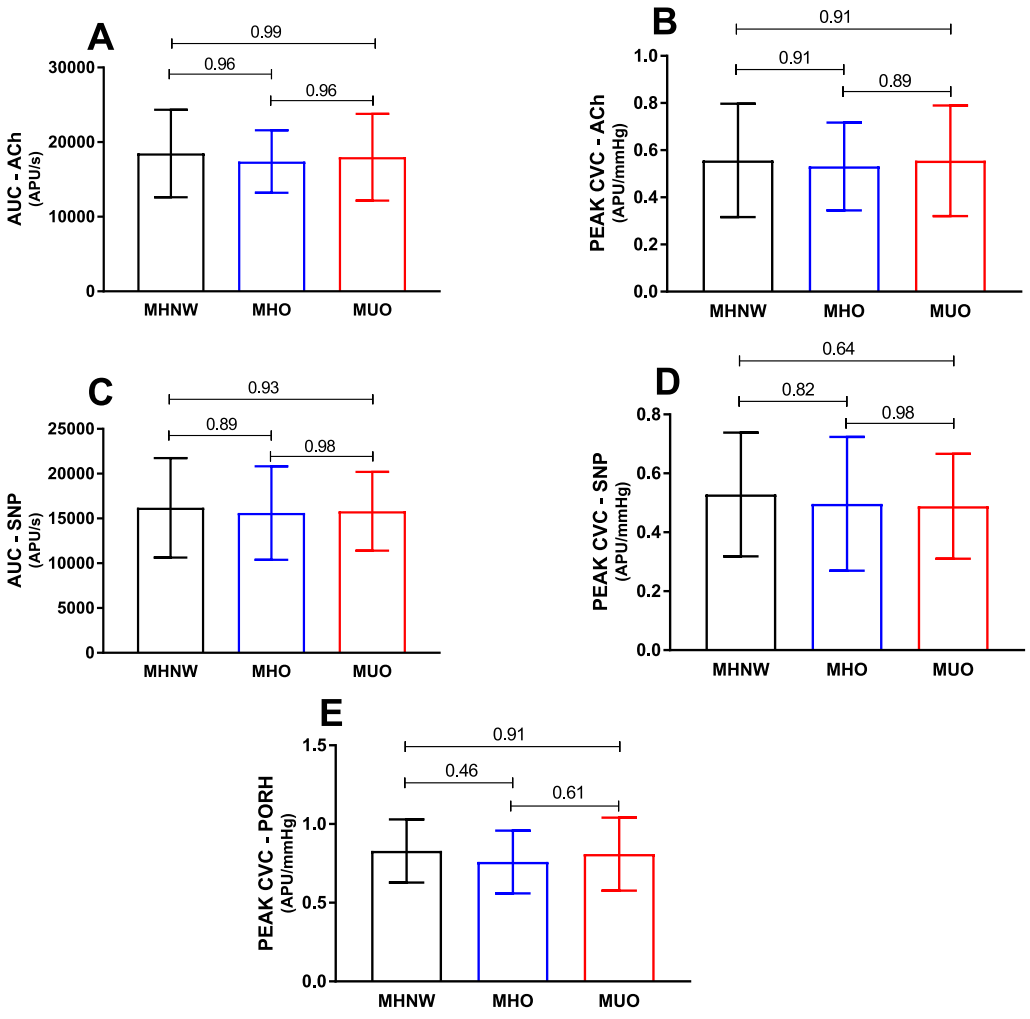


Fig. 4. (A) Area under the curve (AUC) of microvascular vasodilation and (B) peak effects of cutaneous acetylcholine (ACh) iontophoresis on cutaneous microvascular conductance; (C) AUC of microvascular vasodilation and peak effects (D) of cutaneous sodium nitroprusside (SNP) iontophoresis on cutaneous microvascular conductance. (E) Peak effects of postocclusive reactive hyperemia (PORH) on cutaneous microvascular conductance.
Cutaneous microvascular conductance (CVC) is expressed in arbitrary perfusion units (APUs) divided by the mean arterial pressure in mmHg.
Abbreviations: metabolically healthy normal weight (MHNW) controls, individuals with metabolically healthy obesity (MHO) and individuals with metabolically unhealthy obesity (MUO). The results are shown as the mean ± SD.

not different in metabolically healthy and unhealthy young adults compared to their counterparts without obesity; iii) microvascular reactivity is comparable in individuals with normal or high body fat indices.

The lower baseline systemic microvascular flow of individuals with metabolically unhealthy obesity may be explained by several mechanisms. Adipose tissue is also an endocrine organ and not only an energy storage, as it produces both pro- and anti-inflammatory mediators, the adipokines, with local and systemic effects. Leptin, a proinflammatory adipokine, increases in proportion to white adipose tissue mass, and initiates intracellular pathways that activate the innate immune response; it also increases the production of several proinflammatory cytokines, such as IL-6, IL-12, IL-18, and TNFα, induces the production of reactive oxygen intermediates in macrophages, neutrophils, and endothelial cells, enhances platelet aggregation and promotes leukocyte–endothelial cell interactions by increasing the expression of adhesion molecules on myeloid cells and vascular endothelial cells. Therefore, there is a close relationship between obesity, inflammation, and vascular function (Loffreda et al., 1998; Yamagishi et al., 2001; Bouloumie et al., 1999; Konstantinides et al., 2001; Mancuso, 2016).

In this study, individuals with MHO did not display any difference in microcirculatory parameters when compared to control volunteers with MHNW. This may be supportive of prior evidence of a "favorable" phenotype of obesity in the absence of metabolic syndrome (Bluher, 2020; Magkos, 2019). However, the relatively young age of the individuals enrolled in this study might have been an important contributor to this finding, as it has been demonstrated that age strongly influences microvascular function, especially in premenopausal women (Groen et al., 1985; Cordovil et al., 2012; Ungvari et al., 2018). Actually, it is well-known that the structural and functional alterations of the microcirculation are related to aging and several cardiometabolic diseases (Rizzoni et al., 2022; Rizzoni et al., 2019). It is also important to note that the studies reporting the existence of endothelial dysfunction in obese individuals, using methodologies similar to the one used in our study, are characterized by older age associated with different comorbidities, including coronary artery disease (van der Heijden et al., 2017), type 2 diabetes (Yim-Yeh et al., 2011), hypertension (Gryglewska et al., 2020) and in menopausal women (da Silva et al., 2016). Interestingly, macro- and microvascular endothelial functions appear to be are preserved in adolescents with severe obesity (Montero et al., 2014).

Additionally, most volunteers with MHO and MUO had grade I obesity, which might indicate a "healthier" population, even though there was no significant difference in comparison to the MUO subgroup. In addition, most individuals in the MUO group fulfilled only one component for metabolic syndrome. Although individuals with grade III obesity are more complex, the majority of individuals with class III obesity in the MUO group (90 %) had one or two components of the metabolic syndrome. In this context, our study showed that microvascular reactivity is comparable in individuals with normal or high body fat indices.

The absence of a definition of MHO is an inherent problem for the comparison of data from prior research. Studies have described MHO in several ways, from the absence of any component of metabolic syndrome to definitions including the presence of two components (Sabioni

et al., 2021). Other studies included additional criteria such as HOMA-IR, C-reactive protein, glycated hemoglobin, uric acid and fibrinogen levels (Rey-Lopez et al., 2014). Thus, the strict definition adopted in this study (the absence of any metabolic risk factor) might have contributed to the current results. In this context, Kraemer-Aguiar et al. reported that microvascular dysfunction is present in young women with overweight or obesity who present two or three, bur not only one, components of the metabolic syndrome, using the International Diabetes Federation criteria for diagnosis (Kraemer-Aguiar et al., 2010). Of note, the reduced baseline systemic microvascular flow observed in subjects with MUO in our study indicated the presence of subclinical cardiovascular compromise in these individuals, which could be related to microvascular rarefaction in individuals with obesity associated with metabolic syndrome, as demonstrated previously by our research group (Francischetti et al., 2011). The lack of a difference in microvascular reactivity to stimuli (either pharmacological stimuli, such as ACh or SNP, or physiological stimuli, such as PORH) among the groups may be a reflection of a relatively young age, a short duration of obesity, low or well-controlled blood pressure, or blood glucose within the normal limits, among several other possible explanations, including physical activity level, which was not assessed in this study.

In our study, individuals with MUO presented significantly higher plasma levels of the alanine transaminase (ALT) enzyme and of the percentage of glycated hemoglobin (HbA1c), associated with a higher HOMA-IR index value, than MHNW volunteers. This association is suggestive of nonalcoholic fatty liver disease among adults with obesity who are free of manifest metabolic complications (Kuhn et al., 2018).

Of note, the location of fat deposition may also account for differences in the microcirculation of different obesity phenotypes. Grizelj et al. (Grizelj et al., 2015) demonstrated that visceral adipose tissue (VAT) arterioles displayed reduced flow-induced dilation (FID) and ACh-induced vasodilation compared to subcutaneous visceral tissue (SAT) arterioles and that the responses were mediated by different regulatory mechanisms, since nitro-L-arginine methyl ester (a NO synthase inhibitor), indomethacin (a cyclooxygenase inhibitor), and polyethylene glycol catalase (a H_2O_2 scavenger) reduced FID in microvessels from SAT arterioles but not in microvessels from VAT arterioles of the same patients. This might be another substrate for the varied patterns of microcirculatory reactivity among individuals with obesity, who might have a "better" microcirculatory response in the presence of more peripherally distributed fat than in the presence of predominantly visceral fat.

It is worth noting that we chose to exclude the anthropometric measurement of waist circumference due to its collinearity with body mass index. In the LifeLines cohort, >95 % of the obese participants had increased waist circumference according to the NCEP ATP III (van Vliet-Ostaptchouk et al., 2014). Other authors also did not use waist circumference for the definition of MHO, as Karelis et al. (Karelis et al., 2004), Ortega et al. (Ortega et al., 2013) and Chang et al. (Chang et al., 2016).

Finally, MHO may be a transition state from MHNW to MUO. Prior evidence shows that with longer follow-up, it is not infrequent that individuals become metabolically unhealthy (Dai et al., 2020). Therefore, MHO should not be viewed as a definitively "benign" phenotype, and continued attention should be given to individuals with obesity so that efforts toward BMI reduction and a healthy lifestyle can be continuously pursued.

5. Study limitations

This study may support the hypothesis of an MHO status, which, nonetheless, may not be generalizable due to the characteristics of the studied population, especially the relatively young age of the individuals, which might have, at least in part, accounted for the current results. It is also noteworthy that most individuals in the MUO group presented only one component for metabolic syndrome. Additionally, the absence of a universal definition of MHO may limit the comparability of the results to previous studies. Finally, there are no cut-off values concerning microvascular function in healthy individuals using LSCI. Thus, in the present study, the group of metabolically healthy and normal weight individuals was considered a "reference" or control group of individuals, representing microvascular reactivity in healthy individuals without obesity.

6. Conclusions

The study underscores the subclinical derangements of MUO, as demonstrated by the reduced baseline microvascular flow. Thus, we believe that obesity should always be considered a matter of attention, either due to the possible transience of the metabolically healthy phenotype or evidence of subclinical microvascular compromise in individuals with MUO. Further data on the physiological behaviors of the different weight and metabolic phenotypes are desirable to strengthen the knowledge on this theme, which may lead to better patient management at the individual level and more adequate health policies for people with obesity at the population level.

Ethics approval and consent to participate

The present study was conducted in accordance with the Helsinki Declaration, revised in 2013, and was approved by the Institutional Review Board (IRB) of the National Institute of Cardiology, Rio de Janeiro, Brazil, under protocol # CAAE 13959019.0.0000.5272. The informed consent form was also approved was by the Institutional Review Board (IRB) of the National Institute of Cardiology, Rio de Janeiro, Brazil, phone: +55-21-3037-2307, e-mail: cepinclaranjeiras@gmail.com, address: Rua das Laranjeiras 374 – fifth floor, Laranjeiras, Rio de Janeiro, Brazil, zip code: 22.240-006.

Funding

This investigation was supported by grants from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, E.T. grant #311680/2021-6) and FAPERJ (Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro), E.T. grant #E-26/200.976/2022.

CRediT authorship contribution statement

AL, SE and ET contributed to the conception and design of the study, and to the analysis and interpretation of data; SE, DP, MD, VC, AL and ET were involved in the drafting of the manuscript, and literature review. All authors have given final approval of the version to be published and are publicly responsible for its content.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgments

The authors would like to thank Marcio Marinho Gonzalez for his excellent technical assistance.

References

Alberti KG, Zimmet P, Shaw J. Metabolic syndrome–a new world-wide definition. A Consensus Statement from the International Diabetes Federation. Diabet. Med. 2006; 23(5):469–80.

Appleton, S.L., Seaborn, C.J., Visvanathan, R., Hill, C.L., Gill, T.K., Taylor, A.W., et al., 2013. Diabetes and cardiovascular disease outcomes in the metabolically healthy obese phenotype: a cohort study. Diabetes Care 36 (8), 2388–2394.

Bagi, Z., Feher, A., Cassuto, J., 2012. Microvascular responsiveness in obesity: implications for therapeutic intervention. Br. J. Pharmacol. 165 (3), 544–560.

Barata Kasal, D.A., Britto, A., Verri, V., De Lorenzo, A., Tibirica, E., 2021. Systemic microvascular endothelial dysfunction is associated with left ventricular ejection fraction reduction in chronic Chagas disease patients. Microcirculation. 28 (2), e12664.

Barcelos, A., Tibirica, E., Lamas, C., 2018. Evaluation of microvascular endothelial function and capillary density in patients with infective endocarditis using laser speckle contrast imaging and video-capillaroscopy. Microvasc. Res. 118, 61–68.

Bluher, M., 2020. Metabolically healthy obesity. Endocr. Rev. 41, 3.

Bouloumie, A., Marumo, T., Lafontan, M., Busse, R., 1999. Leptin induces oxidative stress in human endothelial cells. FASEB J. 13 (10), 1231–1238.

Chang, Y., Ryu, S., Choi, Y., Zhang, Y., Cho, J., Kwon, M.J., et al., 2016. Metabolically healthy obesity and development of chronic kidney disease: a cohort study. Ann. Intern. Med. 164 (5), 305–312.

Cordovil, I., Huguenin, G., Rosa, G., Bello, A., Kohler, O., de Moraes, R., et al., 2012. Evaluation of systemic microvascular endothelial function using laser speckle contrast imaging. Microvasc. Res. 83 (3), 376–379.

Cracowski, J.L., Roustit, M., 2016. Current methods to assess human cutaneous blood flow: an updated focus on laser-based-techniques. Microcirculation. 23 (5), 337–344.

Dai, H., Alsalhe, T.A., Chalghaf, N., Ricco, M., Bragazzi, N.L., Wu, J., 2020. The global burden of disease attributable to high body mass index in 195 countries and territories, 1990-2017: an analysis of the global burden of disease study. PLoS Med. 17 (7), e1003198.

Danesh, J., Saracci, R., Berglund, G., Feskens, E., Overvad, K., Panico, S., et al., 2007. EPIC-heart: the cardiovascular component of a prospective study of nutritional, lifestyle and biological factors in 520,000 middle-aged participants from 10 European countries. Eur. J. Epidemiol. 22 (2), 129–141.

Duque, A.P., Rodrigues Junior, L.F., Mediano, M.F.F., Tibirica, E., De Lorenzo, A., 2020. Emerging concepts in metabolically healthy obesity. Am. J. Cardiovasc. Dis. 10 (2), 48–61.

Elias-Lopez, D., Vargas-Vazquez, A., Mehta, R., Cruz Bautista, I., Del Razo, Olvera F., Gomez-Velasco, D., et al., 2021. Natural course of metabolically healthy phenotype and risk of developing Cardiometabolic diseases: a three years follow-up study. BMC Endocr. Disord. 21 (1), 85.

Francischetti, E.A., Tibirica, E., da Silva, E.G., Rodrigues, E., Celoria, B.M., de Abreu, V. G., 2011. Skin capillary density and microvascular reactivity in obese subjects with and without metabolic syndrome. Microvasc. Res. 81 (3), 325–330.

Gomes, M.B., Matheus, A.S., Tibirica, E., 2008. Evaluation of microvascular endothelial function in patients with type 1 diabetes using laser-Doppler perfusion monitoring: which method to choose? Microvasc. Res. 76 (2), 132–133.

Grizelj, I., Cavka, A., Bian, J.T., Szczurek, M., Robinson, A., Shinde, S., et al., 2015. Reduced flow-and acetylcholine-induced dilations in visceral compared to subcutaneous adipose arterioles in human morbid obesity. Microcirculation. 22 (1), 44–53.

Groen BB, Hamer HM, Snijders T, van Kranenburg J, Frijns D, Vink H, et al. Skeletal muscle capillary density and microvascular function are compromised with aging and type 2 diabetes. J. Appl. Physiol. (1985). 2014;116(8):998–1005.

Gryglewska, B., Gluszewska, A., Zarzycki, B., Dzieza-Grudnik, A., Fedyk-Lukasik, M., Major, P., et al., 2020. Post-occlusive reactive hyperemic response of skin microcirculation among extremely obese patients in the short and long term after bariatric surgery. Microcirculation. 27 (3), e12600.

Hamer, M., Stamatakis, E., 2012. Metabolically healthy obesity and risk of all-cause and cardiovascular disease mortality. J. Clin. Endocrinol. Metab. 97 (7), 2482–2488.

van der Heijden, D.J., van Leeuwen, M.A.H., Janssens, G.N., Lenzen, M.J., van de Ven, P. M., Eringa, E.C., et al., 2017. Body mass index is associated with microvascular endothelial dysfunction in patients with treated metabolic risk factors and suspected coronary artery disease. J. Am. Heart Assoc. 6 (9).

Hellmann, M., Roustit, M., Cracowski, J.L., 2015. Skin microvascular endothelial function as a biomarker in cardiovascular diseases? Pharmacol. Rep. 67 (4), 803–810.

Holowatz LA, Thompson-Torgerson CS, Kenney WL. The human cutaneous circulation as a model of generalized microvascular function. J. Appl. Physiol. (1985). 2008;105 (1):370–2.

Hosseinpanah, F., Barzin, M., Sheikholeslami, F., Azizi, F., 2011. Effect of different obesity phenotypes on cardiovascular events in Tehran Lipid and Glucose Study (TLGS). Am. J. Cardiol. 107 (3), 412–416.

Karelis, A.D., Brochu, M., Rabasa-Lhoret, R., 2004. Can we identify metabolically healthy but obese individuals (MHO)? Diabetes Metab. 30 (6), 569–572.

Konstantinides, S., Schafer, K., Koschnick, S., Loskutoff, D.J., 2001. Leptin-dependent platelet aggregation and arterial thrombosis suggests a mechanism for atherothrombotic disease in obesity. J. Clin. Invest. 108 (10), 1533–1540.

Kraemer-Aguiar, L.G., Maranhao, P.A., Cyrino, F.Z., Bouskela, E., 2010. Waist circumference leads to prolonged microvascular reactive hyperemia response in young overweight/obese women. Microvasc. Res. 80 (3), 427–432.

Kuhn, T., Nonnenmacher, T., Sookthai, D., Schubel, R., Quintana Pacheco, D.A., von Stackelberg, O., et al., 2018. Anthropometric and blood parameters for the prediction of NAFLD among overweight and obese adults. BMC Gastroenterol. 18 (1), 113.

Lavie, C.J., Laddu, D., Arena, R., Ortega, F.B., Alpert, M.A., Kushner, R.F., 2018. Healthy weight and obesity prevention: JACC health promotion series. J. Am. Coll. Cardiol. 72 (13), 1506–1531.

Loffreda, S., Yang, S.Q., Lin, H.Z., Karp, C.L., Brengman, M.L., Wang, D.J., et al., 1998. Leptin regulates proinflammatory immune responses. FASEB J. 12 (1), 57–65.

Macek, P., Biskup, M., Terek-Derszniak, M., Stachura, M., Krol, H., Gozdz, S., et al., 2020. Optimal body fat percentage cut-off values in predicting the obesity-related cardiovascular risk factors: a cross-sectional cohort study. Diab. Metab. Syndr. Obes. 13, 1587–1597.

Magkos, F., 2019. Metabolically healthy obesity: what's in a name? Am. J. Clin. Nutr. 110 (3), 533–539.

Mancuso, P., 2016. The role of adipokines in chronic inflammation. Immunotargets Ther. 5, 47–56.

Matheus, A.S., Tibirica, E., da Silva, P.B., de Fatima Bevilacqua da Matta M, Gomes MB., 2011. Uric acid levels are associated with microvascular endothelial dysfunction in patients with type 1 diabetes. Diabet. Med. 28 (10), 1188–1193.

Meigs, J.B., Wilson, P.W., Fox, C.S., Vasan, R.S., Nathan, D.M., Sullivan, L.M., et al., 2006. Body mass index, metabolic syndrome, and risk of type 2 diabetes or cardiovascular disease. J. Clin. Endocrinol. Metab. 91 (8), 2906–2912.

Montero, D., Walther, G., Perez-Martin, A., Mercier, C.S., Gayrard, S., Vicente-Salar, N., et al., 2014. Effects of a lifestyle program on vascular reactivity in macro- and microcirculation in severely obese adolescents. J. Clin. Endocrinol. Metab. 99 (3), 1019–1026.

Moraes, R., Van Bavel, D., Moraes, B.S., Tibirica, E., 2014. Effects of dietary creatine supplementation on systemic microvascular density and reactivity in healthy young adults. Nutr. J. 13 (1), 115.

Ogorodnikova, A.D., Kim, M., McGinn, A.P., Muntner, P., Khan, U., Wildman, R.P., 2012. Incident cardiovascular disease events in metabolically benign obese individuals. Obesity (Silver Spring) 20 (3), 651–659.

Ortega, F.B., Lee, D.C., Katzmarzyk, P.T., Ruiz, J.R., Sui, X., Church, T.S., et al., 2013. The intriguing metabolically healthy but obese phenotype: cardiovascular prognosis and role of fitness. Eur. Heart J. 34 (5), 389–397.

Phillips, C.M., 2017. Metabolically healthy obesity across the life course: epidemiology, determinants, and implications. Ann. N. Y. Acad. Sci. 1391 (1), 85–100.

Rey-Lopez, J.P., de Rezende, L.F., Pastor-Valero, M., Tess, B.H., 2014. The prevalence of metabolically healthy obesity: a systematic review and critical evaluation of the definitions used. Obes. Rev. 15 (10), 781–790.

Rizzoni, D., Rizzoni, M., Nardin, M., Chiarini, G., Agabiti-Rosei, C., Aggiusti, C., et al., 2019. Vascular aging and disease of the small vessels. High Blood Press. Cardiovasc. Prev. 26 (3), 183–189.

Rizzoni, D., Mengozzi, A., Masi, S., Agabiti Rosei, C., De Ciuceis, C., Virdis, A., 2022. New noninvasive methods to evaluate microvascular structure and function. Hypertension 79 (5), 874–886.

Roustit, M., Cracowski, J.L., 2012. Non-invasive assessment of skin microvascular function in humans: an insight into methods. Microcirculation 19 (1), 47–64.

Roustit, M., Cracowski, J.L., 2013. Assessment of endothelial and neurovascular function in human skin microcirculation. Trends Pharmacol. Sci. 34 (7), 373–384.

Sabioni, L., De Lorenzo, A., Lamas, C., Muccillo, F., Castro-Faria-Neto, H.C., Estato, V., et al., 2021. Systemic microvascular endothelial dysfunction and disease severity in COVID-19 patients: evaluation by laser Doppler perfusion monitoring and cytokine/chemokine analysis. Microvasc. Res. 134, 104119.

Sabioni, L., De Lorenzo, A., Castro-Faria-Neto, H.C., Estato, V., Tibirica, E., 2023. Long-term assessment of systemic microcirculatory function and plasma cytokines after coronavirus disease 2019 (COVID-19). Braz. J. Infect. Dis. 27 (1), 102719.

da Silva, L.H., Panazzolo, D.G., Marques, M.F., Souza, M.G., Paredes, B.D., Nogueira Neto, J.F., et al., 2016. Low-dose estradiol and endothelial and inflammatory biomarkers in menopausal overweight/obese women. Climacteric 19 (4), 337–343.

Smith, G.I., Mittendorfer, B., Klein, S., 2019. Metabolically healthy obesity: facts and fantasies. J. Clin. Invest. 129 (10), 3978–3989.

Sorop, O., Olver, T.D., van de Wouw, J., Heinonen, I., van Duin, R.W., Duncker, D.J., et al., 2017. The microcirculation: a key player in obesity-associated cardiovascular disease. Cardiovasc. Res. 113 (9), 1035–1045.

Souza, E.G., De Lorenzo, A., Huguenin, G., Oliveira, G.M., Tibirica, E., 2014. Impairment of systemic microvascular endothelial and smooth muscle function in individuals with early-onset coronary artery disease: studies with laser speckle contrast imaging. Coron. Artery Dis. 25 (1), 23–28.

Tew, G.A., Klonizakis, M., Crank, H., Briers, J.D., Hodges, G.J., 2011. Comparison of laser speckle contrast imaging with laser Doppler for assessing microvascular function. Microvasc. Res. 82 (3), 326–332.

Tsatsoulis, A., Paschou, S.A., 2020. Metabolically healthy obesity: criteria, epidemiology, controversies, and consequences. Curr. Obes. Rep. 9 (2), 109–120.

Ungvari, Z., Tarantini, S., Donato, A.J., Galvan, V., Csiszar, A., 2018. Mechanisms of vascular aging. Circ. Res. 123 (7), 849–867.

Van Bavel, D., de Moraes, R., Tibirica, E., 2019. Effects of dietary supplementation with creatine on homocysteinemia and systemic microvascular endothelial function in individuals adhering to vegan diets. Fundam. Clin. Pharmacol. 33 (4), 428–440.

Verri, V., Brandao, A.A., Tibirica, E., 2018. Penile microvascular endothelial function in hypertensive patients: effects of acute type 5 phosphodiesterase inhibition. Braz. J. Med. Biol. Res. 51 (3), e6601.

Verri, V., Nascimento, A.R., Brandao, A.A., Tibirica, E., 2021. Effects of chronic type 5 phosphodiesterase inhibition on penile microvascular reactivity in hypertensive patients with erectile dysfunction: a randomized crossover placebo-controlled trial. J. Hum. Hypertens. 35 (4), 360–370.

van Vliet-Ostaptchouk, J.V., Nuotio, M.L., Slagter, S.N., Doiron, D., Fischer, K., Foco, L., et al., 2014. The prevalence of metabolic syndrome and metabolically healthy obesity in Europe: a collaborative analysis of ten large cohort studies. BMC Endocr. Disord. 14, 9.

WHO, 2016. Obesity. www.who.int/mediacentre/factsheets/fs311/en.

Yamagishi, S.I., Edelstein, D., Du, X.L., Kaneda, Y., Guzman, M., Brownlee, M., 2001. Leptin induces mitochondrial superoxide production and monocyte chemoattractant protein-1 expression in aortic endothelial cells by increasing fatty acid oxidation via protein kinase A. J. Biol. Chem. 276 (27), 25096–25100.

Yim-Yeh, S., Rahangdale, S., Nguyen, A.T., Stevenson, K.E., Novack, V., Veves, A., et al., 2011. Vascular dysfunction in obstructive sleep apnea and type 2 diabetes mellitus. Obesity (Silver Spring) 19 (1), 17–22.

Yvonne-Tee, G.B., Rasool, A.H., Halim, A.S., Wong, A.R., Rahman, A.R., 2008. Method optimization on the use of postocclusive hyperemia model to assess microvascular function. Clin. Hemorheol. Microcirc. 38 (2), 119–133.