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Skin microcirculatory dysfunction is already present in normoglycemic subjects with metabolic syndrome

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Abstract

The role of microcirculatory dysfunction (MD) in metabolic and cardiovascular diseases is not well established. Considering that metabolic syndrome (MS) is an independent risk factor and diabetic patients have microangiopathy, our aim was to investigate if normoglycemic subjects with MS already have detectable skin MD. Thirty-six subjects with MS (National Cholesterol Education Program–Adult Treatment Panel III criteria) (10 men/26 women, 38.8 ± 7.9 years, 35.8 ± 4.9 kg/m^2) with normal glucose tolerance (American Diabetes Association criteria) and 16 controls (11 men/5 women, 33.6 ± 8.4 years, 23.9 ± 3.6 kg/m^2) were studied using nailfold videocapillaroscopy. Afferent, efferent, and apical capillary diameters; functional capillary density; red blood cell velocity (RBCV) at baseline; and $RBCV_{max}$ and time ($TRBCV_{max}$) to reach it during postocclusive reactive hyperemia after 1-minute arterial occlusion were measured. Subjects with MS had smaller afferent, efferent, and apical diameters (4.2 [3.8-4.2] vs 5.6 [4.65-6.25] μm, $P < .001$; 4.8 [4.2-4.8] vs 6.2 [5.6-7] μm, $P < .001$; and 5.2 [4.8-5.55] vs 7.4 [6.2-8] μm, $P < .001$); lower functional capillary density (7.28 [6.37-9.10] vs 10.4 [9.1-11.8] capillaries per square millimeter, $P < .001$), RBCV (0.62 [0.57-0.65] vs 0.79 [0.76-0.89] mm/s, $P < .001$], and $RBCV_{max}$ (1.14 [1.12-1.210] vs 1.57 [1.45-1.62] mm/s, $P < .001$); and longer $TRBCV_{max}$ (10.0 [10-11] vs 4.5 [4-6] seconds, $P < .001$) compared with controls. Microcirculatory dysfunction was associated with body mass index. We concluded that subjects with MS already have nutritive skin MD even within the normoglycemic milieu.

1. Introduction

The current obesity epidemic implies that this disease is becoming an increasingly important risk factor for cardiovascular disease. Hyperinsulinemia and insulin resistance (IR) are common features of obesity in both human and experimental animals. Insulin resistance has been proposed as the metabolic basis of atherogenesis in subjects with metabolic syndrome (MS) based on the concept that reduced insulin sensitivity is the primary abnormality giving rise to dyslipidemia, hypertension, impaired glucose tolerance, or type 2 diabetes mellitus diabetes mellitus (T2DM). Metabolic syndrome, phenotypically associated with abdominal obesity, presents some or all of these features and ultimately increases cardiovascular risks [1]. Although not yet established, endothelial and microcirculatory dysfunctions (MDs), characterized by decreased responses to endothelial-derived relaxing factors (essentially nitric oxide) and alterations of hemodynamic parameters such as number of perfused capillaries and baseline red blood cell velocity (RBCV), respectively, are hypothesized as primary causes of IR in several vascular beds [2,3].

In the microcirculation, the most purposeful functions of circulation occur: transport of nutrients to tissues and removal of cellular excreta. The small arterioles control the blood flow to each tissue area, and local conditions in the tissues themselves control the diameters of the arterioles in turn. Thus, each tissue in most instances controls its own blood flow in relation to its needs. Microvascular morphology and hemodynamics can be studied noninvasively in humans, without disturbing the quantities that are being examined, by nailfold videocapillaroscopy [4]. In T2DM, MD has been well characterized in the coronary bed [5] and in skin [6]; but to our knowledge, there are no data available on MS at the normoglycemic milieu.

There are accumulating evidences of a relationship between impaired glucose tolerance and renal and retinal injuries [7]. Retinopathy has been also associated with blood pressure, lipid

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concentration, and body mass index (BMI) [8], supporting the concept that not only hyperglycemia but also previous metabolic disturbances could impair the microcirculation.

The coexistence of MS on both diabetes mellitus types is considered as a risk indicator of microvascular complications in a recent metascreen [9], reinforcing the role of other risk factors, apart from hyperglycemia, for microcirculatory damage. If these assumptions were correct, microvascular disturbances would appear early on subjects with MS already during normoglycemia or would even exist at its onset. Our aim was to investigate if subjects with MS without any degree of glucose intolerance would already have MD, evaluated by morphologic and functional changes on nailfold capillaries at rest and after an ischemic period.

2. Materials and methods

2.1. Subjects

Thirty-six obese subjects (10 men, 26 women) with MS were selected at the Cardiometabolic Clinic for outpatient care of the State University of Rio de Janeiro. After physical examination, they proceeded to 75-g oral anhydrous glucose tolerance test (fasting and 2 hours), lipid profile, and plasma insulin determinations after 10- to 12-hour fast. All subjects enrolled were first-degree relatives of persons with T2DM, had normal glucose tolerance test according to the American Diabetes Association criteria [10], and had at least 3 criteria for MS according to the National Cholesterol Education Program–Adult Treatment Panel III [11]. Among the 36 obese subjects with MS, 5 were not using antihypertensive drugs. We also invited 16 lean or overweight subjects (11 men, 5 women) to volunteer as controls without MS diagnosis criteria. All subjects gave their written informed consent, and the local ethical committee approved the protocol.

The same trained examiner collected anthropometric measurements in duplicate, waist, height, weight, and blood pressures, as previously reported [12]. *Body mass index* was defined as the ratio between weight in kilograms and squared height in meters.

Main exclusion criteria were pregnancy, T2DM, smoking, major illnesses, a history of previous myocardial infarction or angina pectoris, postmenopause, use of oral contraceptives, and triglyceride (TG) levels greater than 600mg/dL. Except for antihypertensive drugs, no other drug, including aspirin, was accepted for use without previous communication. No therapeutic treatment of dyslipidemia was used.

2.2. Microvascular function assessment

Nailfold videocapillaroscopy was carried out according to a standardized, well-validated methodology [12] on the fourth finger of the left hand. Functional capillary density (FCD), the number of capillaries per square millimeter with flowing red blood cells, was evaluated using a final magnification of ×250 and an area of 3 mm of the distal row of capillaries. Capillary diameters (afferent [AF], apical [AP], and efferent [EF]), RBCV at rest, RBCV after 1-minute arterial occlusion ($RBCV_{max}$), and time taken to reach it ($TRBCV_{max}$) were measured with a final magnification of ×680 during the postocclusive reactive hyperemia (PORH) response. Before RBCV determination on an individual capillary loop, a pressure cuff (1 cm wide) was placed around the proximal phalanx and connected to a mercury manometer. Conceptually, AF, AP, and EF are considered morphologic parameters; and FCD, RBCV, $RBCV_{max}$, and $TRBCV_{max}$ are considered functional parameters. Microcirculatory images were recorded into a super VHS videotape and analyzed using CapImage software [13]. The examination was repeated on 9 subjects in different days; and the interassay coefficient of variation (IECV) ranged from 12.3% to 17.3% and 2.0% to 9.0% between morphologic and functional parameters, respectively.

2.3. Laboratory analysis

All laboratory measurements were performed in duplicate after 10- to 12-hour fast using an automated method (Modular Analytics PP; Roche, Basel, Switzerland). Fasting plasma glucose (FPG), total cholesterol, TG, and high-density lipoprotein (HDL) cholesterol were measured, respectively, by enzyme-colorimetric oxidase-peroxidase method (GOD-PAP; IECV = 1.09%), enzymatic GPO-PAP (IECV = 2.93%), enzymatic GPO-PAP (IECV = 1.29%), and enzyme-colorimetric without pretreatment (IECV = 3.23%). Plasma low-density lipoprotein (LDL) cholesterol was calculated according to the Friedewald equation. Serum insulin levels were analyzed by an automated chemiluminescent method. Homeostasis model assessment of IR (HOMA-IR) was calculated using the following formula: fasting serum insulin (in micro–international units per milliliter) × FPG (in millimoles per liter)/22.5 [14].

2.4. Statistical analysis

Variables are presented as mean ± SD or as median (first-third quartiles), in cases of nonnormal distribution. Comparisons between 2 or more groups were performed using Mann-Whitney *U* test or Kruskal-Wallis analysis of variance by ranks, respectively. Partial correlation and multivariate analyses were used to investigate associations by Spearman rank order test and as data mining because not all variables were normally distributed. All variables were previously converted to *z* scores and subjected to factor analysis to infer contributions of clinical-laboratory and microvascular functional (RBCV, $RBCV_{max}$, and $TRBCV_{max}$) parameters on MS/MD combination. All analyses were adjusted for age and sex. Significant differences were assumed to be present at a 2-tailed *P* less than .05.

3. Results

Anthropometric, clinical, and laboratory data of investigated groups and their differences are described on Table 1.

Table 1
Anthropometric and clinical-laboratory characteristics of groups

	Controls	Subjects with MS
Sex (female/male)	5/11	26/10
Age (y)	33.6 ± 8.4	38.8 ± 7.9
Known hypertension	–	31 (86.1%)
Weight (kg)	72.2 ± 16.9	94.9 ± 18.9*
BMI (kg/m^2)	23.9 ± 3.6	35.8 ± 4.9*
Waist (cm)	82.5 ± 15.1	103.0 ± 10.2*
Hip (cm)	96.6 ± 7.9	115.4 ± 10.9*
Waist-to-hip ratio	0.85 ± 0.1	0.89 ± 0.06
Systolic BP (mm Hg)	107.8 ± 31.5	140 ±16.9*
Diastolic BP (mm Hg)	70.7 ± 13.9	84.2 ± 16.9*
FPG (mg/dL)	87.1 ± 9.2	89.9 ± 7.9
Insulin (μUI/mL)	4.2 (2.9-5.7)	12.9 (10.9-21.6)*
HOMA-IR	0.81 (0.57-0.97)	2.72 (2.29-4.98)*
Postload PG (mg/dL)	–	104 (93-118)
Total cholesterol (mg/dL)	181 (169-189)	200.6 ± 29.1
LDL cholesterol (mg/dL)	110 (95-121)	125.9 ± 25.9
HDL cholesterol (mg/dL)	63 (56-65)	40 (35.547)*
TG (mg/dL)	79 (60-129)	169.3 ± 71.8*

Data as mean ± SD or median (first-third quartiles). BP indicates blood pressure.

* $P < .001$, comparisons between controls and subjects with MS.

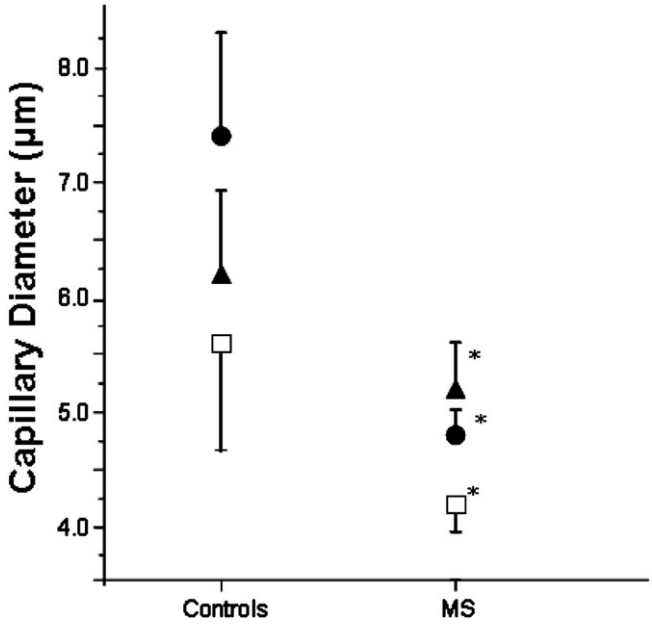

Fig. 1. Afferent (□), AP (●), and EF (▲) capillary diameters in subjects with MS at normoglycemic milieu (MS). Data are the median (first-third quartiles). *$P < .01$, MS vs controls.

Although sex proportion was not the same on controls and subjects with MS (68.8% men and 72.2% women, respectively), no significant intragroup differences on microvasculatory parameters dependent on sex could be found. Several abnormalities were detected on skin microvascular morphology and function on subjects with MS compared with controls (Table 2), expressed by smaller AF, EF, and AP diameters (Fig. 1); lower FCD, RBCV, and $RBCV_{max}$; and longer $TRBCV_{max}$ (Fig. 2).

The investigation was taken 1 step further, and intragroup differences on microvascular parameters were compared with each MS criterion. Fasting plasma glucose and waist circumference were not used for obvious reasons. No difference could be found on microvascular parameters between those that matched the cutoff (n = 7) for HDL cholesterol and the ones who did not (n = 29); but TG cutoff value for MS was able to differentiate subjects according to $RBCV_{max}$, with higher $RBCV_{max}$ (1.16 [1.14-1.28] vs 1.13 [1.12-1.15] mm/s, $P < .04$, respectively) from those with TG greater than the MS criterion (n = 21; 205 [177-232] mg/dL) compared with the ones with TG less than 150 mg/dL (n = 15; 104 [93-117] mg/dL). Although HOMA-IR is not a diagnostic criterion for MS, a cutoff value of 2.71 [15] was used to reclassify subjects with higher (n = 18) and lower (n = 18) levels of IR; and no significant difference could be detected between these groups.

In the pooled group (n = 52, group A; Table 3), morphologic parameters were correlated with clinical-laboratory data; and significant inverse correlations were obtained between (*a*) AF, EF, and AP and BMI, hip circumferences, and systolic and diastolic pressures; (*b*)

Table 2
Microcirculatory derangements on morphology and function in subjects with MS

	Controls	Subjects with MS
AF capillary diameter (μm)	5.6 (4.65-6.25)	4.2 (3.8-4.2)*
EF capillary diameter (μm)	6.2 (5.6-7)	4.8 (4.2-4.8)*
AP capillary diameter (μm)	7.4 (6.2-8)	5.2 (4.8-5.55)*
FCD (capillaries/mm^2)	10.4 (9.1-11.8)	7.28 (6.37-9.10)*
RBCV at baseline (mm/s)	0.79 (0.76-0.89)	0.62 (0.57-0.65)*
RBCV during PORH (mm/s)	1.57 (1.45-1.62)	1.14 (1.12-1.21)*
Time taken to reach $RBCV_{max}$ (s) during PORH	4.5 (4-6)	10.0 (10-11)*

Data as median (first-third quartiles).

* $P < .001$, comparisons between controls and MS.

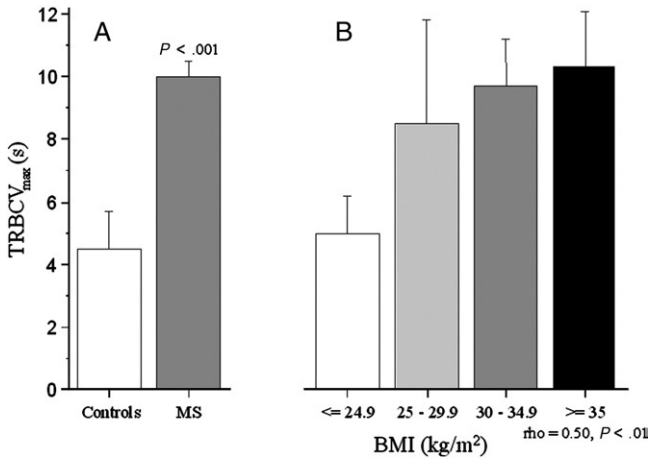

Fig. 2. A, Time taken to reach RBCV ($TRBCV_{max}$) during PORH in subjects with MS at normoglycemic milieu. Data are the median (first-third quartiles). B, Stepwise progression of impaired $TRBCV_{max}$ associated with BMI in the pooled group. Data are the mean ± SD.

Table 3
Correlation between morphologic (AF, AP, and EF) and functional (FCD, RBCV, $RBCV_{max}$, and $TRBCV_{max}$) capillary parameters and clinical-laboratory ones in the pooled group

	AF	AP	EF	FCD	RBCV	$RBCV_{max}$	$TRBCV_{max}$
Weight	−0.28*	–	–	–	−0.41†	–	0.29*
BMI	−0.45†	−0.44†	−0.40†	−0.35*	−0.58‡	−0.48‡	0.50†
Waist	–	–	–	–	−0.41‡	–	0.32*
Hip	−0.39†	−0.34*	−0.31†	–	−0.50‡	−0.38†	0.36*
Waist-to-hip ratio	–	–	–	–	–	–	–
Systolic BP	−0.45†	−0.43†	−0.37†	–	−0.48‡	−0.37†	0.31*
Diastolic BP	−0.44†	−0.39†	−0.32*	–	−0.41†	−0.29*	–
FPG	–	–	–	–	–	–	–
Insulin	−0.36*	–	–	–	−0.42†	−0.37*	0.34*
HOMA-IR	−0.39†	–	–	–	−0.35*	−0.40†	0.30*
Total cholesterol	–	–	−0.31*	–	–	–	0.37*
LDL cholesterol	–	–	−0.32*	–	–	–	0.35*
HDL cholesterol	0.37*	–	–	–	0.47†	0.40†	−0.36†
TG	−0.34*	–	–	–	−0.36*	−0.42‡	0.35*

Data expressed as *R*.
* $P < .05$.
† $P < .01$.
‡ $P < .001$.

AF and weight, insulin, and HOMA-IR; and (*c*) EF and total and LDL cholesterol. Afferent diameter and HDL cholesterol showed a significant direct relationship. Functional parameters were also tested; and significant inverse correlations were detected between (*a*) RBCV and $RBCV_{max}$ and BMI, hip circumferences, systolic and diastolic pressures, insulin, HOMA-IR, and TG; (*b*) RBCV and weight and waist circumference; (*c*) $TRBCV_{max}$ and HDL cholesterol; and (*d*) FCD and BMI. Significant direct relationships were observed between (*a*) RBCV and $RBCV_{max}$ and HDL cholesterol and (*b*) $TRBCV_{max}$ and weight, BMI, waist and hip circumferences, systolic blood pressure, insulin, HOMA-IR, total and LDL cholesterol, and TG. All functional parameters were tested again exclusively on subjects with MS, and only waist and $RBCV_{max}$ were correlated ($R = 0.34$, $P < .05$).

For functional parameters, except for FCD that showed poor correlations with clinical-laboratory data, factor analysis showed large factor load for BMI (−0.847), waist (−0.839), hip (−0.727), systolic (−0.716) and diastolic pressures (−0.675), HDL cholesterol (0.794), and $TRBCV_{max}$ (−0.710), responsible for 39.2% of the total variance (factor 1, eigenvalue = 6.68). Low-density lipoprotein cholesterol (−0.879), total cholesterol (−0.882), RBCV (0.409), and $RBCV_{max}$ (0.457) explained 14.7% of the total variance (factor 2, eigenvalue = 2.51).

4. Discussion

Microcirculatory dysfunction has been described in obesity [8], first-degree relatives of persons with T2DM [16,17], hypertension [18], and diabetes mellitus [6] using different methods. Our study showed that normoglycemic subjects with MS, diagnosed by the National Cholesterol Education Program–Adult Treatment Panel III criteria, already have morphologic abnormalities and, more importantly, MD. Impairments on RBCV and FCD at baseline and on $RBCV_{max}$ and $TRBCV_{max}$ during PORH, recorded by nailfold videocapillaroscopy, have been shown for the first time, to our knowledge, in this investigation. Despite a strong association between BMI and all microvascular parameters, our data support that being overweight, but with MS, is enough to have MD.

Insulin resistance, the main feature of obesity and T2DM, detected also in hypertension and atherogenic dyslipidemia [19,20], is a risk factor for atherosclerosis and coronary heart disease [21] and plays a central role on MS. However, the importance of MD on these diseases is not yet fully established. Recent experimental and prospective studies have shown impaired capillary recruitment in IR and hypertension [18,22] and a contribution of truncal distribution of adiposity to MD in adulthood, probably associated with adipocytokines [23].

In nutritive skin capillaries, local mediators regulate functional parameters. When the pressure cuff is released after occlusion, there is a sharp rise in blood flow, the so-called reactive hyperemia, followed by a gradual return to resting level. This response is not dependent on vasomotor nerves, but is influenced by nitric oxide; accumulated metabolites, normally washed out or destroyed by circulating blood; reactive oxygen species; and smooth muscle cell reactivity. After occlusion release and sudden increase in intraluminal pressure a rapid stretch of vascular smooth muscle cells followed by a strong and short-lasting arteriolar constriction, the myogenic response, could negatively influence $RBCV_{max}$. Because several mediators could play a role on the reactive hyperemia response, it is possible to consider that our patients might have impairments on other vascular beds. To reinforce this hypothesis, we have

previously reported concomitant improvement on skin MD [12] and on endothelial reactivity [24] with an insulin-sensitizing agent given to normoglycemic subjects with MS.

Data obtained using nailfold videocapillaroscopy have been already associated with cardiovascular risk [25] and to cardiac syndrome X [26,27]. Functional parameters (RBCV, $RBCV_{max}$, and $TRBCV_{max}$) showed better reproducibility than morphologic ones because of smaller day-to-day IECV. They represent direct measurements of vasodilation at the precapillary level, and their impairments could be interpreted as MD.

There is mounting evidence suggesting that obesity per se exerts its deleterious effects on the cardiovascular system by inducing an inflammatory state that targets both large and small blood vessels through adipokines released from the adipose tissue and IR [28]. A major consequence of adipocyte-derived products and IR is oxidative stress, leading to nuclear factor–κB activation and subsequent up-regulation of inflammatory genes, including cell adhesion molecules, in endothelial cells. Such events could provoke vasoconstriction at the precapillary level, endothelial cell swelling [29], and consequently the decrease in blood flow observed on nailfold capillaries.

Triglyceride level was the only MS criterion able to differentiate microvascular function during the reactive hyperemia response. A predictive value of TG for development and progression of retinal and renal microvascular complications of type 1 diabetes mellitus has been already described [30]. Endothelial dysfunction might play a role in the evolution of atherogenic changes related to lipoprotein concentration through impaired action of endothelial-bound lipoprotein lipase. Lipoprotein lipase dysfunction leads to increased plasma levels of TG, reduced concentrations of HDL cholesterol, and perhaps premature atherosclerosis [31]. From a pathophysiologic point of view, this might be a plausible hypothesis because reduction of FCD, and hence endothelial surface [32], could lead to higher concentration of lipoprotein by impaired clearance, decreased diffusion area, and reduced lipoprotein lipase activity. In our data, RBCV and $RBCV_{max}$ were inversely related and $TRBCV_{max}$ was directly related to TG levels, suggesting impairment on arteriolar vasodilator capacity because capillary hemodynamics reflect the precapillary segment. Unfortunately, the exact cause/effect of this association could not be established by the cross-sectional design used in this study.

Other relationships were also observed between clinical-laboratory variables and microvascular ones. Body mass index was the only one related to all microvascular parameters, with higher BMI being associated with morphologic abnormalities (smaller diameters) and impaired function (lower FCD, RBCV, and $RBCV_{max}$ and longer $TRBCV_{max}$). Studies on Zucker rats have shown structural and functional defects: increased permeability to macromolecules [33]; capillary rarefaction, independently of prevailing hypertension [34]; smaller arteriolar diameter in muscle [35]; and activated sympathetic nervous system [36]. Because of elevated adrenergic tone and structural vessel narrowing, reactive hyperemia was impaired. In obese humans, capillary rarefaction in skeletal muscle has also been reported [37].

Waist circumference is a clinical marker of visceral adiposity directly associated with IR, with some functional parameters of MD, and with lower value of $RBCV_{max}$ after correlation tests on subjects with MS. In fact, bigger waist circumference could be associated with lower $RBCV_{max}$ when heterogeneity was no longer a problem. Impaired microvascular function, positively associated with adiposity [38], was described already in normal children, suggesting that risk factors for adult cardiovascular diseases begin to cluster early in life. Factor analysis of functional parameters showed that BMI, waist and hip circumferences, systolic and diastolic pressures, HDL cholesterol, insulin, HOMA-IR, and $TRBCV_{max}$ contributed to an expressive change for the total variance. Low-density lipoprotein cholesterol, total cholesterol, RBCV, and $RBCV_{max}$ were combined on another group of variables and were not as important. This interpretation deserves caution because not all variables were normally distributed.

Recently, it has been hypothesized that insulin's metabolic and vasodilatory actions are functionally coupled [39] and play an important role on microcirculatory function [40]. Its vasodilatory activity, also seen in the skin, can favor insulin and glucose availability to cells, associated with insulin sensitivity during fasting hyperglycemia [41]. It should be pointed out that skin could not be considered as primary target for insulin-mediated glucose uptake. During hyperinsulinemia, skin capillary recruitment increases in healthy subjects [42]; and in obese ones, impaired microvascular function occurs, associated with decreased insulin sensitivity [43]. Cellular defects on insulin signaling pathways [44] and microcirculatory and endothelial dysfunctions have been described or hypothesized as causes of IR [2,3]. Our data could not establish the exact cause/effect mechanism for such relationship, but suggest that IR markers were associated with skin MD at precocious states, where glucose normotolerance was still present. Whether MD expresses mainly a causal effect of IR or even the opposite remains to be determined. Although restricted to our data, we could suggest that MD parallels the IR state, clinically manifested as MS, and precedes glucose intolerance/diabetes. The fact that MS and MD influence each other negatively and are closely associated makes it difficult to distinguish from cause and effect. It is probably better to consider it a vicious circle. Unfortunately, skin microcirculatory function has its own particularities and does not express entirely what happens on coronary or other end-organ microcirculation. Its comparison with end-organ microcirculation needs to be investigated with diagnostic techniques. However, emphasis should be given to the fact that microvascular coronary derangements are also involved in progressive contractile dysfunction and heart failure [45]; and there are evidences of a relationship between MD and large vessel diseases [46].

Thus, although learning more about relationships between MD and IR is crucial for our knowledge, the association between MD, metabolic diseases, and cardiovascular risk should not be forgotten but reinforced in clinical practice.

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