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Dynamic nailfold videocapillaroscopy may be used for early detection of microvascular dysfunction in obesity

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ABSTRACT

Objectives: It has been hypothesized that obesity is the primary cause of microvascular dysfunction (MD), which could be a pathway to increase blood pressure and decrease insulin sensitivity. Due to the high prevalence of this metabolic disorder in the world today, the aim of this study was to investigate which is the most appropriate videocapillaroscopic method, between nailfold and dorsal finger, to assess microvascular function in obese patients since both techniques are non-invasive and could be used for early detection as well as for follow-up.
Methods: Eighteen lean [27.8 ± 6.2 years, body mass index (BMI) 21.8 ± 1.8 kg/m^2] and nineteen obese (30.8 ± 4.6 years; BMI 32.3 ± 1.5 kg/m^2) women participated in the study. Dynamic nailfold videocapillaroscopy assessed morphological (capillary diameters) and functional [functional capillary density (FCD); red blood cell velocity (RBCV) at baseline and peak and time (TRBCV$_{max}$) taken to reach it during the post-occlusive reactive hyperemia (PORH) response, after 1-min ischemia] parameters; while dorsal finger videocapillaroscopy assessed FCD at rest and capillary recruitment during PORH and post-venous occlusion.
Results: RBCV (0.32 ± 0.01 vs. 0.30 ± 0.01 mm/s; $p < 0.0001$) and RBCV$_{max}$ (0.32 ± 0.01 vs. 0.30 ± 0.015 mm/s; $p = 0.0020$) were significantly higher in control subjects compared to the obese group. Moreover, TRBCV$_{max}$ was prolonged in the obese group compared to control one (3.5 ± 1.4 vs. 5.5 ± 1.3 s; $p = 0.0001$). Multiple regression analysis showed that these variables were influenced by some others, especially those related to adiposity and metabolic disease. On the other hand, dorsal finger videocapillaroscopy did not show any significant differences between groups.
Conclusion: Our results strongly suggest that microvascular dysfunction consequent to obesity could be better detected by dynamic nailfold videocapillaroscopy than by dorsal finger videocapillaroscopy.
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Introduction

Obesity is a major public health problem and its prevalence has increased during the last decades, turning it a worldwide pandemia. It has been hypothesized elsewhere (de Jongh et al., 2004) that obesity is the primary cause of microvascular dysfunction (MD), which could be a pathway to increase blood pressure and decrease insulin sensitivity. Therefore, early detection of MD could help in prevention of obesity associated disorders, such as hypertension and type 2 diabetes mellitus (T2DM).

Microvascular function can be assessed noninvasively in human skin through videocapillaroscopy. Using this technique, capillaries can be seen parallel to the skin using nailfold videocapillaroscopy (Kraemer-Aguiar et al., 2010) or perpendicular to it by dorsal finger capillaroscopy (Ijzerman et al., 2006; Serne et al., 2002). The choice of the most appropriate methodology to observe skin capillaries relies on research purposes. Nailfold videocapillaroscopy (NVC) allows the study of capillary morphology; assessment of hemodynamic variables, such as red blood cell velocity at rest (RBCV), maximal red blood cell velocity (RBCV$_{max}$) and time taken to reach it (TRBCV$_{max}$) during the post-occlusive reactive hyperemia (PORH) response; and also functional capillary density [(FCD), number of perfused capillaries per unit of tissue area]. On the other hand dorsal finger videocapillaroscopy allows the evaluation of FCD (de Jongh et al., 2006) during resting state and capillary recruitment (increment in the number of capillaries per unit of tissue area) during PORH response as well as after venous occlusion.

We believe that derangements in microvascular hemodynamics are the earliest sign of MD in obesity, preceding alterations in FCD. Since NVC assesses microvascular hemodynamic variables we have hypothesized that it could be the most appropriate videocapillaroscopic method to detect MD in obesity, before diagnosis of hypertension and T2DM. In order to investigate our hypothesis, we have used both

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videocapillaroscopic methodologies to evaluate microvascular function in normotensive glucose tolerant young obese women without other comorbidities and compared the results to ones obtained from age-matched lean control group.

Material and methods

Subjects

Obese women (n = 19) aged between 19 to 40 years were selected from the Unit Care Center for outpatients of the State University of Rio de Janeiro. Healthy volunteers (n = 18) were recruited through newspaper and internet advertisements. All participants were selected after clinical and laboratory assessments. For the obese group, the main inclusion criterion was body mass index (BMI) between 30.0–34.9 kg/m^2 and the main exclusion criteria were being on any nutritional or pharmacological intervention; the presence of chronic diseases (T2DM and hypertension) and more than 5% weight reduction six months before recruitment appointment. Lean women (control group) were in the normal BMI range (18.5–24.9 kg/m^2). The written informed consent was obtained from all participants; the study protocol was approved by the local Ethics Committee (CAAE: 0190.0.228.000-10) and performed according to principles outlined in the Declaration of Helsinki (Clinical Trials.gov registration no. NCT01692327).

Study design

This was a pilot study with cross-sectional design in which participants were subjected to a screening phase before being eligible to participate in the study. This phase comprised anthropometric measures and biochemical tests such as fasting glucose, insulin, total cholesterol (TC), triglycerides (TG) and HDL-c assessed after 12-hour overnight fast. A 75-g oral glucose tolerance test (OGTT) was also performed. Subjects presenting any degree of glucose intolerance according to the American Diabetes Association criteria (ADA, 2013) were excluded. In the day of the trial, participants arrived at the laboratory after 12-hour overnight fast and evaluations started after 30-min acclimatization period. Blood pressure was checked using the standard auscultatory method and after all procedures, volunteers were subjected to two different methods of videocapillaroscopy.

Laboratory analysis

In order to certify that obese subjects did not have any co-morbidity, after venous blood collection, samples were centrifuged for 10-min at 22 °C and 3000 rpm, for plasma glucose and serum TC, TG and HDL-c assessments using enzymatic colorimetric methods. For glucose analysis IECV (inter-assay coefficient of variation) and IACV (intra-assay coefficient of variation) were <5%, and for TC, TG and HDL-c were <8%. Oral glucose tolerance (OGTT) test was assessed by 75 g oral glucose for all patients. LDL-c concentration was calculated by Friedewald's equation (Friedewald et al., 1972).

Microvascular function assessment

NVC was carried out and analyzed according to a standardized, well-validated methodology on the 4th finger of the left hand to assess dynamic microvascular reactivity (Kraemer-Aguiar et al., 2010) and on the dorsal area of the 3rd finger (3 mm below nailfold bed) to assess dorsal finger capillaries (Buss et al., 2012). Both exams were carried out as previously reported (Kraemer-Aguiar et al., 2010; Maranhao et al., 2011; Buss et al., 2012).

NVC was performed to evaluate the following functional microvascular variables: FCD, number of capillaries with flowing red blood cells per unit of tissue area (mm^2) (Kraemer-Aguiar et al., 2008) at rest, red blood cell velocity before (RBCV) and during the post-occlusive reactive hyperemia (PORH) response after 1-min ischemia, ($RBCV_{max}$) and time to reach peak red blood cell velocity ($TRBCV_{max}$), with magnification of ×680. Before RBCV assessment on an individual capillary loop, a pressure cuff (1 cm wide) was placed around the proximal phalanx and connected to a mercury manometer. The percentage of $RBCV_{max}$ increment was calculated using the equation ($RBCV_{max}$ / RBCV × 100) − 100 (Kraemer-Aguiar et al., 2010).

Dorsal finger videocapillaroscopy was performed to evaluate FCD at rest (recorded during 2-min); after the cuff was inflated to 180 mm Hg during 4-min to evaluate it during PORH; and after 5-min at rest, the cuff was inflated to 60 mm Hg during 2-min to assess FCD after 2-min occlusion (FCD post-venous occlusion). The percentage of recruitment was calculated as previously described (de Jongh et al., 2006; Buss et al., 2012).

Statistical analysis

For statistical analysis, GraphPad Prism® 5 (GraphPad Software, Inc., USA) was used. Normal Gaussian distribution was checked using Shapiro–Wilk normality test. For parametric and non-parametric variables, data are expressed as mean ± SD and median [1st–3rd quartiles], respectively. Inter group comparisons for parametric and non-parametric variables were performed by unpaired t-test and Mann Whitney U test, respectively. In the pooled group, a multiple regression analysis was used to test whether some clinical–anthropometrical–laboratorial data have impact on microvascular function tested by both methods. Values of $p < 0.05$ were considered statistically significant.

Results

Baseline

The studied sample comprised of obese (n = 19) and healthy (n = 18) women, aged 30.8 ± 4.6 and 27.8 ± 6.2 years, respectively (non-statistically significant difference). As expected, obese women showed significantly higher body weight [82.2 ± 7.8 vs. 57.8 ± 6.2 kg; $p < 0.0001$]; BMI [32.2 ± 1.5 vs. 21.8 ± 1.8 kg/m^2; $p < 0.0001$]; waist circumference [104.2 ± 0.3 vs. 77.1 ± 5.5 cm; $p < 0.0001$]; systolic blood pressure (SBP) [117.8 ± 10.3 vs. 109.1 ± 7.5 mm Hg; $p < 0.01$]; diastolic blood pressure (DBP) [76.6 ± 8.5 vs. 69.1 ± 6.1 mm Hg; $p < 0.01$]; fasting glucose [85.0 ± 4.8 vs. 81.0 ± 6.0 mg/dl; $p < 0.05$]; insulin levels [12.8 ± 4.7 vs. 8.2 ± 4.2 mU/l; $p < 0.01$]; TC [195.9 ± 29.5 vs. 169.4 ± 32.2 mg/dl; $p < 0.05$] and TG [110.1 ± 55.9 vs. 73.7 ± 37.8 mg/dl; $p < 0.05$] compared to the control group.

Functional capillary density in obese and control groups

FCD was evaluated at baseline at the nailfold and the dorsum of the finger. Due to technical difficulties with videocapillaroscopy, during PORH only FCD at the dorsal site of the finger could be assessed. Furthermore, we were unable to evaluate microvascular parameters in one lean and two obese participants due to their skin phototype (amount of melanin pigment in the skin) IV or V, according to Fitzpatrick classification (Treu et al., 2011) that impaired the observation of the cutaneous microcirculation.

Videocapillaroscopic variables are presented on Table 1. As demonstrated, FCD at baseline, using both techniques, did not show any significant differences between controls and obese women. Using the dorsal finger technique, FCD during PORH and post-venous occlusion was not significantly different between groups. Similar results were obtained within group comparing FCD at rest, during PORH and post venous occlusion. Moreover, the percentage of capillary recruitment was not significantly different between groups. It was also possible to note that

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