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Short Communication

Evaluation of systemic microvascular endothelial function using laser speckle contrast imaging

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ABSTRACT

Objective: The aim of this study was to compare cutaneous microvascular function in young healthy subjects (n = 50) with that of cardiometabolic diseased patients (n = 50) using laser speckle contrast imaging (LSCI) coupled with transdermal iontophoretic delivery of acetylcholine (ACh) and post-occlusive reactive hyperemia (PORH).
Methods: Cutaneous blood flow was assessed in the forearm using LSCI at rest, during PORH and during iontophoresis of ACh with increasing anodal currents of 30, 60, 90, 120, 150 and 180 μA during 10-second intervals spaced 1 min apart.
Results: Endothelium-dependent skin microvascular vasodilator responses induced by both ACh and PORH were significantly reduced in cardiometabolic diseased patients compared to healthy subjects. Vasodilator responses induced by ACh were significantly higher in young women than in young men. Iontophoresis charges up to 1.5 mC do not induce nonspecific effects on skin microvascular flux.
Conclusion: LSCI appears to be a promising noninvasive technique for evaluating systemic microvascular endothelial function.

Introduction

The evaluation of microvascular endothelial function is essential for investigating the pathophysiology of cardiometabolic diseases, including arterial hypertension, diabetes, dyslipidemia and obesity (Struijker-Boudier et al., 2007). Moreover, in clinical research and practice, the study of microcirculation is of great importance in the assessment of the effects of medical interventions and the monitoring of disease progression. Laser speckle contrast imaging (LSCI) is a newly-developed noninvasive technique that allows for the continuous recording of skin microvascular blood flow (Rousseau et al., 2011; Roustit and Cracowski, 2011). It has already been shown that the reproducibility of LSCI coupled with post-occlusive reactive hyperemia (PORH) is superior to that of laser Doppler flowmetry (LDF) and laser Doppler imaging (Roustit et al., 2010; Tew et al., 2011). In the present study, we compared the cutaneous microvascular function of young healthy subjects to that of cardiometabolic diseased patients using LSCI coupled with physiological (PORH) or pharmacological (iontophoresis of acetylcholine) local vasodilator stimuli. The skin of the forearm was chosen as a recording site of systemic microvascular reactivity because it has been used in most studies that use LSCI in the evaluation of microvascular blood flow (Mahé et al., 2011; Millet e al., 2011; Rousseau et al., 2011; Roustit et al., 2010; Tew et al., 2011). Our primary aim was to test whether this technology could distinguish between cutaneous microvascular responses of patients with endothelial dysfunction and that of young normoglycemic individuals with normal plasma lipid levels. A secondary objective was to investigate the nonspecific effects of the electrical charges introduced during skin iontophoresis, a method used to enhance transdermal drug delivery, on microvascular responses.

Research design and methods

This cross-sectional study included 50 medical students and 50 outpatients with arterial hypertension and dyslipidemia who were treated at the National Institute of Cardiology, Rio de Janeiro, Brazil (the clinical characteristics are described in Table 1). The study was approved by the local ethics committee, and all participants gave written informed consent (protocol # 0345/24). Venous blood samples were obtained for the biochemical testing in the morning after twelve hour fasting and microcirculatory tests were performed after

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Table 1
Clinical characteristics of young healthy controls and dyslipidemic patients.

Characteristics	Healthy subjects (n = 50)	Dyslipidemic patients (n = 50)	*P* value
Age (years)	24.2 ± 0.46	62.2 ± 1.4	<0.0001
Male n (%)	25 (50)	26 (52)	0.84
Smokers n (%)	3 (6)	2 (4)	0.66
Diabetes n (%)	0 (0)	19 (38)	<0.0001
Weight (kg)	68.7 ± 1.9	73.9 ± 2.4	0.10
Body mass index (kg/m²)	23.5 ± 0.43	29.8 ± 1.7	0.0006
Waist circumference (cm)	82.5 ± 1.2	99.5 ± 1.9	<0.0001
Systolic blood pressure (mm Hg)	116.9 ± 1.4	138.5 ± 3.4	<0.0001
Diastolic blood pressure (mm Hg)	74.6 ± 1.1	84.6 ± 1.9	<0.0001
Mean arterial pressure (mm Hg)	88.3 ± 1.2	102.5 ± 2.2	<0.0001
Creatinine (mg/dl)	0.97 ± 0.03	0.98 ± 0.04	0.77
Urea (mg/dl)	28.2 ± 1.4	38.7 ± 1.9	0.0002
Total cholesterol (mg/dl)	170.9 ± 5.3	212.8 ± 8.6	0.0007
Triglycerides (mg/dl)	85.3 ± 3.7	200.7 ± 18.6	<0.0001
HDL-C (mg/dl)	51.6 ± 2.6	40.6 ± 1.6	0.0002
LDL-C (mg/dl)	102.5 ± 5.2	132.4 ± 7.2	0.0040
Glucose (mg/dl)	88.6 ± 1.3	121.4 ± 6.2	<0.0001

Results are expressed as mean ± SEM. HDL-C: high-density lipoprotein; LDL-C: low-density lipoprotein. *P* values were estimated by unpaired *t* tests or Chi-squared tests when appropriate.

a 20-minute rest in the supine position in a temperature-controlled room (23 ± 1 °C) approximately 1 h after a light breakfast. Patients took their usual medication on the morning of the tests. The brachial systolic (SAP) and diastolic (DAP) blood pressures were measured twice, 1 min apart, using a mercury sphygmomanometer, and the mean values were recorded as the patients' clinical blood pressure. Mean arterial pressure (MAP) was calculated as DAP + 1/3 (SAP-DAP). Microvascular reactivity was evaluated using an LSCI system with a laser wavelength of 785 nm (PeriCam PSI system, Perimed, Järfälla, Sweden) in combination with iontophoresis of acetylcholine (ACh) for noninvasive and continuous measurement of cutaneous microvascular perfusion changes (in arbitrary perfusion units, APU). The image acquisition rate was 8 images/s, and the distance between the laser head and the skin surface was fixed at 20 cm, as recommended by the manufacturer's manual. Images were analyzed using the manufacturer's software (PIMSoft, Perimed, Järfälla, Sweden). Two skin sites approximately 5 cm apart were randomly chosen on the ventral surface of the forearm avoiding hair, broken skin, areas of skin pigmentation and visible veins; and two drug-delivery electrodes were installed by means of adhesive discs (LI 611, Perimed, Järfälla, Sweden). Three measurement areas (circular regions of interest) of approximately 80 mm^2 were determined. Two of the measurement areas were within the electrodes (ACh, and H_2O), and the third (PORH) was adjacent to the electrodes. A vacuum cushion (AB Germa, Kristianstad, Sweden) was used to reduce recording artifacts generated by arm movements. ACh 2% w/v (Sigma Chemical CO, USA) or H_2O (as a control of current administration) iontophoresis was performed using a Microphamacology system (PF 751 Perilont USB Power Supply, Perimed, Sweden) with increasing anodal currents of 30, 60, 90, 120, 150 and 180 μA for 10-second intervals spaced 1 min apart (the total charges were 0.3, 0.6, 0.9, 1.2, 1.5 and 1.8 mC, respectively; Fig. 1). The dispersive electrode was attached approximately 15 cm away from the electrophoresis chamber. During the PORH test, arterial occlusion was performed with suprasystolic pressure (50 mm Hg above SAP) using a sphygmomanometer for 3 min. Following the release of pressure, the maximum flux was measured. Measurements of skin blood flux were divided by MAP to give cutaneous vascular conductance (CVC) in APU/mm Hg. The amplitude of the PORH responses was expressed as the peak CVC minus the baseline CVC. The results are presented as mean ± SEM and were analyzed using two-way repeated measures analysis of variance (ANOVA) followed by the Newman–Keuls multiple-comparison test or unpaired Student's *t* test when appropriate. *P* values <0.05 were considered statistically significant.

Results

Mean resting flux did not differ between control subjects and patients (Fig. 2). ACh iontophoresis in control subjects induced marked and current-dependent increases in CVC (with a maximum of 271.0 ± 24.8%; $P<0.001$) relative to the mean basal value of 0.20 ± 0.01 APU/mm Hg. Iontophoresis using only the solvent induced mild, but significant, effects at the highest current (13.3 ± 3.3%; $P<0.05$) relative to the basal values of 0.20 ± 0.01 APU/mm Hg. PORH induced a marked increase in CVC in control subjects, reaching a maximum of 241.3 ± 25.7% ($P<0.001$) compared to the basal values of 0.31 ± 0.02 APU/mm Hg. Additionally, CVC values measured during iontophoresis of ACh were significantly higher in female ($P<0.05$) than male control subjects in the range of iontophoresis currents from 90 to 180 μA (Fig. 2). However, PORH responses expressed as the peak CVC minus the baseline CVC were not significantly different between males and females (0.53 ± 0.04 and 0.57 ± 0.04 respectively, $P>0.05$). Patients' microvascular responses to both ACh and PORH were significantly reduced compared to healthy control subjects (Fig. 2). The maximum increases in CVC induced by ACh and PORH in patients were 143.7 ± 14.5 7% and 123.9 ± 7.4 7%, respectively ($P<0.001$ for both responses). As expected, the measured values of arterial pressure, blood glucose level, plasma lipid levels (total and LDL-cholesterol) and body mass index were significantly higher in arterial hypertension and dyslipidemia patients than control subjects. Plasma levels of HDL-cholesterol were lower in patients than in control subjects (Table 1).

Conclusions

The main findings of this study are as follows: (i) endothelium-dependent skin microvascular vasodilator responses investigated using LSCI coupled with ACh iontophoresis and PORH are significantly reduced in patients with cardiometabolic diseases compared to healthy subjects; (ii) skin vasodilator responses induced by ACh are significantly higher in young women than in young men; (iii) iontophoresis charges up to 1.5 mC do not induce nonspecific effects on skin microvascular flux.

Using the newly-developed LSCI technique, the present study confirmed that endothelium-dependent skin microvascular reactivity is reduced in patients with cardiometabolic diseases, as described previously with LDF (de Jongh et al., 2007). In addition, the obviously higher age of the patients, when compared to young healthy volunteers, undoubtedly contributed to the observed endothelial dysfunction. In fact, aging is well-known to be associated with progressive deterioration in endothelial function (Rajagopalan et al., 2002; Taddei et al., 2006). LSCI provides a microvascular perfusion measure in real time that is proportional to the average velocity of red blood cells (Boas and Dunn, 2010). An advantage of LSCI compared to LDF is that the blood flow response is measured over the whole area of drug delivery, thus reducing the variability of the measurements due to the spatial heterogeneity of the skin microvasculature (Roustit et al., 2010). In our experimental protocol of transdermal iontophoresis, a current-induced response was observed only at the highest charge of 1.8 mC. This nonspecific increase in blood flow has previously been attributed to membrane hyperpolarization, and pH changes in the iontophoretic chamber induced by the ions present in the vehicle (Ferrell et al., 2002). The responses of skin microcirculation to pharmacological interventions can also be assessed using microdialysis fiber insertion coupled either with LDF (Hodges et al., 2009) or LSCI (Cracowski et al., 2011). The main advantages of this procedure are that it is reproducible, provides two-dimensional

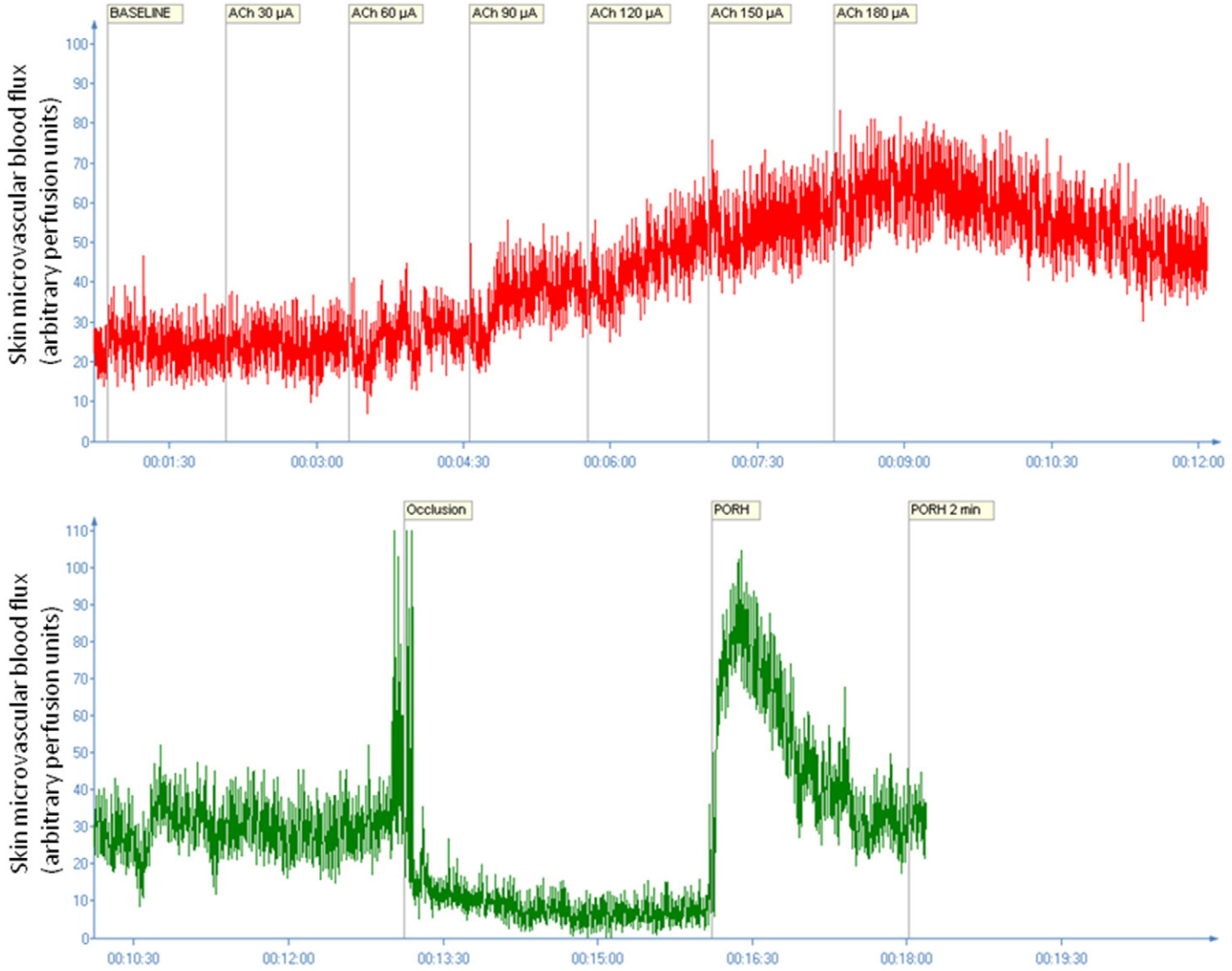

Fig. 1. Microvascular blood flux recorded with laser speckle contrast imaging during iontophoresis of ACh 2% w/v using increasing anodal currents of 30, 60, 90, 120, 150 and 180 µA during 10-second intervals spaced 1 min apart (A) and during post-occlusive reactive hyperemia (B).

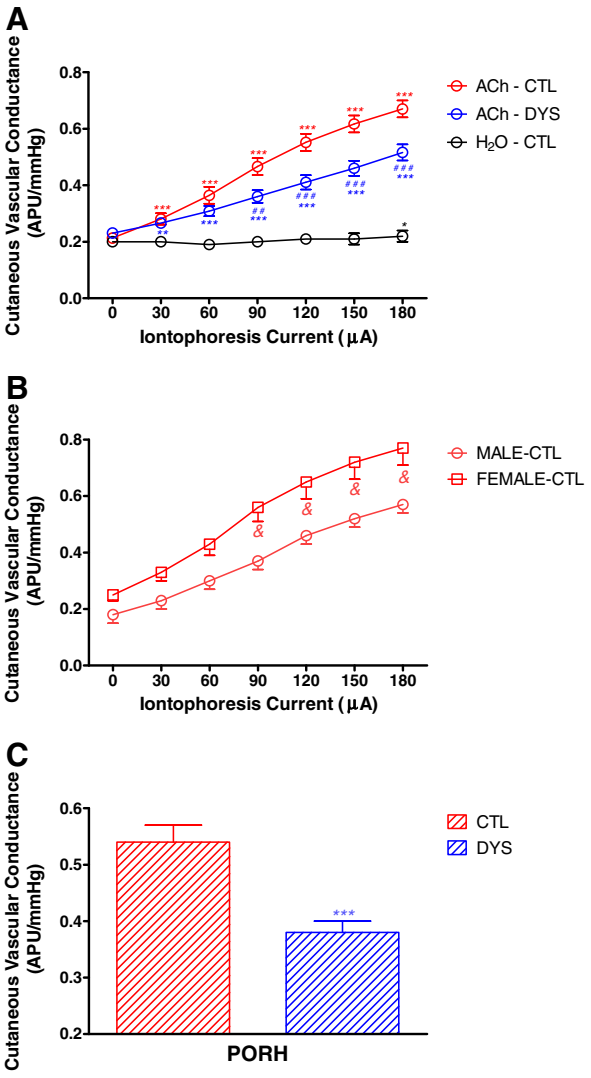

Fig. 2. (A) A comparison of the effects of cutaneous iontophoresis of acetylcholine (ACh) or distilled water (H_2O) on cutaneous microvascular conductance (CVC, expressed in arbitrary perfusion units, APU, divided by mean arterial pressure in mm Hg) of young healthy volunteers (CTL) or dyslipidemic patients (DYS) and (B) a comparison between healthy volunteers of both sexes; (C) a comparison of peak microvascular responses to post-occlusive reactive hyperemia (PORH) in healthy volunteers and patients. The amplitudes of PORH responses are expressed as peak CVC minus baseline CVC. The values are mean ± SEM. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ compared to basal values; $^{\#\#}P<0.01$ and $^{\#\#\#}P<0.001$ compared to ACh-CTL; $^{\&}P<0.05$ compared to MALE subjects.

assessment of the microcirculation and substances can be applied locally without systemic effects (Cracowski et al., 2011; Hodges et al., 2009). Although we did not account for the different stages of female subjects' menstrual phases, as most of them were taking oral contraceptives, we observed a gender-specific difference in the microvascular endothelial function of young healthy subjects. These results confirm the well-known beneficial effects of estrogen on cardiovascular functions, mainly through the enhancement of nitric oxide and endothelium-derived hyperpolarizing factor production (Huang and Kaley, 2004). In conclusion, laser speckle contrast imaging appears to be a promising noninvasive technique in the evaluation of systemic microvascular endothelial function. Nevertheless, several technical aspects related to the physiological and pharmacological reactivity tests require further investigation. Actually, the gender-related differences in microvascular reactivity were observed using transdermal iontophoretic delivery of acetylcholine but not during post-occlusive reactive hyperemia, suggesting the involvement of dissimilar physiological mechanisms in these different vasodilator stimuli.

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