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Assessment of microvascular endothelial function in type 1 diabetes using laser speckle contrast imaging

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ABSTRACT

Objective: To test whether laser speckle contrast imaging (LSCI) coupled with physiological post-occlusive reactive hyperemia (PORH) and pharmacological iontophoresis of acetylcholine (ACh) as local vasodilator stimuli could distinguish between cutaneous microvascular responses of Type 1 Diabetes (T1DM)'s patients with endothelial dysfunction and that of healthy controls.
Methods: Patients with T1DM aged ≥12 years completed a clinical-epidemiological questionnaire. Data detailing patients' such as daily insulin dose, duration of diabetes, and use of pharmaceuticals such as antihypertensive drugs and statins that could interfere with endothelial function were obtained. Vascular reactivity was assessed in the forearm by LSCI and PORH at baseline and during iontophoresis of ACh using increasing anodic currents of 30, 60, 90, 120, 150 and 180 μA in 10 second intervals.
Results: This study included 50 patients with T1DM and 30 control subjects. The mean resting flux did not differ between patients and control subjects. T1DM patients exhibited endothelial dysfunction upon challenge with physiological or pharmacological stimuli. The microvascular response to both ACh and PORH (i.e., maximum response at peak and amplitude) were significantly reduced in patients with diabetes compared with control subjects ($p < 0.001$).
Conclusion: We demonstrated that endothelium-dependent skin microvascular vasodilator responses are significantly impaired in patients with T1DM compared to healthy subjects investigated using LSCI coupled with ACh iontophoresis and PORH. Additionally, we find that LSCI is a promising methodology for studying physiological vascular reactivity in T1DM.

1. Introduction

Type 1 diabetes (T1DM) presents a substantial risk of early morbidity and mortality due to its associated micro- and macrovascular complications (Astrup et al., 2008; Bertoluci, Ce, da Silva, & Punales, 2008; Skrha, Prazny, Haas, Kvasnicka, & Kalvodova, 2001; Walsh, Zgibor, Borch-Johnsen, & Orchard, 2004). Endothelial dysfunction appears to be a common denominator in the pathophysiology of these chronic complications.

Due to the increasing relevance of long term cardiovascular risk prediction, there is growing interest in the use of non-invasive techniques to assess endothelial function (Cohn, Quyyumi, Hollenberg, & Jamerson, 2004; Vita & Keaney, 2002). Numerous techniques have potential for this application, and laser speckle contrast imaging (LSCI) has emerged as a promising noninvasive technique for the reproducible measurement of blood flow in tissue in real time. (Roustit, Millet, Blaise, Dufournet, & Cracowski, 2010).

In a previous study, we demonstrated that the endothelium-dependent pharmacological response to acetylcholine (ACh) was significantly reduced in T1DM patients compared to controls using laser Doppler flowmetry (LDF) (Matheus, Tibirica, da Silva, de Fatima Bevilacqua da Matta & Gomes, 2011). However, the results obtained by this technique are highly variable. Thus, we decided to assess the microvascular endothelial function of the T1DM population using LSCI, which offers excellent spatial and temporal resolution and better reproducibility (Boas & Dunn, 2010). Moreover, this new technique has not been tested on the T1DM population thus far.

In the present study, we aimed to test whether LSCI coupled with physiological post-occlusive reactive hyperemia (PORH) and pharmacological iontophoresis of acetylcholine (ACh) as local vasodilator stimuli could distinguish between cutaneous microvascular responses of T1DM patients with endothelial dysfunction and that of healthy controls.

Abbreviations: T1DM, type 1 diabetes; PORH, post-occlusive reactive hyperemia; AUC, area under the curve; Ach, acetylcholine; LDF, laser Doppler flowmetry; LSCI, laser speckle contrast imaging; ACE, angiotensin-converting enzyme; LSPU, laser speckle cutaneous arbitrary perfusion units.

Conflict of interest: None.

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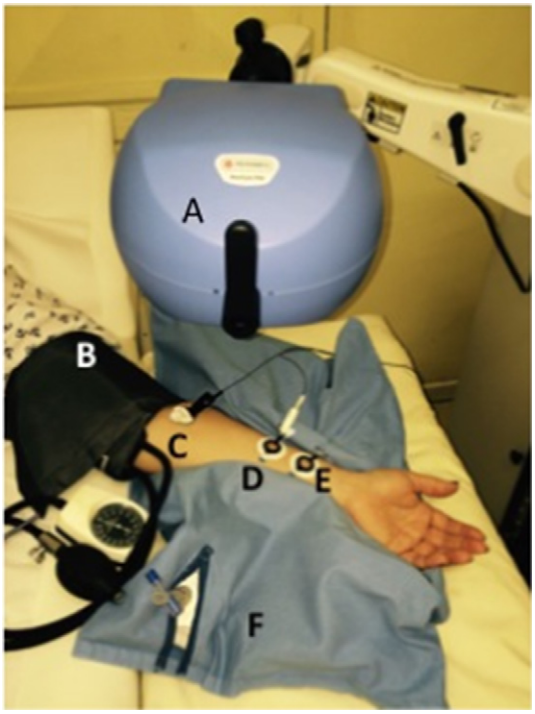

Fig. 1. Laser speckle contrast imaging studies skin microcirculation on the forearm. A, Laser head; B, Sphygmomanometer for occlusion; C, Electrode (−); D, Electrode (+) filled with Acetylcholine; E-Electrode control filled with NaCl; F, Vacuum cushion.

2. Material and methods

We conducted a cross-sectional study including 50 T1DM patients attending the diabetes outpatient unit at the University Hospital Pedro Ernesto and 30 age-, sex- and weight-matched healthy controls. The study was approved by the local ethics committee, and written informed consent was obtained prior to enrollment from all participants. Patients underwent a clinical evaluation of age at diagnosis, disease duration, daily insulin dose, blood pressure and body mass index (BMI, kg/m2), as well as their use of drugs that could interfere with endothelial function, such as angiotensin-converting enzyme (ACE) inhibitors, statins and angiotensin receptor blockers. To account for the potentially confounding effects of these medications, a dichotomous 'endothelium-interfering drug' variable was created (i.e., use vs. no use) as the only categorical variable.

Inclusion criteria for healthy volunteers were an age of 12 years or older and no significant medical history or use of any drug. The inclusion criteria for T1DM patients were an age of 12 years or older and a duration of diabetes greater than 1 year. For all subjects, exclusion criteria included dermatological disease affecting the arms and the consumption of tobacco or caffeine, the performance of physical exercise, or any hypoglycemic event on the morning of the exam.

A microvascular assessment was performed in the morning after a 20-minute rest period in the supine position in a temperature-controlled room (23 ± 1 °C) approximately 1 hour after a light breakfast, such as a glass of milk and a piece of bread with light cheese.

The brachial systolic arterial (SAP) and diastolic arterial (DAP) blood pressures were measured twice, 1 min apart, using an electronic sphygmomanometer (ONROM), 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 at a laser wavelength of 785 nm (PeriCam PSI system, Perimed, Järfälla, Sweden) in combination with iontophoresis of ACh for noninvasive and continuous measurement of changes in laser speckle cutaneous microvascular perfusion units (LSPU) coupled with PORH. The image acquisition rate was 1 image/s, and the distance between the laser head and the skin surface was fixed at 20 cm, because it has been documented that the distance between 10 and 30 cm could not influence on skin blood flow recordings at rest or at peak's microvascular responses. (Mahe, Humeau-Heurtier, Durand, Leftheriotis, & Abraham, 2012) The resolution was 0.53 mm, and the resulting images were 10 × 7 cm in size. The manufacturer's software (PIMSoft, Perimed, Järfälla, Sweden) was used to analyze the images. Two skin sites approximately 5 cm apart were randomly chosen on the ventral surface of the left forearm, avoiding hair, broken skin, areas of skin pigmentation and visible veins; 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 15 mm diameter were chosen. Two of the measurement areas encompassed the electrodes (ACh, and NaCl), and the third (PORH) was adjacent to the electrodes. A vacuum cushion (AB Germa, Kristianstad, Sweden) was used to avoid recording artifacts associated with arm movements (Fig. 1).

Cutaneous basal blood flow was measured for 3 minutes, after which iontophoresis was performed with ACh 2% *w/v* (Sigma Chemical CO, USA) or NaCl (to control for current administration) using a Micropharmacology system (PF 751 Perilont USB Power Supply, Perimed, Sweden) with increasing anodal currents of 30, 60, 90, 120, 150 and 180 μA in 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). The dispersive electrode was attached approximately 10 cm away from the electrophoresis chamber. After iontophoresis, blood flow

Table 1
Clinical characteristics of Type 1 diabetes patients and young healthy controls.

	Type 1 diabetes	Controls	p value
Age (years)	32.8 ± 13	26.2 ± 5	0.01
Female (%)	29 (58)	18 (60)	0.86
Weight (kg)	65.6 ± 12.4	70.6 ± 15.4	0.11
BMI (kg/m^2)	24.1 ± 4	24.5 ± 4	0.66
Systolic blood pressure (mmHg)	125 ± 15	117 ± 10	0.02
Diastolic blood pressure (mmHg)	74 ± 10	71 ± 6	0.09
Mean arterial pressure (mmHg)	91 ± 10	86 ± 6	0.02
Duration of type 1 diabetes (years)	16 ± 11		
Daily insulin dose (UI/kg/day)	0.7 ± 0.2		
Age at diagnosis (years)	16 ± 9		
A1c: diabetes group (%)	9.4 ± 2.4		
Cutaneous temperature (°C)	23.0 ± 0.7	22.9 ± 0.9	0.42

Data are expressed in mean ± SD.

Table 2
Microvascular reactivity of the studied population.

	Type 1 DM	Controls	p value
PORH Baseline (LSPU)	36.93 ± 8.1	37.72 ± 7.6	0.66
Baseline CVC (LSPU/mmHg)	0.40 ± 0.09	0.43 ± 0.08	0.13
Peak (LSPU)	76.15 ± 23.6	94.20 ± 28.81	0.004*
Peak CVC (LSPU/mmHg)	0.83 ± 0.26	1.08 ± 0.33	<0.001*
Amplitude	0.42 ± 0.21	0.65 ± 0.31	<0.001*
ACh Baseline (LSPU)	30.12 ± 7	30.19 ± 5	0.96
Baseline CVC (LSPU/mmHg)	0.32 ± 0.07	0.31 ± 0.12	0.44
Peak (LSPU)	63.45 ± 18.86	78.40 ± 16.3	0.001
Peak CVC (LSPU/mmHg)	0.70 ± 0.22	0.90 ± 0.21	<0.001
Amplitude	0.36 ± 0.20	0.55 ± 0.19	<0.001

PORH: Post occlusive reactive hyperemia; ACh: Acetylcholine; CVC: Cutaneous vascular conductance; Amplitude Peak CVC - Basal CVC: LSPU: laser speckle cutaneous arbitrary perfusion units.

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