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ORIGINAL ARTICLE

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Evaluation of systemic microvascular reactivity in adults with congenital heart disease

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Abstract

Objective: Adults with congenital heart disease share some features with those with chronic heart failure. Although microvascular endothelial dysfunction has been described in chronic heart failure, evaluation of the microcirculation in adults with congenital heart disease is lacking. The present study aimed to investigate systemic microvascular reactivity in adults with congenital heart disease.

Interventions: The patients initially underwent cardiopulmonary exercise testing. Then, the cutaneous microvascular reactivity was evaluated in these patients using a laser speckle contrast imaging system coupled with skin iontophoresis of endothelial-dependent (acetylcholine) or -independent (sodium nitroprusside) vasodilators and postocclusive reactive hyperemia (PORH) and compared with healthy controls matched for age and sex.

Results: Thirty-one patients and 29 healthy controls were evaluated. The basal microvascular flow ($P < .0001$) and area under the curve in response to acetylcholine ($P < .0001$) were higher in the patients than in the healthy volunteers. The increase in cutaneous vascular conductance in response to sodium nitroprusside was reduced in the patients compared to the healthy volunteers ($P = .0031$). No difference in the microvascular response was observed during postocclusive reactive hyperemia. The basal microvascular flow of patients with peak oxygen consumption below 16.0 mL kg^{-1} min^{-1} was superior to that of patients with values greater than 16.0 mL kg^{-1} min^{-1} ($P = .0046$).

Conclusions: Adults with congenital heart disease present a higher baseline cutaneous microvascular blood flow than healthy controls and do not present systemic microvascular endothelial dysfunction. Nevertheless, endothelium-independent microvascular reactivity is blunted, suggesting an altered vascular smooth muscle response or vascular structural alterations. Finally, patients with a lower functional capacity presented a greater microvascular basal blood flow than subjects with a higher functional capacity.

KEYWORDS

congenital, endothelium, heart defects, laser speckle contrast imaging, microcirculation, microvascular reactivity

Abbreviations: Ach, acetylcholine; ACHD, adults with congenital heart disease; APU, arbitrary perfusion units; BNP, B-type natriuretic peptide; CVC, cutaneous vascular conductance; HF, heart failure; IRB, institutional review board; LDF, laser Doppler flowmetry; LSCI, laser speckle contrast imaging; PAT, peripheral arterial tonometry; PORH, postocclusive reactive hyperemia; SD, standard deviation; SNP, sodium nitroprusside; $V'O_2$, oxygen consumption.

1 | INTRODUCTION

The prevalence of congenital heart disease has increased over the past five decades due to significant progress in the treatment of these pathologies in childhood.[1] At present, congenital heart disease affects more than 1 000 000 adults in the United States.[2,3] The affected individuals have a lower functional capacity[4–7] and worse prognosis[8–14] than healthy individuals of the same age group and usually evolve with heart failure (HF), which is the main cause of cardiovascular mortality in this population.[15]

Congenital heart disease in adults shares some characteristics with HF caused by other etiologies, including effort intolerance,[4–7] ventricular dysfunction (left or right),[16] cardiac arrhythmia,[17] myocardial fibrosis,[18] ventilatory inefficiency,[1,11,19] increased inflammatory cytokine levels,[20] and neurohormonal activation.[21] Among the pathophysiological changes in HF, endothelial dysfunction is highlighted and is present even in the early stages.[22] However, the presence of endothelial dysfunction in patients with congenital heart disease is controversial because some studies have demonstrated the occurrence of endothelial dysfunction in these patients,[23–27] whereas other studies have not confirmed this condition.[28–31] Research in individuals with HF has shown that these individuals present compromised systemic endothelial function of the cutaneous microcirculation.[32–34] In contrast, the literature on the endothelial function of the cutaneous microcirculation of adults with congenital heart disease (ACHD) is limited, and the results are conflicting.[23,25,26,28] Indeed, cutaneous microcirculation in individuals with congenital heart disease was investigated in only one study.[35]

Considering that endothelial dysfunction may be one cause of effort intolerance in patients with HF[36,37] and ACHD,[27] aerobic training may represent an effective therapeutic option, as has been shown for HF.[38] In addition, the presence of microvascular endothelial dysfunction may allow identification of ACHD with an unfavorable prognosis similar to HF, in which this prognostic condition is associated with higher morbidity and mortality.[39]

Laser speckle contrast imaging (LSCI) is a newly developed noninvasive technique that allows continuous recording of the skin microvascular blood flow.[40] Additionally, cutaneous microvascular reactivity has been correlated with microvascular function in different vascular beds both in intensity and regarding the underlying pathophysiological mechanisms.[41]

The primary objective of this study is to evaluate the systemic microvascular endothelial function in ACHD. The secondary objective is to investigate endothelium-independent microvascular reactivity, which represents the function of vascular smooth muscle and structural changes in the microvasculature.

2 | METHODS

This observational, cross-sectional study evaluated 31 adult patients with congenital heart disease (aged >18 years) who were acyanotic or cyanotic and were treated clinically, surgically, or percutaneously and 29 healthy volunteers (reference group for microcirculatory parameters). The patients were recruited from April 2016 to August 2017. The group of healthy individuals originated from an earlier study by our research group.[42] This study was approved by the Institutional Review Board (IRB) of our institution under Protocol No. 47563315.2.0000.5272. The patients were informed of the nature of the study and signed the informed consent form previously approved by the IRB. This study complied with the guidelines of the World Health Organization and the Declaration of Helsinki. The exclusion criteria were patients aged <18 years, refusal to sign the informed consent form, and a diagnosis of diabetes mellitus.

3 | CARDIOPULMONARY EXERCISE TEST

The study patients underwent a cardiopulmonary exercise test to assess their aerobic function, degree of functional limitation, and clinical severity. A maximum exercise test (limited by symptoms) was performed always between 8 and 12 AM. on a treadmill (Inbramed, Porto Alegre, RS, Brazil) with a ramp protocol and an expected duration of 8 to 12 min. Patients were encouraged to continue the effort to exhaustion. For gas measurement, the patients had their noses sealed with a clip and used a mouthpiece with a saliva collector coupled to a pneumotachograph; this device was connected to a gas analysis transducer (model VO2000; MedGraphics, St Paul, Minnesota). The device was connected to a computer running the Ergo PC Elite software (Micromed, Brasília, Federal District, Brazil), which measured breath-to-breath data using the means obtained at 20-s intervals. The oxygen consumption ($V'O_2$) peak was considered the highest value identified over the past 20 s of exercise or the first measurement of recovery. The patients were classified according to Weber et al into four classes by the $V'O_2$ peak values determined by direct measurement as follows: A—>20.0 mL kg^{-1} min^{-1}; B—16.0-20.0 mL kg^{-1} min^{-1}; C—10.0-15.9 mL kg^{-1} min^{-1}; and D—<10.0 mL kg^{-1} min^{-1}.[43]

4 | CUTANEOUS MICROVASCULAR REACTIVITY

The microcirculatory tests were performed after a 20-minute rest with the patients in a supine position in an environment with a controlled temperature (23 ± 1°C) approximately 60 minute after a light breakfast, according to a previously validated standard experimental protocol.[44–47] Endothelium-dependent microvascular reactivity was evaluated using a recently standardized and validated LSCI laser system[47] at a wavelength of 785 nm (PeriCam PSI system, Perimed, Järfälla, Sweden) combined with iontophoresis of acetylcholine (ACh) for the continuous and noninvasive measurement of cutaneous microvascular perfusion in arbitrary perfusion units (APUs). Endothelial-independent cutaneous microvascular reactivity was analyzed using the same system but combined with sodium nitroprusside (SNP) iontophoresis. The image acquisition rate was

eight images per second, and the distance between the origin of the laser and the skin surface was set at 20 cm as recommended by the manufacturer. The images were analyzed using PIM-Soft software (Perimed). Two areas of the skin on the ventral surface of the forearm (separated by 5 cm) were randomly chosen to record the cutaneous blood flow. Areas with excess pigmentation, excess hair, visible blood vessels, or any lesion were avoided, and two microiontophoresis electrodes were glued with double-sided adhesive (LI611, Perimed). Three areas (circular regions of interest) of approximately 80 mm^2 were measured. Two of these areas were located inside the electrode area (for measurement of ACh and SNP), and the third area was located adjacent to the electrode to measure postocclusive reactive hyperemia (PORH). A vacuum cushion (a specially constructed cushion filled with polyurethane foam and shaped to any desired shape by creating a vacuum)(AB Germa, Kristianstad, Sweden) was used to limit the possible generation of noise in the recordings by movement of the upper limbs. After 5-minute recordings of the baseline microvascular blood flow were collected, iontophoresis with 2% ACh w/v or 2% SNP w/v (Sigma Chemical Co., St. Louis, Missouri) was performed using a micropharmacological system (PF 751 Perilont USB Power Supply, Perimed) with anode currents (ACh) or cathode currents (SNP) of increasing intensities of 30, 60, 90, 120, 150, and 180 μA that were applied for 10 s and separated from each other by 60-s intervals. The total loads were 0.3, 0.6, 0.9, 1.2, 1.5, and 1.8 mC, respectively. For current dispersion, the neutral electrode was installed 15 cm above the infusion electrodes. The protocol did not include the administration of injectable drugs, and the drugs mentioned above were maintained in contact only with the epidermis of the volunteers. For the PORH tests, brachial artery occlusion was performed using a pneumatic cuff inflated at suprasystolic pressure (50 mm Hg above the systolic pressure of the volunteers) for 3 minutes. Maximum cutaneous blood flow was measured after release of the cuff. Blood pressure (systolic, diastolic, and mean) and heart rate were determined using an automatic oscillometer (Dinamap PRO 100, General Electric, Jackson, Tennessee) with a cuff of appropriate size. The cutaneous blood flow values were divided by the mean arterial pressure to determine the cutaneous vascular conductance (CVC) in APU/mm Hg. The following parameters were evaluated: area under the curve, in APU/s; CVC peak, in APU/mm Hg; and amplitude (or increase) of the CVC, in APU/mm Hg. The latter measurement was defined as the difference between the peak and baseline CVC. The area under the curve is automatically calculated

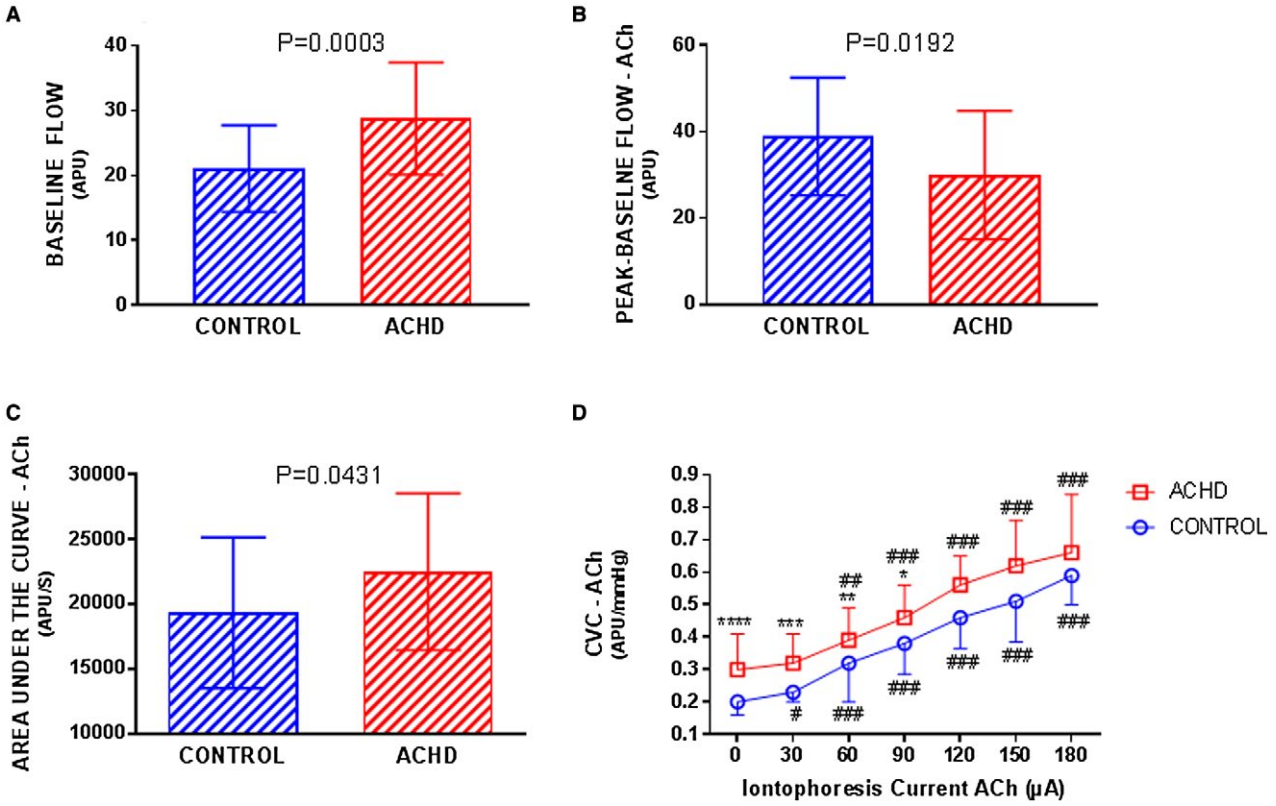

FIGURE 1 Baseline microvascular flow (A), peak-baseline values of microvascular flow (B), area under the curve for acetylcholine (ACh) iontophoresis (C), and cumulative effects of ACh iontophoresis (D) on the cutaneous microcirculation of healthy volunteers (CONTROL, $n = 29$) and adult patients with congenital heart disease (ACHD, $n = 31$). Values represent the means ± SDs. P values were calculated using the two-tailed unpaired Student's t test (Figures 1A, 1B and 1C). $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$, and $^{****}P < .0001$ compared to the CONTROL, using the two-tailed unpaired Student's t test; $^{\#}P < .05$; $^{\#\#}P < .01$, and $^{\#\#\#}P < .001$ compared to the baseline values, using repeated-measures ANOVA followed by the Newman-Keuls multiple comparisons test (Figure 1D). Abbreviations: APU, arbitrary perfusion units; CVC, cutaneous vascular conductance [Colour figure can be viewed at wileyonlinelibrary.com]

by the PIM-Soft software. It represents the integral response of flow values versus the dose of ACh or SNP and is calculated as the area situated between the flow curve and the x-axis. For instance, in Figure 1C, the area under the curve is the area below the dose-response curve (y-axis) and the dose (x-axis).

5 | STATISTICAL ANALYSIS

We have performed a sample size calculation to confirm that the number of patients included in the study would be appropriate to verify potential statistical differences between groups. Sample size calculation was performed using GPower (version 3.0.10, University of Kiel, Kiel, Germany), based on the difference between groups for microvascular responses to acetylcholine of 0.11 APU/mm Hg (cutaneous microvascular conductance), with a standard deviation of 0.17 APU/mm Hg.[44] Assuming 70% of power and 5% significance level, a minimum of 29 patients in each group was necessary.

The normality of the distributions of data was assessed using the Shapiro-Wilk test; according to the results of the test, the data are presented as the mean ± standard deviation (SD), for values with parametric (normal) distribution, or the median (interquartile range), for values with nonparametric (nonnormal) distribution. Comparisons between groups were made using a two-tailed unpaired Student's *t* test (for data with a normal distribution) or the Mann-Whitney test (for data with a nonnormal distribution). Categorical variables were compared using Fisher's exact test. The null hypothesis was rejected at $P < .05$. The ACh curves were analyzed using repeated-measures ANOVA followed by the Newman-Keuls multiple comparisons test (parametric data). The SNP curves were analyzed using Friedman's test followed by Dunn's multiple comparisons test (for nonparametric data). Prism, version 7.0 (GraphPad Software Inc, La Jolla, California), was used for the statistical analysis.

6 | RESULTS

6.1 | Clinical characteristics and medications

Thirty-one adults with congenital heart disease aged 34.0 (22.0-48.0) years were evaluated, including 17 women (54.84%) and 14 men (45.16%) (Table 1). The control group consisted of 29 healthy volunteers aged 41.5 (34.7-44.0) years ($P = .0781$ vs. patients), including 17 women and 12 men ($P = .7997$ vs. patients).

The most common pathology in the study sample was tetralogy of Fallot, with nine cases (29.03%). A total of 27 acyanotic patients (87.1%) and 4 cyanotic patients (12.9%) were analyzed (Table 1). Twenty-four patients (77.4%) underwent surgical treatment, one patient (3.2%) was subjected to percutaneous treatment only, and the remaining six patients (19.4%) underwent clinical treatment with no history of previous interventions. The most common associated comorbidities were systemic arterial hypertension in three patients (9.67%), dyslipidemia in two patients (6.45%), hyperuricemia in two patients (6.45%), coronary artery disease in one patient (3.22%), and hypothyroidism in one patient (3.22%). The clinical characteristics of the patients are shown in Table 1, and the drugs used are described in Table 2.

TABLE 1 Clinical characteristics of the patients

Characteristics	*n*	%
Female/male	17/14	54.84/45.16
Age (years)	34.0 (22.0–48.0)	–
Acyanotic/cyanotic	27/4	87.09/12.91
Main diagnosis		
Tetralogy of Fallot	9	29.03
Single ventricle	4	12.90
VSD	3	9.67
Ebstein's anomaly	2	6.45
ASD	2	6.45
TGA	2	6.45
Noncompacted myocardium	1	3.22
Tricuspid insufficiency	1	3.22
Tricuspid stenosis	1	3.22
Pulmonary stenosis	1	3.22
Coarctation of the aorta	1	3.22
Truncus arteriosus	1	3.22
TGA cc	1	3.22
CASD	1	3.22
ALCAPA	1	3.22

Abbreviations: VSD, ventricular septal defect; ASD, atrial septal defect; TGA, transposition of the great arteries; TGAcc, congenitally corrected transposition of the great arteries; CASD, complete atrioventricular septal defect; ALCAPA, anomalous origin of the left coronary artery from the pulmonary artery.

6.2 | Cardiopulmonary exercise test

The peak $V'O_2$ was 19.13 ± 1.33 mL/kg/min, corresponding to 44.86% ± 18.01% of the predicted maximum $V'O_2$ for age and gender according to the prediction equation of Jones and Campbell.[48] The patients were grouped according to the classification of Weber et al into classes A and B or C and D.[43] When the difference in the maximum aerobic power between groups was considered, the peak $V'O_2$ was 18.75 (17.56-28.64) mL/kg/min in the 17 patients from classes A and B and 10.86 (9.55-15.12) mL/kg/min in the 14 patients from classes C and D ($P < .0001$). The cardiovascular parameters measured during cardiopulmonary exercise tests are described in Table 3.

6.3 | Cutaneous microvascular reactivity

The absolute values of the microvascular parameters, before and after pharmacological or physiological stimulation, as well as arterial blood pressure values obtained during microvascular evaluation,

are described in Table 4 and Figures 1 and 2. The table shows that there are prominent statistically significant differences in the basal microvascular parameters, as well as in the vasodilator responses to pharmacological and physiological stimulation, between healthy controls and patients. Moreover, arterial blood pressure was significantly lower in ACHD patients, compared to healthy controls.

The baseline CVC was 0.32 ± 0.02 APU/mm Hg in the group of patients and 0.22 ± 0.01 APU/mm Hg in the control group ($P < .0001$). In response to ACh, the area under the curve was 22 500 ± 1086 APU/s in the group of patients and 19 331 ± 1078 APU/s in the control group ($P = .0431$) (Figure 1C). The CVC amplitude was 0.34 ± 0.03 APU/mm Hg in the group of patients and 0.39 ± 0.03 APU/mm Hg in the control group ($P = .1779$), and the CVC peak was 0.66 ± 0.03 APU/mm Hg in the group of patients and 0.61 ± 0.03 APU/mm Hg in the control group ($P = .3507$).

The responses to increasing doses of ACh in the intervention and control groups starting at baseline are shown in Figure 1D. The curves are practically parallel; the largest difference between the groups was in the baseline blood flow, and this difference was significant up to a current of 90 μA. Relative to baseline, the difference was significant beginning at 60 μA in the group of patients and 30 μA in the control group.

In response to PORH, the CVC peak was 0.78 ± 0.03 APU/mm Hg in the group of patients and 0.76 ± 0.03 APU/mm Hg in the control group ($P = .5797$), whereas the CVC amplitude was 0.44 ± 0.03 APU/mm Hg in the group of patients and 0.48 ± 0.03 APU/mm Hg in the control group ($P = .3734$).

The responses to SNP iontophoresis were available for operational reasons for only 24 patients (77.42%). The area under the curve for the SNP stimulus was 17 810 ± 943 APU/s in the group of patients and 15 760 ± 893 APU/s in the control group ($P = .1219$) (Figure 2C). The CVC amplitude was 0.18 ± 0.02 APU/mm Hg in the group of patients and 0.31 ± 0.03 APU/mm Hg in the control group ($P = .0031$), and the CVC peak was 0.46 (0.37-0.61) APU/mm Hg in the group of patients and 0.51 (0.39-0.67) APU/mm Hg in the control group ($P = .5857$).

The responses to progressive doses of SNP starting at baseline are shown in Figure 2D. Similar to the response curve for ACh, the difference between the two groups starting at baseline remained significant up to a current of 90 μA. Relative to baseline, the difference was significant starting at 120 μA in both groups.

The difference in the area under the curve between the two groups in response to ACh iontophoresis was maintained even when the four cyanotic individuals were excluded from the analysis and the 27 acyanotic patients were compared with the control group (22 630 ± 1201 APU/s in the acyanotic patients and 19 330 ± 1078 APU/s in the controls, $P = .454$). A similar result was observed when the five patients taking sildenafil were excluded from the analysis. The area under the curve in response to ACh iontophoresis considering only the 26 patients not using this vasodilator was 22 680 ± 1253 APU/s in the intervention group and 19 330 ± 1078 APU/s in the control group ($P = .0466$).

The division of the patients according to Weber's classification into functional classes A and B with a $V'O_2$ peak ≥16.0 mL.kg^{-1}.min^{-1} or classes C and D with a $V'O_2$ peak <16.0 mL.kg^{-1}.min^{-1} indicated that the patients with lower maximum aerobic power (classes C and D) had a comparatively higher baseline CVC (0.38 ± 0.3 APU/mm Hg vs. 0.27 ± 0.22 APU/mm Hg, $P = .0046$) (Figure 3A) and a tendency to a larger area under the curve in response to ACh iontophoresis (20 620 ± 1124 APU/s in classes A and B vs. 24 780 ± 1 124 APU/s in classes C and D, $P = .0554$) (Figure 3B).

TABLE 2 Medications used by the patients

Medications	*n*	%
Beta-blockers	19	61.29
Diuretic	15	48.38
ACEI	12	38.71
ARB	8	25.81
Warfarin	7	22.58
Sildenafil	5	16.13
Folic acid	4	12.90
Ferrous sulfate	4	12.90
AAS	3	9.68
Levothyroxine	3	9.68
Digoxin	2	6.45
Amiodarone	2	6.45
Amlodipine	2	6.45
Statin	2	6.45
Allopurinol	2	6.45
Prednisone	1	3.22
Trimetazidine	1	3.22
Metformin	1	3.22

Abbreviations: ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; ASA, acetylsalicylic acid.

TABLE 3 Cardiovascular parameters of adult patients with congenital heart disease (*n* = 31) during the cardiopulmonary exercise test

Hemodynamic parameter	
Resting systolic arterial pressure (mm Hg)	114.1 ± 17.5
Peak systolic arterial pressure (mm Hg)	145.2 ± 26.6
Resting heart rate (bpm)	76.3 ± 13.4
Peak heart rate (bpm)	143.1 ± 37.4

The results are presented as the mean ± SD.

TABLE 4 Microvascular data and arterial blood pressure of healthy volunteers (CONTROL, *n* = 29) and adult patients with congenital heart disease (ACHD, *n* = 31 for ACh and PORH data; *n* = 24 for SNP data)

	Control	ACHD	*P* value
Systolic arterial pressure (mm Hg)	128.7 ± 139	122.0 ± 18.6	.1026
Diastolic arterial pressure (mm Hg)	80 (75–86)	75.2 ± 9.9	.0074
Mean arterial pressure (mm Hg)	97 (93–103)	90.8 ± 11.9	.0027
Microvascular flow before ACh iontophoresis (APU)	21.1 ± 6.7	28.8 ± 8.6	.0003
Peak microvascular flow after ACh iontophoresis (APU)	59.9 ± 17.2	58.7 ± 16.4	.7849
Variation of microvascular flow (peak-baseline) after ACh iontophoresis (APU)	38.9 ± 13.6	29.9 ± 14.8	.0192
Microvascular flow before SNP iontophoresis (APU)	21.7 ± 6.5	29.2 ± 7.6	.0009
Peak microvascular flow after SNP iontophoresis (APU)	52.3 ± 19.3	41 (36–54)	.1792
Variation of microvascular flow (peak-baseline) after SNP iontophoresis (APU)	30.5 ± 15.5	16.6 ± 10.3	.0019
Microvascular flow before PORH (APU)	27.3 ± 5.5	30.4 ± 6.9	.0643
Peak microvascular flow after PORH (APU)	74.2 ± 16.4	69.7 ± 13.9	.2550
Variation of microvascular flow (peak-baseline) after PORH (APU)	46.7 ± 14.9	39.3 ± 12.7	.0395

Abbreviations: ACh, acetylcholine; APU, arbitrary perfusion units; PORH, postocclusive reactive hyperemia; SNP, sodium nitroprusside.
The assessment of the normality of data was performed using the Shapiro-Wilk Normality test. The results of parametric data are presented as the mean ± SD and analyzed using the two-tailed unpaired Student's *t* test. For nonparametric data, the results are presented as the median (interquartile range) and analyzed using the Mann-Whitney test. The analyses of parameters that include both parametric and nonparametric data (CONTROL vs. ACHD) were performed using the nonparametric test (Mann-Whitney).

The comparison of the patients' responses according to gender indicated an area under the curve of 24 227 ± 1561 APU/s in the female patients and 20 404 ± 1329 APU/s in the male patients (*P* = .0794).

7 | DISCUSSION

The results of the cutaneous microvascular reactivity analysis in ACHD did not confirm the initial hypothesis that these individuals presented microcirculatory endothelial dysfunction. The vasodilatory response to iontophoresis with ACh in ACHD was similar to that in healthy subjects because no significant differences were found in the amplitude and maximal CVC value after transdermal administration of ACh. In contrast, the areas under the curve for ACh-induced vasodilation were higher in ACHD than in the controls, possibly because the baseline microvascular blood flow was increased in the group of patients in combination with a sustained response to ACh. No significant difference in the response to PORH was observed between the ACHD and the healthy volunteers. In contrast, with respect to the response to SNP iontophoresis, the healthy controls demonstrated a stronger vasodilatory response than the ACHD with a higher CVC amplitude, suggesting that ACHD had a lower microvascular smooth muscle-dependent vasodilator response than healthy individuals; this result is similar to that observed in patients with coronary artery disease[42] and HF.[32,33] Alternatively, ACHD may present microvascular structural changes, including vascular smooth muscle hypertrophy, which in turn may reduce the endothelium-independent microcirculatory vasodilation.

The results of studies on HF using a methodology similar to ours, including laser Doppler flowmetry (LDF) and iontophoresis with ACh, were different from our study results. The reactivity of cutaneous microcirculation was lower in patients with HF than in healthy individuals.[32–34] Additionally, patients with HF required only nitrate suspension for 6 hours before the intervention. Although none of the evaluated participants of the present study used nitrates, five patients (16.13%) used sildenafil. However, the responses of these patients to the ACh stimulus were not significantly changed even when they were excluded from the analysis.

The results available in the literature on the microvascular endothelial function of ACHD are conflicting. Cordina et al evaluated the vascular reactivity of individuals with cyanotic congenital heart disease and reported the presence of endothelial dysfunction when

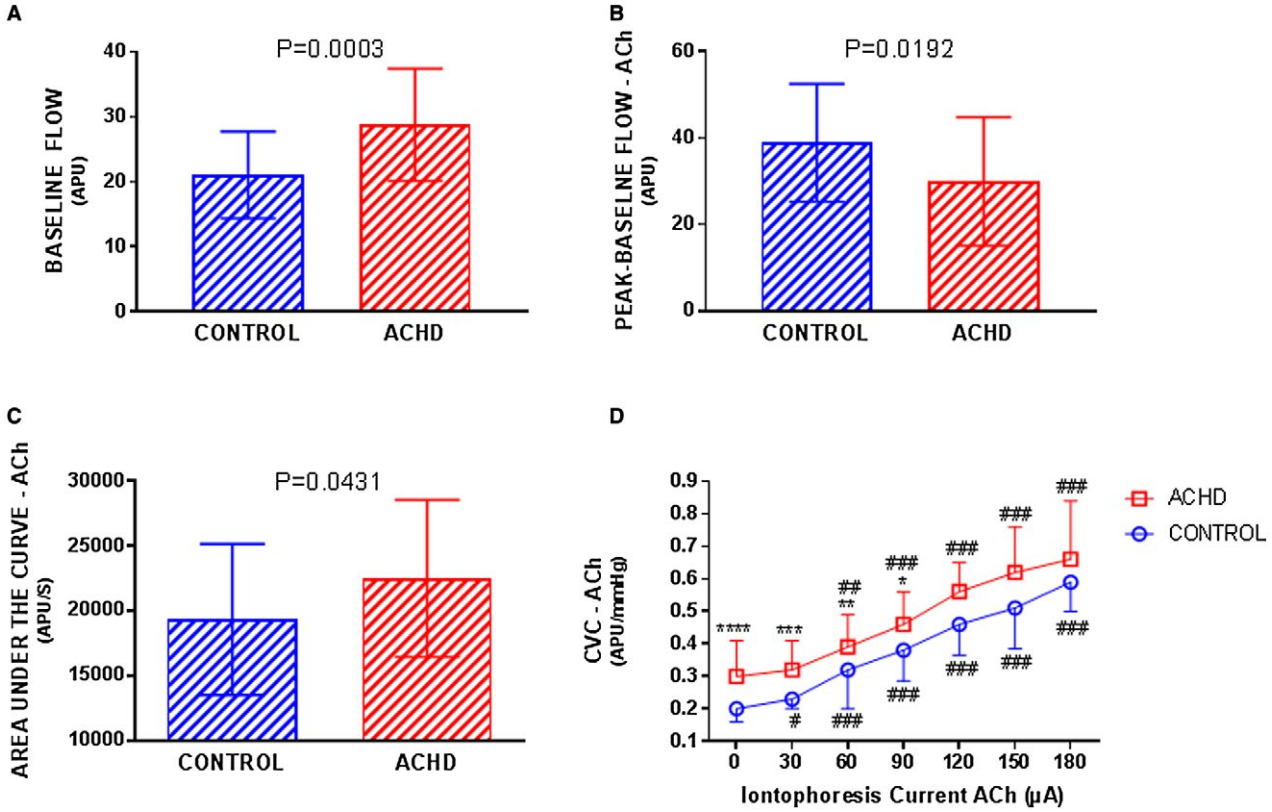

FIGURE 2 Baseline microvascular flow (A), peak-baseline values of microvascular flow (B), area under the curve for sodium nitroprusside (SNP) iontophoresis (C), and cumulative effects of SNP iontophoresis on the cutaneous microcirculation of healthy volunteers (CONTROL, $n = 29$) and adult patients with congenital heart disease (ACHD, $n = 24$). Values represent the means ± SDs. P values were calculated using the two-tailed unpaired Student's t test (Figures 1A, 1B and 1C). $^{**}P < .01$; $^{***}P < .001$, and $^{****}P < .0001$ compared to the CONTROL, using the two-tailed unpaired Student's t test; $^{\#\#}P < .01$ and $^{\#\#\#}P < .001$ compared to the baseline values, using Friedman's test followed by Dunn's multiple comparisons test (Figure 2D) [Colour figure can be viewed at wileyonlinelibrary.com]

patients were evaluated for flow-mediated dilatation but not for peripheral arterial tonometry (PAT), suggesting the presence of endothelial dysfunction in conductance vessels but not in the microcirculation.[24] Lambert et al used the PAT technique and observed microvascular endothelial dysfunction in patients with Fontan circulation.[26] Oechslin et al employed venous occlusion plethysmography and observed the occurrence of microvascular endothelial dysfunction in cyanotic congenital cardiopathies.[25] In addition, these authors found a correlation between lower oxygen saturation and a lower vasodilator response. However, venous occlusion plethysmography evaluates muscle blood flow in the forearm, including the microcirculation and large blood vessels.[49]

The results of previous studies from our group indicated that patients with systemic arterial hypertension and dyslipidemia[47] and patients with coronary artery disease[42,50] had lower microvascular cutaneous reactivity than healthy controls when evaluated by LSCI coupled to ACh iontophoresis. To the best of our knowledge, no studies to date have used this method in patients with HF or congenital heart disease.

Notably, the baseline blood flow was higher in the cutaneous microcirculation of the ACHD than in the healthy controls. Previous studies from our group using LSCI found no significant difference in the baseline microvascular blood flow in patients with coronary artery disease[50] or systemic arterial hypertension and dyslipidemia[47] compared to the control group. Similarly, studies with LDF found no significant difference between the baseline cutaneous microcirculation in patients with HF and healthy individuals.[32,34,51] In contrast to our assumptions, the baseline cutaneous microcirculation and reactivity to ACh in ACHD were not similar to that of patients with HF. Corroborating these findings, ACHD with lower peak $V'O_2$ values (Weber functional classes C and D), who might have the most severe conditions and greater functional limitation, had higher baseline microcirculatory blood flows than patients with higher $V'O_2$ peaks (Weber functional classes A and B).

The few studies that have analyzed baseline blood flow in ACHD have reported discordant results. Oechslin et al reported that the baseline blood flow was lower in patients with cyanotic congenital heart disease than in the control group.[25] Pedersen et al evaluated cyanotic patients and found no significant differences in the baseline blood flow between these patients and healthy individuals.[30] Similarly, Brili et al found no significant differences in patients with coarctation of the aorta.[23] However, contrary to the present study, none of these studies exclusively investigated cutaneous microcirculation, which is currently considered a surrogate marker of systemic

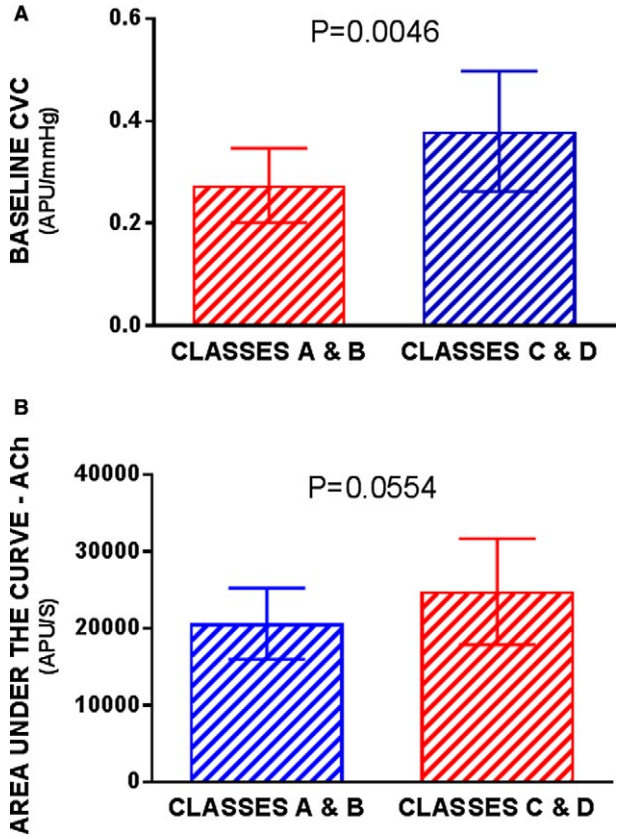

FIGURE 3 (A) Baseline cutaneous vascular conductance (CVC) and area under the curve for acetylcholine (ACh) iontophoresis (B) of adult patients with congenital heart disease according to Weber's functional classes (see Methods). APU, arbitrary perfusion units. *P* values obtained using the two-tailed unpaired Student's *t* test [Colour figure can be viewed at wileyonlinelibrary.com]

circulation.[41] Only one study published in the 1990 s found results similar to those we described. Chang et al evaluated children and adolescents with tetralogy of Fallot using LDF and observed that the baseline blood flow was higher in the cutaneous microcirculation of these patients than in the healthy controls, particularly in patients with hemoglobin below 19 g/dL.[35]

The literature available to date only allows us to speculate on the pathophysiological mechanisms involved. The use of drugs is an unlikely hypothesis if we consider that patients with hypertension, dyslipidemia, coronary heart disease, and HF certainly use cardiovascular drugs and therefore do not present a baseline blood flow in the cutaneous microcirculation that is significantly different from that of healthy individuals.[32,34,47,50,51] In addition, our results did not change significantly when the patients taking sildenafil were withdrawn from the analysis. Another explanation is that ACHD may develop an adaptive compensatory mechanism that is different from the situation in patients with HF, resulting in accumulation of vasodilators in the cutaneous microvasculature. In fact, contrary to the traditional HF model in which the left ventricle is usually involved, impairment occurs predominantly in the right cavities and in the pulmonary circulation in most congenital heart defects. In contrast to congenital heart disease, HF primarily affects older individuals with associated comorbidities, including systemic arterial hypertension, dyslipidemia, diabetes mellitus, and coronary artery disease, and these conditions are associated with endothelial dysfunction. Future studies are necessary to corroborate these findings and elucidate the underlying mechanisms.

7.1 | Limitations and strengths of the study

The limitations of this study include the relatively small sample size, which is common studies involving pathologies with a lower prevalence. Moreover, there was a rather large variation in the type of congenital heart disease of patients included in the present study. Unlike studies that investigated endothelial function in ACHD, this study was not restricted to a specific pathology but instead evaluated a heterogeneous group of congenital heart diseases. However, other studies performed in ACHD found that these individuals usually presented characteristics in common, including increased effort intolerance,[4–6] lower ventilatory efficiency,[1,19] and higher levels of B-type natriuretic peptide (BNP) compared to healthy individuals of the same age group. The clinical conditions associated with a lower peak $V'O_2$,[8] lower ventilatory efficiency,[13] and higher BNP levels[6] include cyanotic congenital heart disease and Fontan circulation. The low percentage of individuals with cyanosis and Fontan circulation in our sample indicated that the sample was composed of patients with less severe disease, which might partially explain the results. In contrast, no significant correlation was found between the severity and degree of microvascular endothelial dysfunction in patients with HF.[32,33] These authors found that the main factor associated with endothelial dysfunction was age because elderly individuals with HF presented the strongest microcirculatory impairment. In addition, progressive deterioration of microvascular endothelial function with aging has been reported.[52] However, most of the analyzed patients in the present study were young.

Importantly, the present study is the first to evaluate microcirculation in young adults with congenital heart disease using a state-of-the-art noninvasive laser-based technology.

8 | CONCLUSION

In contrast to patients with HF, the ACHD in our sample did not present endothelial dysfunction of cutaneous microcirculation. However, the ACHD had higher baseline blood flows and sustained vasodilator responses to the ACh stimulus than did the healthy individuals. Moreover, patients with a higher degree of effort intolerance (Weber functional classes C and D) presented increased microvascular baseline blood flows than those with higher maximum aerobic powers (Weber functional classes A and B).

AUTHOR CONTRIBUTIONS

All authors read and approved the final version of the manuscript.

Concept/design: Marino, Kopiler, Tibiriçá.

Data collection: Marino, Lopes.

Data analysis/interpretation: Marino, Kopiler, Borges, Tibiriçá.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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REFERENCES

1. Khan AM, Paridon SM, Kim YY. Cardiopulmonary exercise testing in adults with congenital heart disease. *Expert Rev Cardiovasc Ther*. 2014;12(7):863-872.
2. What Are Congenital Heart Defects? 2011; https://www.nhlbi.nih.gov/health/health-topics/topics/chd/, 2017.
3. Hoffman JI, Kaplan S, Liberthson RR. Prevalence of congenital heart disease. *Am Heart J*. 2004;147(3):425-439.
4. Kempny A, Dimopoulos K, Uebing A, et al. Reference values for exercise limitations among adults with congenital heart disease. Relation to activities of daily life–single centre experience and review of published data. *Eur Heart J*. 2012;33(11):1386–1396.
5. Buys R, Cornelissen V, Van De Bruaene A, et al. Measures of exercise capacity in adults with congenital heart disease. *Int J Cardiol*. 2011;153(1):26–30.
6. Trojnarska O, Gwizdała A, Katarzyński S, et al. Evaluation of exercise capacity with cardiopulmonary exercise test and B-type natriuretic peptide in adults with congenital heart disease. *Cardiol J*. 2009;16(2):133–141.
7. Miliaresis C, Beker S, Gewitz M. Cardiopulmonary stress testing in children and adults with congenital heart disease. *Cardiol Rev*. 2014;22(6):275–278.
8. Inuzuka R, Diller GP, Borgia F, et al. Comprehensive use of cardiopulmonary exercise testing identifies adults with congenital heart disease at increased mortality risk in the medium term. *Circulation*. 2012;125(2):250–259.
9. Diller GP, Dimopoulos K, Okonko D, et al. Exercise intolerance in adult congenital heart disease: comparative severity, correlates, and prognostic implication. *Circulation*. 2005;112(6):828–835.
10. Diller GP, Giardini A, Dimopoulos K, et al. Predictors of morbidity and mortality in contemporary Fontan patients: results from a multicenter study including cardiopulmonary exercise testing in 321 patients. *Eur Heart J*. 2010;31(24):3073–3083.
11. Giardini A, Specchia S, Tacy TA, et al. Usefulness of cardiopulmonary exercise to predict long-term prognosis in adults with repaired tetralogy of Fallot. *Am J Cardiol*. 2007;99(10):1462–1467.
12. Giardini A, Specchia S, Berton E, et al. Strong and independent prognostic value of peak circulatory power in adults with congenital heart disease. *Am Heart J*. 2007;154(3):441–447.
13. Dimopoulos K, Okonko DO, Diller GP, et al. Abnormal ventilatory response to exercise in adults with congenital heart disease relates to cyanosis and predicts survival. *Circulation*. 2006;113(24):2796–2802.
14. Diller GP, Dimopoulos K, Okonko D, et al. Heart rate response during exercise predicts survival in adults with congenital heart disease. *J Am Coll Cardiol*. 2006;48(6):1250–1256.
15. Zomer AC, Vaartjes I, Uiterwaal CS, et al. Circumstances of death in adult congenital heart disease. *Int J Cardiol*. 2012;154(2):168–172.
16. Broberg CS, Burchill LJ. Myocardial factor revisited: the importance of myocardial fibrosis in adults with congenital heart disease. *Int J Cardiol*. 2015;189:204–210.
17. Bouchardy J, Therrien J, Pilote L, et al. Atrial arrhythmias in adults with congenital heart disease. *Circulation*. 2009;120(17):1679–1686.
18. Broberg CS, Chugh SS, Conklin C, Sahn DJ, Jerosch-Herold M. Quantification of diffuse myocardial fibrosis and its association with myocardial dysfunction in congenital heart disease. *Circ Cardiovasc Imaging*. 2010;3(6):727–734.
19. Giardini A, Specchia S, Gargiulo G, Sangiorgi D, Picchio FM. Accuracy of oxygen uptake efficiency slope in adults with congenital heart disease. *Int J Cardiol*. 2009;133(1):74–79.
20. Sharma R, Bolger AP, Li W, et al. Elevated circulating levels of inflammatory cytokines and bacterial endotoxin in adults with congenital heart disease. *Am J Cardiol*. 2003;92(2):188–193.
21. Bolger AP, Sharma R, Li W, et al. Neurohormonal activation and the chronic heart failure syndrome in adults with congenital heart disease. *Circulation*. 2002;106(1):92–99.
22. Bank AJ, Lee PC, Kubo SH. Endothelial dysfunction in patients with heart failure: relationship to disease severity. *J Card Fail*. 2000;6(1):29–36.
23. Brili S, Tousoulis D, Antoniades C, et al. Evidence of vascular dysfunction in young patients with successfully repaired coarctation of aorta. *Atherosclerosis*. 2005;182(1):97–103.
24. Cordina RL, Nakhla S, O'Meagher S, Leaney J, Graham S, Celermajer DS. Widespread endotheliopathy in adults with cyanotic congenital heart disease. *Cardiol Young*. 2015;25(3):511–519.
25. Oechslin E, Kiowski W, Schindler R, Bernheim A, Julius B, Brunner-La Rocca HP. Systemic endothelial dysfunction in adults with cyanotic congenital heart disease. *Circulation*. 2005;112(8):1106–1112.
26. Lambert E, d'Udekem Y, Cheung M, et al. Sympathetic and vascular dysfunction in adult patients with Fontan circulation. *Int J Cardiol*. 2013;167(4):1333–1338.
27. Inai K, Saita Y, Takeda S, Nakazawa M, Kimura H. Skeletal muscle hemodynamics and endothelial function in patients after Fontan operation. *Am J Cardiol*. 2004;93(6):792–797.
28. Radke RM, Diller GP, Duck M, et al. Endothelial function in contemporary patients with repaired coarctation of aorta. *Heart*. 2014;100(21):1696–1701.
29. Cuypers J, Leirgul E, Larsen TH, Berg A, Omdal TR, Greve G. Assessment of vascular reactivity in the peripheral and coronary arteries by Cine 3T-magnetic resonance imaging in young normotensive adults after surgery for coarctation of the aorta. *Pediatr Cardiol*. 2013;34(3):661–669.
30. Pedersen CM, Schmidt MR, Mortensen B, et al. Preserved flow-mediated dilation in adults with cyanotic congenital heart disease. *Pediatr Cardiol*. 2009;30(7):965–970.
31. Cuypers J, Leirgul E, Samnøy S, et al. Assessment of coronary flow reserve in the coronary sinus by cine 3T-magnetic resonance imaging in young adults after surgery for tetralogy of Fallot. *Pediatr Cardiol*. 2012;33(1):65–74.
32. Andersson SE, Edvinsson ML, Edvinsson L. Cutaneous vascular reactivity is reduced in aging and in heart failure: association with inflammation. *Clin Sci (Lond)*. 2003;105(6):699–707.
33. Edvinsson ML, Uddman E, Andersson SE. Deteriorated function of cutaneous microcirculation in chronic congestive heart failure. *J Geriatr Cardiol*. 2011;8(2):82–87.

34. Edvinsson ML, Uddman E, Edvinsson L, Andersson SE. Brain natriuretic peptide is a potent vasodilator in aged human microcirculation and shows a blunted response in heart failure patients. *J Geriatr Cardiol*. 2014;11(1):50–56.
35. Chang CH, Yu HS, Chen GS, Wu JR, Huang TY, Yu CL. Deterioration of cutaneous microcirculatory status and its clinical correlation in tetralogy of Fallot. *Microvasc Res*. 1996;51(1):59–68.
36. Piepoli MF, Guazzi M, Boriani G, et al. Exercise intolerance in chronic heart failure: mechanisms and therapies. *Part I. Eur J Cardiovasc Prev Rehabil*. 2010;17(6):637–642.
37. Wisløff U, Støylen A, Loennechen JP, et al. Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: a randomized study. *Circulation*. 2007;115(24):3086–3094.
38. Pearson MJ, Smart NA. Aerobic training intensity for improved endothelial function in heart failure patients: a systematic review and meta-analysis. *Cardiol Res Pract*. 2017;2017:2450202.
39. Fujisue K, Sugiyama S, Matsuzawa Y, et al. Prognostic significance of peripheral microvascular endothelial dysfunction in heart failure with reduced left ventricular ejection fraction. *Circ J*. 2015;79(12):2623–2631.
40. Cracowski JL, Roustit M. Current methods to assess human cutaneous blood flow: an updated focus on laser-based-techniques. *Microcirculation*. 2016;23(5):337–344.
41. 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):370–372.
42. Souza EG, De Lorenzo A, Huguenin G, Oliveira GM, Tibiriçá E. 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*. 2014;25(1):23–28.
43. Weber KT, Kinasewitz GT, Janicki JS, Fishman AP. Oxygen utilization and ventilation during exercise in patients with chronic cardiac failure. *Circulation*. 1982;65(6):1213–1223.
44. Borges JP, Mendes F, Lopes GO, Sousa AS, Mediano M, Tibirica E Is endothelial microvascular function equally impaired among patients with chronic Chagas and ischemic cardiomyopathy? *Int J Cardiol*. 2018;265:35–37.
45. Barcelos A, Tibirica E, Lamas C. Evaluation of microvascular endothelial function and capillary density in patients with infective endocarditis using laser speckle contrast imaging and video-capillaroscopy. *Microvasc Res*. 2018;118:61–68.
46. Huguenin GV, Moreira AS, Siant'Pierre TD, et al. Effects of dietary supplementation with Brazil nuts on microvascular endothelial function in hypertensive and dyslipidemic patients: a randomized crossover placebo-controlled trial. *Microcirculation*. 2015;22(8):687–699.
47. Cordovil I, Huguenin G, Rosa G, et al. Evaluation of systemic microvascular endothelial function using laser speckle contrast imaging. *Microvasc Res*. 2012;83(3):376–379.
48. Jones NL, Campbell E. *Clinical exercise testing*, 2nd ed. Philadelphia: Saunders; 1982.
49. Wilkinson IB, Webb DJ. Venous occlusion plethysmography in cardiovascular research: methodology and clinical applications. *Br J Clin Pharmacol*. 2001;52(6):631–646.
50. Borges JP, Lopes GO, Verri V, et al. A novel effective method for the assessment of microvascular function in male patients with coronary artery disease: a pilot study using laser speckle contrast imaging. *Braz J Med Biol Res*. 2016;49(10):e5541.
51. Andersson SE, Edvinsson ML, Alving K, Edvinsson L. Vasodilator effect of endothelin in cutaneous microcirculation of heart failure patients. *Basic Clin Pharmacol Toxicol*. 2005;97(2):80–85.
52. Khalil A, Humeau-Heurtier A, Mahé G, Abraham P. Laser speckle contrast imaging: age-related changes in microvascular blood flow and correlation with pulse-wave velocity in healthy subjects. *J Biomed Opt*. 2015;20(5):051010.

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