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The impact of exercise frequency upon microvascular endothelium function and oxidative stress among patients with coronary artery disease

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Summary

Purpose This study compared the effects of low and high weekly exercise frequencies on microvascular endothelium function and oxidative stress among patients with coronary artery disease.

Methods Thirty-four male patients completed a 6-month cardiac rehabilitation programme, from which 23 performed exercise with a high frequency (HF) and 11 with a low frequency (LF). Systemic microvascular blood flow, maximal aerobic capacity, blood lipids, oxidative stress and anthropometric data were assessed prior to and after the cardiac rehabilitation programme. Microvascular blood flow was assessed in the skin of the forearm using laser speckle contrast imaging coupled with iontophoresis of acetylcholine.

Results Maximal aerobic capacity, biochemical analysis and anthropometric data were similar between groups prior to and after the cardiac rehabilitation programme ($P>0{\cdot}05$). However, after 6 months of cardiac rehabilitation performed with HF, there was an increase in the peak response to acetylcholine compared with LF ($83{\cdot}5 \pm 58{\cdot}5$ versus $21{\cdot}8 \pm 22{\cdot}4\%$; $P<0{\cdot}05$). Changes in lipid peroxidation (HF: $-5{\cdot}5 \pm 9{\cdot}4$ versus LF: $2{\cdot}2 \pm 12{\cdot}0$ pmol MDA mg^{-1}; $P = 0{\cdot}19$), catalase activity (HF: $0{\cdot}07 \pm 0{\cdot}17$ versus LF: $0{\cdot}04 \pm 0{\cdot}08$ U mg^{-1}; $P = 0{\cdot}74$) and nitric oxide levels (HF: $1{\cdot}8 \pm 15{\cdot}3$ versus LF: $-3{\cdot}2 \pm 12{\cdot}3$ μM; $P = 0{\cdot}36$) were similar between groups after cardiac rehabilitation.

Conclusion Six months of aerobic exercise training performed with high frequency is preferable to low frequency aiming endothelium microvascular function increases in patients with coronary artery disease. The mechanisms involved in this response are unclear and warrant additional research.

Introduction

Despite numerous therapeutic advances during the past decade, coronary artery disease (CAD) remains the single most common cause of death globally (Anderson *et al.*, 2016). It is well known that CAD is mainly caused by atherosclerosis that comprises a series of inflammatory and oxidative damages resulting in endothelial dysfunction (Ross, 1999; Borges *et al.*, 2016a,b). We have recently demonstrated, using laser-based technology, that endothelial-dependent systemic microvascular vasodilation is significantly impaired in patients with CAD compared to healthy subjects (Borges *et al.*, 2016a).

Exercise training is a well-established non-pharmacological approach for patients with cardiovascular diseases (Nieuwland *et al.*, 2000; Naci & Ioannidis, 2015). Several mechanisms have been proposed to explain the benefits promoted by exercise among these patients, including improvements in peripheral vascular function, peak oxygen uptake (VO_{2peak}), muscle function, exercise tolerance, and reduction in body mass (Wisloff *et al.*, 2007; Borges & Lessa, 2015; Borges *et al.*, 2016b). However, few studies have investigated the influence of exercise

training on microcirculatory impairments observed in CAD (Olsen *et al.*, 2015).

In addition, several important aspects regarding exercise prescription could influence the effectiveness of an exercise programme. In this sense, a key decision is how often exercise should be performed (Kemmler & von Stengel, 2013). For instance, although traditional cardiac rehabilitation guidelines have suggested an optimal exercise frequency of three to five times per week (Price *et al.*, 2016), supplementary studies have questioned this recommendation and reported no influence of exercise frequency on improvements of clinical parameters (e.g. VO_{2peak}) in patients with CAD (Nieuwland *et al.*, 2000). In this context, the influence of different exercise training protocols (e.g. frequency) on cardioprotective exercise-induced physiological mechanisms is still a matter of debate. Therefore, the purpose of this study was to investigate the effects of dissimilar weekly exercise frequencies on systemic microvascular endothelium function and oxidative stress in patients presenting with CAD.

Methods

Study design

This observational longitudinal study was conducted between October 2013 and November 2015 in a tertiary hospital in Rio de Janeiro, Brazil. During this period, 83 consecutive male outpatients were enrolled in a cardiac rehabilitation (CR) programme, of which 23 were excluded from the study for not presenting CAD. Of the 60 remaining patients, 26 were excluded because they did not complete 6 months of exercise sessions with attendance of at least 75%. Therefore, a total of 34 patients who completed the 6-month CR programme were included in this study. For the present analysis, the weekly attendance rate of each patient for the 6-month intervention was calculated, and then, patients were divided into two groups: (i) overall exercise frequency of ≤ two sessions/week (low frequency; LF; $n = 11$) and (ii) overall exercise frequency of > two sessions/week (high frequency; HF; $n = 23$).

Coronary artery disease was defined by the occurrence of any acute coronary syndrome, including ST elevation, non-ST elevation myocardial infarction and unstable angina (all defined by characteristic histories and electrocardiographic and cardiac enzyme abnormalities) or by the diagnosis of obstructive coronary artery disease based on coronary angiography (defined as ≥50% stenosis of any epicardial coronary artery) in patients with stable angina (Matsuzawa & Lerman, 2014).

All procedures described in this study were conducted in accordance with the Declaration of Helsinki of 1975 as revised in 2000 and were approved by the local Institutional Review Board (IRB) under protocol number CAAE 38701614·8·0000·5272. Once considered eligible, all of the subjects read and signed an informed consent form that was approved by the IRB. The protocol of the study was registered and made public on ClinicalTrials.gov (identifier: NCT02514564).

Procedures

Prior to and after the 6-month cardiac rehabilitation training, patients underwent cutaneous microvascular reactivity testing, maximal exercise testing and anthropometric measurements. In the morning scheduled for the test, the patients presented in 12-h fasted condition for blood collection. The patients should not have smoked or ingested caffeine from the night before until the completion of the tests. Patients were advised to take their usual medications on the morning of the tests, except direct vasodilators, which were administered immediately after the microcirculatory tests.

Cardiac rehabilitation programme

The CR programme consisted of 6 months of exercise training complemented by nutritional and psychosocial counselling. The exercise sessions consisted of 60 min, including 5 min of warm-up with stretching and callisthenic exercises, 30 min of walking on treadmill or pedalling on cycle ergometer, 20 min of local strength exercises (sit-ups, push-ups, and pull-ups), and 5 min cool down with stretching exercises. The aerobic exercise intensity was established according to heart rate corresponding to the individual ventilatory threshold, determined during a maximal exercise testing performed before and after 3 months of exercise intervention. All patients were recommended to avoid additional regular exercise (either supervised or unsupervised) during the CR programme.

Progressive cardiopulmonary exercise testing

Treadmill cardiopulmonary exercise testing was performed using a ramp protocol designed to elicit maximal volitional effort within 8–12 min (Balady *et al.*, 2010). Respiratory gas analysis was performed using breath-by-breath analysis of O_2 and CO_2 with a VO2000 metabolic cart (Medical Graphics™, Saint Louis, MO, USA) (Laterza *et al.*, 2007). The VO_{2peak} was defined as the peak O_2 consumption reached during exertion with respiratory exchange ratios of at least 1·01.

The incremental test was interrupted when the patient reported any discomfort preventing him/her from continuing the exercise. The ventilatory threshold was estimated by the V-slope method, as the point where the relationship between VCO_2 and VO_2 production was no longer linear, or at the point where the oxygen ventilatory equivalent or end-tidal oxygen partial pressure curves reached their minimum values and began to rise during the progressive exercise (Schneider *et al.*, 1993). Ventilatory threshold detection was performed by two experienced exercise physiologists, independently and blindly. If the intensity corresponding to ventilatory threshold was not within 3% agreement across investigators, a third trained researcher analysed data to adjudicate its determination. The adjudicated

ventilatory threshold was then compared with those previously obtained and averaged using the closest value obtained in the initial assessment.

Anthropometric measurements

Anthropometric evaluation included measurements of height and body mass. Body mass index (BMI) was computed as body mass in kilograms divided by the square of height in metres (Kg m^{-2}). Height was measured by a wall-mounted stadiometer, and body mass using a calibrated digital scale. Height and body mass were recorded to the closest 0·01 m and 0·1 kg, respectively.

Evaluation of skin microvascular reactivity

Microcirculatory tests were performed after a 20-minute rest with the patients in the supine position in a temperature-controlled room (23 ± 1°C) approximately 1 h after a light breakfast. Microcirculatory test was performed as previously described by our group (Cordovil *et al.*, 2012; Souza *et al.*, 2014; Borges *et al.*, 2016a). Briefly, microvascular reactivity was assessed using a laser speckle contrast imaging system with a laser wavelength of 785 nm (PeriCam PSI system, Perimed, Järfälla, Sweden) in combination with the iontophoresis of acetylcholine (ACh) for non-invasive and continuous measurements of cutaneous microvascular perfusion changes (in comparison with baseline, %). Skin blood flow measurements were also divided by the mean arterial pressure to yield the cutaneous vascular conductance (CVC) in APU $mmHg^{-1}$ and expressed as the peak response to ACh minus baseline.

Images were analysed using the manufacturer's software (PIMSoft, Perimed, Järfälla, Sweden). ACh (2% w/v; Sigma Chemical Co., St. Louis, MO, USA) iontophoresis was performed using a microphamacology system (PF 751 PeriIont USB Power Supply, Perimed, Sweden) with six increasing doses obtained through anodal currents of 30, 60, 90, 120, 150 and 180 μA applied 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).

Lipids, nitric oxide levels and oxidative stress

Total cholesterol and HDL cholesterol were determined by means of a photometric colorimetric optical system (Cobas™ Mira systems, Roche Diagnostic Corporation, Indianapolis, IN, USA). The LDL cholesterol fraction was calculated by the Friedewald's formula, as detailed elsewhere (Friedewald *et al.*, 1972).

Nitric oxide bioavailability, lipid peroxidation and catalase activity were assessed as previously described (Huguenin *et al.*, 2015; Medeiros-Lima *et al.*, 2017). Briefly, the evaluation of total plasma NOx (NO2 + NO3) concentrations was performed by a colorimetric assay (Cayman Inc., USA) with a sensitivity of 2·5 μM and a 2·7% intra-assay coefficient of variation. Catalase activity was assessed according to Aebi (Aebi, 1984), while oxidative damage was assessed by determination of by-products of lipid peroxidation (malondialdehyde, MDA), through the reaction with thiobarbituric acid, which results in a pinkish substance that was subsequently measured by spectrophotometry at 532 nm.

Statistical analysis

Sample size calculation using GPower (version 3·0·10, University of Kiel, Kiel, Germany) was based on the difference between groups for microvascular responses to ACh of 0·12 APU $mmHg^{-1}$, with a standard deviation of 0·14 APU $mmHg^{-1}$ (Borges *et al.*, 2016a). Assuming 70% of power and 5% significance level, a minimum of 11 patients in each group was necessary.

The results are expressed as mean ± standard deviation (continuous variables) or percentages (categorical). Normal sample distributions were verified with the Shapiro–Wilk test. Baseline clinical characteristics and longitudinal changes between groups were compared using either Student's t-test or chi-squared test. Maximal exercise capacity, anthropometric, lipid profile, microvascular reactivity and oxidative stress between groups prior to and after the 6-month CR programme were evaluated using a two-way analysis of variance. The Bonferroni post-test was used to localize the significant differences. In all cases, the significance level was set at $P<0·05$.

Results

Baseline characteristics

Table 1 shows that the baseline clinical characteristics of the patients who completed the study were similar between groups. Concerning the use of cardiovascular drugs, patients in the LF and HF groups were being treated, respectively, with: (i) angiotensin-converting enzyme inhibitors (43% and 54%, $P = 0·50$), (ii) β-blockers (87% and 91%, $P = 0·70$), (iii) lipid-lowering drugs (83% and 91%, $P = 0·50$), (iv) nitrates (52% and 45%, $P = 0·70$), (v) angiotensin II receptor blockers (48% and 27%, $P = 0·20$) and (vi) diuretics (52% and 54%, $P = 0·90$).

Maximal exercise capacity, lipids levels and anthropometrical variables

Data for maximal exercise capacity, lipids levels and anthropometrical variables are depicted in Table 2. VO_{2peak} increased by approximately 6% after LF or HF exercise sessions without significant between-group difference ($P = 0·86$). Although HDL cholesterol was higher at baseline in patients who exercised with LF versus HF ($P = 0·01$), no difference was detected for changes from baseline ($P = 0·85$).

Table 1 Baseline clinical characteristics of patients.

	High frequency (n = 23)	Low frequency (n = 11)	*P*-value*
Age (years)	62·5 ± 9·9	63·8 ± 12·4	0·20
Smokers, n (%)	2 (7)	1 (9)	0·90
Diabetes, n (%)	11 (48)	6 (54)	0·70
Hypertension, n (%)	20 (87)	10 (91)	0·70
Dyslipidemia, n (%)	15 (65)	7 (64)	0·90
Acute myocardial infarction, n (%)	17 (74)	7 (64)	0·50
Coronary bypass surgery, n (%)	8 (35)	4 (36)	0·90
Coronary angioplasty, n (%)	6 (26)	3 (27)	0·90
Left ventricle ejection fraction (%)	51·1 ± 16·4	58·8 ± 18·2	0·20
Resting heart rate (bpm)	66·1 ± 13·0	66·1 ± 8·2	0·90
Systolic blood pressure (mmHg)	132·6 ± 16·9	127·6 ± 13·8	0·40
Diastolic blood pressure (mmHg)	74·8 ± 10·6	69·8 ± 4·2	0·10
Mean blood pressure (mmHg)	94·1 ± 11·5	89·1 ± 6·4	0·20

The results are expressed as the mean ± SD.
*Unpaired Student's t-test or chi-squared test when appropriate.

Skin microvascular reactivity

Before the cardiac rehabilitation programme, there was no difference between groups for ACh-induced percentage increases in skin microvascular blood flow (HF: 65·8 ± 41·5 versus LH: 62·7 ± 45·1%; P>0·05; Fig. 1) and peak increases in CVC relative to baseline (HF: 0·17 ± 0·11 versus LF: 0·18 ± 0·12 APU $mmHg^{-1}$; P>0·05; Fig. 2). However, 6 months of cardiac rehabilitation performed with HF induced greater responses to ACh compared with LF at dose 4 (44·0 ± 42·9 versus 11·8 ± 17·5%; P<0·05; Fig. 1), 5 (60·6 ± 48·7 versus 13·8 ± 23·5%; P<0·05; Fig. 1) and 6 (83·5 ± 58·5 versus 21·8 ± 22·4%; P<0·05; Fig. 1). Peak increases in CVC relative to baseline was also greater in HF group than in LF after the cardiac rehabilitation programme (0·19 ± 0·13 versus 0·08 ± 0·09 APU $mmHg^{-1}$; P<0·05; Fig. 2).

Oxidative stress and nitric oxide levels

Figure 3 presents lipid peroxidation, catalase activity and nitric oxide bioavailability data. At baseline, no difference between groups was found for lipid peroxidation (HF: 48·8 ± 8·7 versus LF: 45·2 ± 10·9 pmol MDA mg^{-1}; P = 0·50), catalase activity (HF: 0·05 ± 0·05 versus LF: 0·03 ± 0·03 U mg^{-1}; P = 0·47) and NOx levels (HF: 34·6 ± 18·1 versus LF: 25·3 ± 16·5 μM; P = 0·16). Changes induced by cardiac rehabilitation for lipid peroxidation (HF: −5·5 ± 9·4 versus LF: 2·2 ± 12·0 pmol MDA mg^{-1}; P = 0·19), catalase activity (HF: 0·07 ± 0·17 versus LF: 0·04 ± 0·08 U mg^{-1}; P = 0·74) and NOx levels (HF: 1·8 ± 15·3 versus LF: −3·2 ± 12·3 μM; P = 0·36) were similar between HF and LF groups.

Table 2 Maximal exercise capacity, anthropometric and lipid levels before and after the 6-month cardiac rehabilitation programme.

	Mean ± SD		
	High frequency	Low frequency	*P*-value*
Peak oxygen uptake (ml kg^{-1} min)			
Baseline	16·1 ± 2·6	18·0 ± 3·2	0·10
6 months	17·1 ± 3·8	19·2 ± 2·4	0·18
Difference	0·99 ± 3·54	1·21 ± 2·83	0·86
Body weight (kg)			
Baseline	80·4 ± 13·3	72·6 ± 10·2	0·10
6 months	80·8 ± 14·1	74·0 ± 10·6	0·16
Difference	0·48 ± 3·48	1·37 ± 2·72	0·46
Body mass index (kg m^{-2})			
Baseline	27·6 ± 3·4	26·2 ± 2·6	0·36
6 months	27·7 ± 3·8	26·4 ± 2·4	0·95
Difference	0·16 ± 1·24	0·24 ± 1·00	0·86
Abdominal circumference (cm)			
Baseline	27·5 ± 9·8	24·6 ± 4·8	0·38
6 months	24·2 ± 6·0	24·7 ± 5·0	0·70
Difference	−0·12 ± 3·15	1·86 ± 4·99	0·17
Total cholesterol (mg dl^{-1})			
Baseline	161·8 ± 42·0	168·3 ± 39·9	0·67
6 months	150·4 ± 29·1	156·0 ± 34·3	0·63
Difference	−11·35 ± 37·12	−12·36 ± 18·58	0·93
LDL cholesterol (mg dl^{-1})			
Baseline	93·1 ± 29·6	98·6 ± 32·6	0·63
6 months	83·6 ± 28·1	83·7 ± 27·1	0·99
Difference	−9·52 ± 28·51	−14·95 ± 15·51	0·56
HDL cholesterol (mg dl^{-1})			
Baseline	35·7 ± 8·2	47·1 ± 11·9	0·01
6 months	35·7 ± 7·2	47·6 ± 12·1	0·01
Difference	0·0 ± 7·37	0·45 ± 5·34	0·85

*Unpaired Student's t-test.

Discussion

This study investigated the impact of two different exercise frequencies on systemic microvascular endothelium function

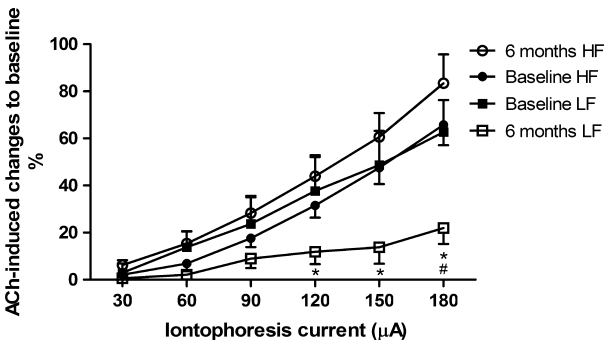

Figure 1 Acetylcholine-induced percentage changes to baseline in microvascular skin blood flow before and after 6 months follow-up of a high-frequency (HF) and low-frequency (LF) cardiac rehabilitation programme. Values represent means ± SDs. *P<0·05 versus 6 months HF; #P<0·05 versus baseline LF.

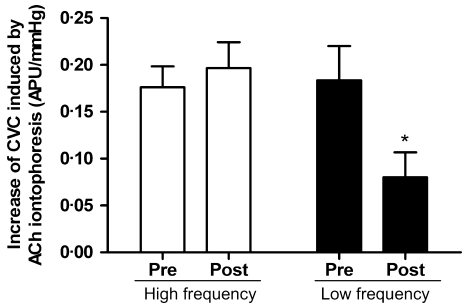

Figure 2 Increase of cutaneous microvascular conductance (CVC, expressed in arbitrary perfusion units; APU, divided by mean arterial pressure in mmHg) induced by the iontophoresis of acetylcholine (ACh) before (Pre) and after (Post) cardiac rehabilitation with high and low frequency. Values represent means ± SDs. *P<0·05 versus post-high frequency cardiac rehabilitation.

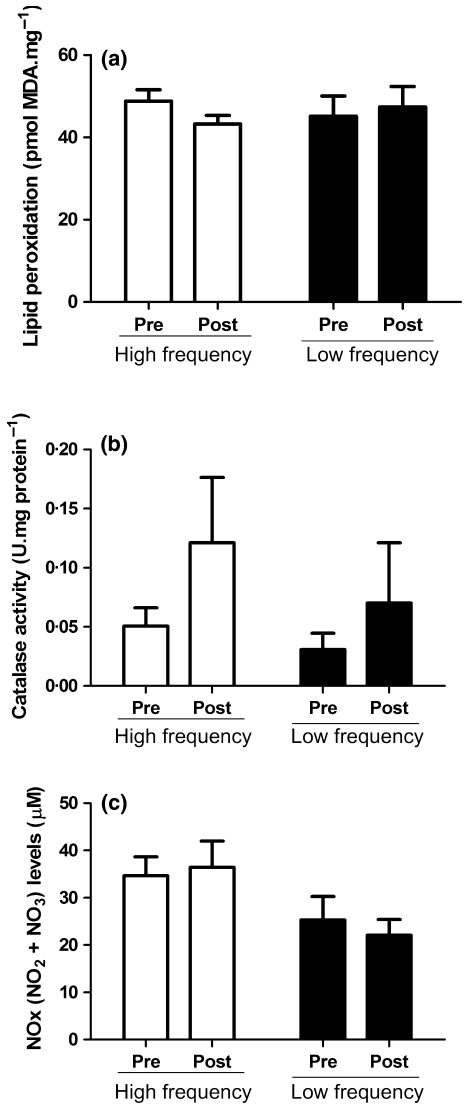

Figure 3 Lipid peroxidation measured by thiobarbituric acid-reactive substances (a), catalase activity (b) and total plasma NOx (NO_2^-/NO_3^-) levels (c) before (Pre) and after (Post) cardiac rehabilitation with high and low frequency. Values represent means ± SDs.

and oxidative stress in patients presenting with coronary artery disease. Our main finding is that a 6-month cardiac rehabilitation programme performed three times per week induces a greater increase in microvascular endothelial function than one to two hebdomadary sessions. Intriguingly, the lower-frequency group showed a reduced microvascular endothelial function after exercise intervention compared to the higher-frequency group, suggesting that less than three weekly exercise sessions for 6 months do not prevent CAD to induce further damage in microvascular function.

Evidence indicates that patients presenting with CAD exhibit a reduction of approximately 36% of systemic microvascular blood flow response to ACh compared with healthy controls (Borges *et al.*, 2016a). Moreover, Souza *et al.* (2014) demonstrated that younger adult patients with early-onset CAD already present reduced systemic microvascular density and reactivity. Although clearly CAD impairs microvascular endothelial function, data regarding how fast CAD affects vascular function are lacking. In this sense, our data suggest that endothelial-dependent microvascular function of patients with CAD gets worse within 6 months. Most patients included in our study had suffered a myocardial infarction in the year prior to their inclusion in the cardiac rehabilitation programme, which may have contributed to the fast reduction in endothelial microvascular function despite the low-frequency exercise training attendance. In accordance with this rationale, Khalepo *et al.* (2009) have indicated a drastic decrease in skin microvascular endothelial reactivity to vasoactive drugs following myocardial infarction development. In this sense, the inclusion of a control sedentary group with CAD in the present study would provide more detailed and useful information about the role of exercise training with less than three weekly sessions in preventing CAD-induced damages on microvascular function. Although the lack of a control group represents our major limitation, we could not preclude patients to engage in cardiac rehabilitation due to ethical reasons.

Most studies demonstrate enhanced endothelium-dependent vasomotor function of cutaneous microvasculature after exercise training (Simmons *et al.*, 2011). Hodges *et al.* (2010), for instance, observed that three exercise sessions/week for 48 weeks in postmenopausal women increased peak cutaneous endothelial-dependent vasodilation in response to acetylcholine iontophoresis measured by laser-Doppler flowmetry. In respect to cardio-metabolic diseases, de Moraes *et al.* (2016) reported that skin microvascular density and endothelium-dependent capillary recruitment were increased in patients with diabetes after twelve weeks of exercise training performed four times per week. On the other hand, Olsen *et al.* (2015) demonstrated that three weekly sessions of aerobic interval exercise for 12 weeks did not change systemic vascular function measured by arterial tonometry in patients with CAD. Regardless of these contradictory results, to the best of our knowledge, this is the first evidence indicating that there may be a threshold above which physical training should be performed in terms of exercise frequency to achieve

benefits in the microcirculatory function. Considering that endothelial dysfunction is involved in the aetiology of most cardiovascular diseases, this finding is relevant for practitioners when designing cardiac rehabilitation programmes. Existing studies comparing the effect of exercise frequency in cardiac rehabilitation settings are limited to clinical outcomes, mainly aerobic conditioning (Dressendorfer *et al.*, 1995; Nieuwland *et al.*, 2000). In this context, our results are consistent with previous evidence demonstrating no effects of exercise training frequency upon cardiorespiratory fitness (Dressendorfer *et al.*, 1995; Nieuwland *et al.*, 2000).

Endothelium-dependent pathways involved in acetylcholine-induced vasodilatation in the skin are complex and only partially understood. Changes in prostanoids and nitric oxide levels, density of vessels and redox status seem to be involved in this response (Simmons *et al.*, 2011). However, it is still unclear which of these pathways are changed by exercise training that, in turn, lead to increased microvascular function. In the present study, increments in microcirculatory function induced by high-frequency over low-frequency exercise were not accompanied by significant changes in redox status and nitric oxide bioavailability. Actually, changes from baseline in oxidative stress, catalase activity and nitric oxide availability after cardiac rehabilitation were similar between high-frequency and low-frequency exercise groups. The quite large but non-significant differences between groups suggest that the study may be underpowered due to the relatively small number of subjects or large inter-individual variability for most of the variables related to oxidative stress, and this must be acknowledged. Sample size was calculated to achieve statistically significant differences only for the primary outcome (microcirculation response). Studies regarding the effect of exercise training on redox status are controversial. Previous studies conducted in clinical and experimental settings have shown increment in antioxidant enzymes' levels and reduced oxidative stress after exercise training (Takahashi *et al.*, 2013; Conti *et al.*, 2015; Lawler *et al.*, 2016), while others have not (Balakrishnan & Anuradha, 1998; Chang *et al.*, 2002; Finkler *et al.*, 2014). Therefore, further research with larger sample sizes on the mechanisms by which exercise training benefits microvascular function is warranted.

In conclusion, our data suggest that cardiac rehabilitation with at least three weekly exercise sessions is preferable over two times per week aiming endothelium microvascular function increases of CAD patients. The mechanisms involved in this response are unclear and warrant additional research.

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Conflict of interest

The authors declare no conflict of interest.

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