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High-intensity interval training or continuous training, combined or not with fasting, in obese or overweight women with cardiometabolic risk factors: study protocol for a randomised clinical trial

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ABSTRACT

Introduction Physical inactivity and increased caloric intake play important roles in the pathophysiology of obesity. Increasing physical activity and modifying eating behaviours are first-line interventions, frequently hampered by lack of time to exercise and difficulties in coping with different diets. High-intensity interval training (HIIT) may be a time-efficient method compared with moderate-intensity continuous training (CT). Conversely, diets with a fasting component may be more effective than other complex and restrictive diets, as it essentially limits caloric intake to a specified period without major diet composition changes. Therefore, the combination of HIIT and fasting may provide incremental benefits in terms of effectiveness and time efficiency in obese and sedentary populations. The aim of this study is to determine the effect of HIIT versus CT, combined or not with fasting, on microcirculatory function, cardiometabolic parameters, anthropometric indices, cardiorespiratory fitness and quality of life in a population of sedentary overweight or obese women with cardiometabolic risk factors.

Methods and analysis Sedentary women aged 30–50 years, with a body mass index ≥25 kg/m^2 and cardiometabolic risk factors, will be randomised to HIIT performed in the fasting state, HIIT performed in the fed state, CT in the fasting state or CT in the fed state. Cardiometabolic parameters, anthropometric indices, cardiorespiratory fitness, quality of life and microvascular function (cutaneous capillary density and microvascular reactivity evaluated by laser speckle contrast imaging) will be evaluated before initiation of the interventions and 16 weeks thereafter.

Ethics and dissemination The trial complies with the Declaration of Helsinki and has been approved by the local ethics committee (Instituto Nacional de Cardiologia, Rio de Janeiro, Brazil). All patients provide written informed consent before enrolment and randomisation. The study's results will be disseminated to the healthcare community by publications and presentations at scientific meetings.

Trial registration number NCT03236285.

Strengths and limitations of this study

- This study will provide novel data on the benefits of high-intensity interval training combined with fasting.
- The randomised nature of this study and the number of subjects are strengths, since most previous investigations on the effects of exercise on microvascular function were small and non-randomised.
- There are inherent difficulties of assessing adherence to diet, since that depends on the accuracy of each patient's report.
- This is a study of otherwise healthy, premenopausal, overweight/obese sedentary women, and therefore its results may not be generalisable to other populations.
- Future studies are warranted to compare the results of the present study with those obtained in individuals with higher cardiovascular risk of both genders.

INTRODUCTION

Obesity is a growing worldwide public health concern that is associated with cardiovascular disease and death. The increasing prevalence of obesity is largely due to overall increased food consumption, especially of high-calorie foods, and decreased energy expenditure associated with work or leisure.[1–5]

The relationships between physical inactivity, weight gain and metabolic abnormalities are well established. Sedentary habits combined with increased energy intake promote adipose tissue expansion and insulin resistance.[6] In overweight or obese individuals, skeletal muscle insulin resistance plays a key role in the development of diabetes mellitus and metabolic syndrome,[7] and physical inactivity is an important determinant of this effect.[8] Importantly, it has been

demonstrated that regular exercise improves insulin sensitivity.[9] Additionally, exercise improves cardiorespiratory fitness, muscular strength, endurance and body composition and reduces visceral fat.[10–13] In this context, increases in physical activity and modification of eating behaviours are first-line interventions, given their low costs and low risks of complications.[14] However, there are several barriers to regular physical activity, with lack of time being one of the most common.[15] Traditionally, moderate intensity, continuous forms of exercise performed several days of the week have been used.[16] However, different forms of training, which aim at reducing time spent exercising by increasing exercise intensity, have been considered alternatives to increase adherence to the training programmes, with evidence suggesting that higher intensity exercise may promote greater benefit.[17]

High-intensity interval training (HIIT) can be described as short periods of exercise performed at high intensity (>80%–85% peak oxygen uptake), alternated with periods of active or passive rest.[18] HIIT has been explored as an option in place of moderate-intensity continuous training (CT) to improve time efficiency.[19] HIIT and other high-intensity exercise training programmes have favourable effects on metabolic control in several populations, including the obese[20 21] and patients with metabolic disease.[22 23] HIIT increases cardiorespiratory fitness,[24] an important marker of cardiovascular health in the general adult population.[25] Importantly, low cardiovascular fitness is a risk factor for all-cause mortality and cardiovascular disease, independent of body fatness,[26 27] and increasing cardiorespiratory fitness may reduce the risks associated with dyslipidaemia, obesity and type 2 diabetes mellitus.[28] HIIT also improves vascular function, leading to increases in muscle capillary density[29] and endothelial nitric oxide synthase (eNOS) levels.[30] However, studies to date have assessed the vascular effects of HIIT in large vessels, mostly using flow-mediated vasodilation of the brachial artery.[31–33] The effects of HIIT on microcirculation are still largely unknown.

Similar to exercise protocols, the search for optimal dietary interventions for obesity is ongoing. Fasting using different protocols, such as intermittent (5:2), Ramadan and others, all of them essentially restricting caloric intake to a specified period of time, has been a matter of recent interest due to convenience and beneficial health effects,[34–38] including body weight reduction and improvement of insulin sensitivity.[39–41] In this context, previous studies have already shown that the combination of regular exercise training with a Mediterranean diet improves microvascular reactivity in a high-risk population of postmenopausal women.[42 43] It is worth noting that increases in insulin, resulting from meal ingestion, lead to insulin-mediated activation of eNOS and subsequent increases in nitric oxide (NO) production in the terminal arterioles of human skeletal muscle. This process promotes vasodilatation and an increase in the blood supply to the capillaries served by this terminal arteriole.[44] However, this mechanism is impaired in obese, sedentary, elderly individuals and in insulin-resistant states and type 2 diabetes.[45 46] Insulin also stimulates the expression of the potent vasoconstrictor endothelin 1, which opposes the vasodilator action of NO and, consequently, the balance between NO production and endothelin-1 expression; this is key to insulin-mediated capillary recruitment.[47]

Regarding the combination of exercise and fasting, Terada *et al* showed that exercise performed in the fasted state reduced postprandial glycaemic increments to a greater extent than postbreakfast exercise, indicating that HIIT under fasting conditions might be an advantageous approach in that scenario.[48] Restricting carbohydrates and/or training in a fasted state has shown beneficial metabolic effects in glucose and fat metabolism.[49] Increased resting fat oxidation and cardiorespiratory fitness were reported following a 2-week HIIT plus carbohydrate-restricted diet intervention in obese men.[50] Conversely, exercising before or after a high-fat meal has been shown to ameliorate the detrimental effects of fat on endothelial function.[51] Thus, the relationships between exercise, diet and vascular function arise as an important field for investigation.

Due to the inherent difficulties of studying muscle capillaries and the importance of systemic microcirculation in the pathophysiology of cardiovascular disease,[52] the non-invasive evaluation of systemic microvascular function by laser speckle contrast imaging (LSCI), using cutaneous microcirculation as an accessible and representative vascular bed for the assessment of microcirculatory reactivity is a useful approach, with superior repeatability and reproducibility compared with other techniques.[53] We have recently demonstrated that endothelial-dependent and endothelial-independent vasodilation assessed by LSCI are directly associated with serum adiponectin, which is reduced in obesity.[54] The evaluation of functional capillary density in the skin (the number of spontaneously perfused capillaries per mm^2 of skin area) assessed by high-resolution intravital colour microscopy also adds information regarding the status of the microcirculation. Microvascular rarefaction has been closely correlated with cardiovascular and metabolic diseases, including arterial hypertension, diabetes, obesity and the metabolic syndrome.[55–57] Therefore, these methods provide an opportunity to non-invasively study the effects of exercise and diet on systemic microvascular function.

Given the reported benefits of HIIT and fasting, it may be hypothesised that the combination of both may maximise health benefits. It is worth noting that most studies of the vascular effects of HIIT, on either trained or sedentary subjects who are healthy or diseased, have been small and non-randomised.[58] This manuscript describes the protocol of a study comparing CT and HIIT when either is combined with fasting. Due to the aforementioned reasons, we believe that it is appropriate to determine, in a randomised controlled trial, the effect of HIIT associated with fasting on microcirculatory

function, cardiometabolic parameters, anthropometric indices, cardiorespiratory fitness and quality of life in a population of overweight or obese sedentary women with cardiometabolic risk factors. We also included an evaluation of nitric oxide systemic bioavailability, which correlates with endothelial dysfunction. Plasma levels of NO, which reflect changes in endothelial NO synthase activity in humans, have been used as markers of systemic NO bioavailability in humans and are associated with cardiovascular risk factors and atherosclerosis.[59 60]

STUDY RATIONALE AND OBJECTIVES

We aim to investigate the effects of HIIT versus CT, combined or not with fasting, on capillary density, microvascular function, cardiometabolic risk markers, functional capacity and quality of life in overweight or obese sedentary women with cardiometabolic risk factors. We postulate that HIIT will promote greater improvements in these parameters than CT. Furthermore, we believe that the positive effects of exercise may increase when it is performed in the fasting state compared with exercise performed in the fed state. Indeed, a recent study showed that HIIT attenuated postprandial endothelial dysfunction measured by brachial artery flow-mediated vasodilation.[61] Thus, it is possible to hypothesise that the combination of HIIT and fasting may indeed cause incremental benefits on endothelial function, among other effects.

DESIGN

The HIIT-FAST trial is a randomised, controlled trial, open-label (with blinded evaluation of microvascular parameters), conducted at a single centre in Rio de Janeiro, Brazil. All items from the WHO Trial Registration Data Set are described in online supplementary appendix 1. Our protocol follows the recommendations provided by the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) 2013 statement.

ELIGIBILITY

Volunteers will be recruited among the workers of the National Institute of Cardiology, a tertiary-care, public hospital located in the city of Rio de Janeiro, Brazil. Convenience sampling will be employed, with recruitment among the hospital's workers, involved in healthcare or not, by local advertising through posters. The inclusion and exclusion criteria are shown in box 1. We decided to recruit only women due to their reported higher rate of attempts to lose weight compared with men.[62] Premenopausal status was considered necessary to minimise variations of the influence of oestrogen status on vascular function and other study variables[63] and because of female-specific differences in microvascular endothelial function.[64]

Box 1 Inclusion and exclusion criteria

Inclusion criteria

- Female gender.
- Age ≥30 years and ≤50 years.
- Premenopausal status.
- Body mass index >25 kg/m^2.
- At least one of the following: waist circumference ≥80 cm; triglycerides ≥150 mg/dL or treatment for lipid abnormality; high-density lipoprotein cholesterol <50 mg/dL or treatment for this abnormality; fasting plasma glucose≥100 mg/dL.[66]

Exclusion criteria

- Chronic pulmonary disease.
- Any systemic disease or condition that might reduce the adherence or tolerance to exercise or fasting.
- Orthopaedic or neurological conditions that might impair exercise training.
- Pregnancy or breastfeeding.
- Abnormalities elicited at exercise treadmill testing that preclude the initiation of exercise training.
- Current exercise training protocol or any other exercise engagement.

STUDY PROCEDURES

The study will be conducted at the National Institute of Cardiology, a public hospital in Rio de Janeiro, Brazil. Volunteers will be recruited among the hospital's workers, involved in healthcare or not, by local advertising through posters. Figure 1 depicts recruitment, randomisation and follow-up interventions.

After verification of eligibility, the investigators will obtain written, informed consent from all participants. Randomisation and allocation to study arm will be performed by one of the investigators not involved with the training of the study subjects. The randomisation list will be generated using STATA V.13 (StatCorp). The randomisation strategy uses permuted blocks of random sizes. Block sizes will not be disclosed to ensure concealment. The allocation sequence will be implemented with sealed, opaque envelopes. After assignment to interventions, two of the study's investigators (AL and ET) will be blinded to patient allocation and will be responsible for data analysis.

Subjects will undergo clinical data collection and physical examination, a quality of life questionnaire (36-item short-form health survey),[65] blood sampling and an exercise treadmill test. Cardiometabolic risk factors will be defined according to the International Diabetes Federation definition.[66] Collected data will include age, gender, the presence of systemic hypertension, diabetes mellitus or dyslipidaemia (any abnormality of serum low-density lipoprotein (LDL), high-density lipoprotein (HDL) or triglycerides), medical history and active medication use. Current smoking status will be defined as self-reported active smoking at the time of the stress test. Family history of coronary artery disease will be defined as compatible history in a first-degree relative. Hypertension will be defined as a prior diagnosis of hypertension or use of antihypertensive medications. Dyslipidaemia will be defined by prior diagnosis of any

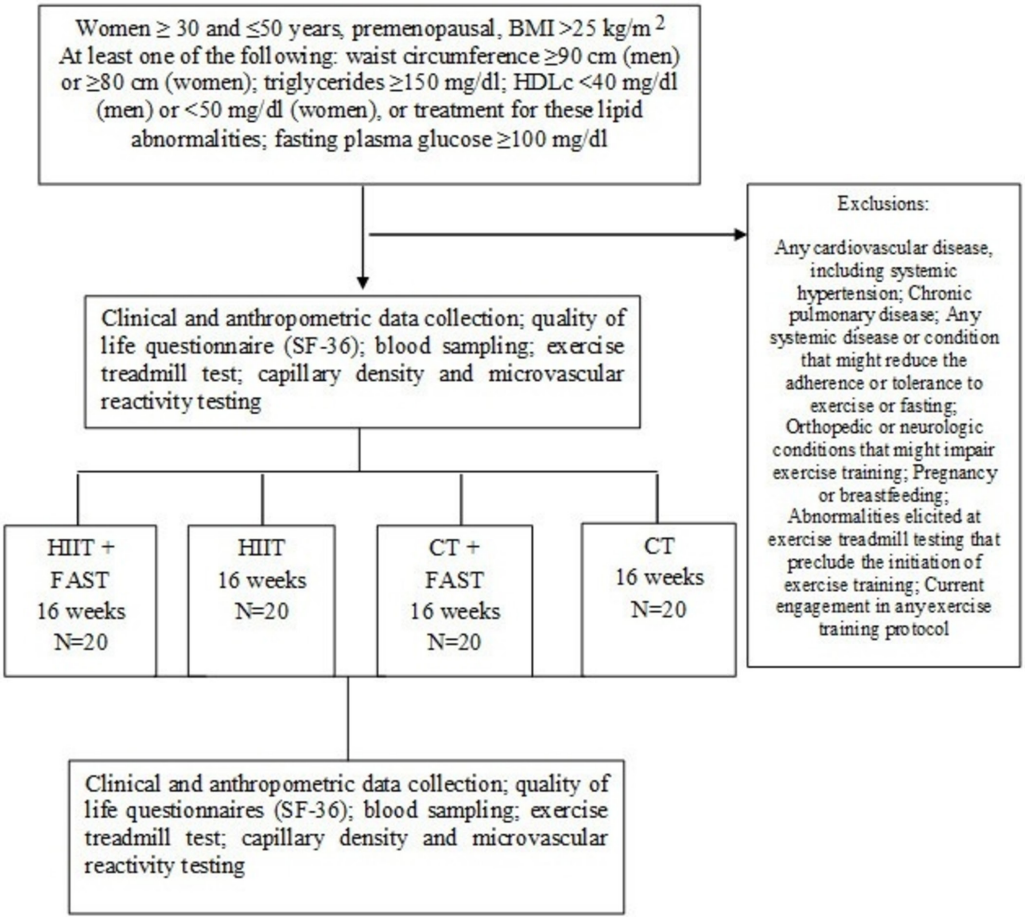

Figure 1 Patient recruitment, randomisation and follow-up interventions. BMI, body mass index; CT, continuous training; HDL-c, high-density lipoprotein cholesterol; HIIT, high-intensity interval training; SF-36, 36-item short-form health survey.

major lipid abnormality or use of lipid-lowering medications. Diabetes will be defined as prior diagnosis of diabetes or use of hypoglycaemic medication. Known cardiovascular disease will be considered as prior myocardial infarction, coronary angioplasty, coronary artery bypass surgery, any documented obstructive coronary artery disease, congestive heart failure, valvular heart disease, cardiac arrhythmias or cardiomyopathies, diagnosed at any time before consideration for inclusion in the study.

Weight in kilograms, height in metres and waist circumference in centimetres will be measured at the initial visit. Body composition will be assessed by bioimpedance (Inbody 720, Biospace, USA). Weight will be measured to the nearest 0.1 kg, with participants dressed in underwear and height will be measured to the nearest 0.5 cm, both measured using a scale with stadiometer (Filizola, Brazil). Waist circumference will be measured to the nearest 0.5 cm, with waist circumference defined as the midline between the lowest border of rib cage and the upper border of iliac crest.

Laboratory data

Peripheral venous blood will be collected after a 12 hours fast for the measurement of glucose, insulin, LDL-cholesterol, HDL-cholesterol, triglycerides, C reactive protein (CRP), adiponectin and leptin. Fasting glucose, total cholesterol, HDL cholesterol, triglycerides and CRP will be determined by a photometric colourimetric optical system (Cobas Mira systems, Roche Diagnostic Corporation, USA). LDL cholesterol will be calculated using Friedewald's formula. Insulin, adiponectin and leptin will be determined in serum by ELISA, using commercial kits (Merck Millipore, Germany). The evaluation of total plasma NO (NO_2+NO_3) concentrations will be performed by a colourimetric assay (Cayman, USA) with a sensitivity of 2.5 µM and a 2.7% intra-assay coefficient of variation.

Exercise testing

All subjects will undergo a symptom-limited, ramp protocol[67 68] on a treadmill (Micromed, Brasilia, Brazil). The exercise testing protocol will be employed to (1) verify if there is any medical issue which might preclude exercise training, such as ischaemic responses to exercise and (2) obtain estimated metabolic equivalent (MET) and maximal oxygen consumption max values. Speed and inclination will be individually adjusted according to the ramp protocol.[67 68] Subjects will have continuous ECG monitoring. A 12-lead ECG will be obtained at rest, every 3 min during exercise, every minute during a 5 min recovery period and additionally whenever necessary. Blood pressure will be measured and recorded every 3 min. Exercise will be terminated due to limiting

dyspnoea or fatigue, anginal symptoms, a fall in blood pressure >20 mm Hg or sustained arrhythmias. An ischaemic exercise treadmill test (ETT) will be defined as that with ≥1 mm ST segment depression, 80 ms after the J-point, independent of the occurrence of exercise-induced angina. Functional capacity will be expressed in MET (1 MET=3.5 mL O_2/kg/min).[68]

All anthropometric measures, blood sampling, exercise testing and measurements of capillary density and microvascular reactivity will be performed before (within 7 days) the beginning of the exercise and dietary interventions and after the end of the interventions (within 7 days of the end of the training protocol). A schedule of interventions is depicted in online supplementary appendix 2. Data collection will be performed by two of the investigators (DVB and RM) as well as trained research personnel from the National Institute of Cardiology.

MEASUREMENTS OF CAPILLARY DENSITY AND MICROVASCULAR REACTIVITY

Microcirculatory tests will be performed, after a 20 min rest in the supine position, in a quiet room with a stable temperature (23°C±1°C) and sea-level air pressure (1 atmosphere or approximately 1000 hPa). Humidity in the air-conditioned rooms of our clinical research setting is typically approximately 60%. The dorsum of the non-dominant middle phalanx will be used for image acquisition while the patient sits comfortably. The arm will be positioned at the level of the heart and immobilised using a vacuum cushion (AB Germa, Kristianstad, Sweden). Capillary density, that is, the number of perfused capillaries per square millimetre of skin area, will be assessed by high-resolution intravital colour microscopy (Moritex, Cambridge, UK), as previously described and validated.[56 69 70] A video-microscopy system with an epi-illuminated fibreoptic microscope containing a 100 W mercury vapour lamp light source and an M200 objective with a final magnification of 200× will be used. Images will be acquired and saved for posterior offline analysis using a semiautomatic integrated system (Microvision Instruments, Evry, France). The mean capillary density for each patient will be calculated as the arithmetic mean of visible (ie, spontaneously perfused) capillaries in three contiguous microscopic fields of 1 mm^2 each. For postocclusive reactive hyperaemia (PORH), a blood pressure cuff will be applied around the patient's arm and inflated to suprasystolic pressure (50 mm Hg greater than systolic arterial pressure) to completely interrupt blood flow for 3 min. This duration of occlusion has been shown to effectively recruit capillaries in an endothelium-dependent manner. After cuff release, images will again be acquired and recorded over the subsequent 60–90 s, during which time the maximal hyperaemic response is expected to occur. The mean number of spontaneously perfused capillaries at rest is considered to represent the functional capillary density, as previously described.[71 72] On the other hand, the number of perfused capillaries during postocclusive reactive hyperaemia represents functional capillary recruitment, resulting from the release of endothelial mediators and consequent arteriolar vasodilation.[71 72]

Cutaneous microvascular reactivity will be evaluated using LSCI (PeriCam PSI System; Perimed, Jarfalla, Sweden) with pharmacological local vasodilator stimuli. Endothelial microvascular function will be evaluated using the transdermal iontophoretic delivery of acetylcholine (ACh), whereas endothelium-independent vasodilation will be studied using the iontophoresis of sodium nitroprusside (SNP). Images will be analysed using the manufacturer's software (PIMSoft; Perimed). Skin sites on the ventral surface of the forearm will be randomly chosen and drug-delivery electrodes will be installed by means of adhesive discs (LI 611; Perimed). ACh 2% w/v or SNP 2% w/v (Sigma Chemical Co, St Louis, Missouri, USA) iontophoresis will be performed using a microphar-macology system (PF 751 PeriIont USB Power Supply; Perimed). Cutaneous microvascular perfusion changes will be measured in arbitrary perfusion units (APU) and as cutaneous vascular conductance (APU divided by mean arterial pressure, values expressed in APU/mm Hg).[69–71] The results of the pharmacological tests will be expressed both as peak values, representing the maximal vasodilation observed after the highest dose of ACh or SNP and area under the curve of vasodilation.

EXERCISE TRAINING PROTOCOLS

The individuals will be allocated to exercise training protocols designed for HIIT or CT, which will be performed in the morning, either after a ≥12 hour fast or in the fed state (according to subject allocation), three times a week for 16 weeks. Exercise will be supervised and performed at a gym located close to the hospital. The HIIT protocol will consist of 40 min on a treadmill: a 10 min warm-up at 70% of maximal predicted heart rate (MPHR, considered as 220 age[73]), followed by four cycles of 4 min at 90% of MPHR, with 3 min of active recovery at 70% of MPHR between each cycle and a 5 min cool down at the end of exercise.[74] The CT protocol will consist of a 47 min session of moderate intensity exercise on a treadmill at 70% of MPRH. Time commitment will equalise energy expenditure between HIIT and CT.[75] Heart rate will be continuously monitored during exercise (Polar V800, Polar Electro Finland Oy, Espoo, Finland) to ensure that the subjects train at the desired intensity. The investigators are trained in cardiopulmonary resuscitation, and all facilities have the necessary apparatus to perform emergency procedures.

Patients will be instructed not to engage in any other exercise training during the study period and will be encouraged to adhere to the study protocol by weekly reminder phone calls.

DIETARY INTERVENTIONS

All subjects will undergo an individualised advice session with a nutritionist, who will discuss healthy food

choices, portion sizes and regular meal times. Individuals randomised to HIIT +FAST or CT +FAST will be instructed to have the last meal of the day at least 12 hours before the time of the training sessions, which will be performed during fasting. During fasting, zero-calorie coffee, tea and water intake will be permitted. Subjects will be instructed not to consume any kind of dietary supplement (eg, protein supplements) and caffeine intake will be controlled. During feeding time, participants will be allowed to eat within the individualised kcal count, calculated according to the 2015 dietary guidelines.[76] Subjects randomised to 'non-fasting exercise' (either HIIT or CT) will be allowed to have breakfast before the training sessions. Dietary intake will be monitored by retrospective methods including 24 hours recall and a food frequency questionnaire.

ADHERENCE ASSESSMENTS

Treatment adherence will be evaluated every 15 days by counting the number of training sessions performed and by in-person dietary evaluation, including a questionnaire of food frequency and a timetable of feeding times. Compliance rates ≥85% with the exercise sessions and with the fasting prescription throughout the study period will be regarded as protocol adherence.

STUDY ENDPOINTS

Primary endpoint

The primary endpoint of this study is the change of capillary density, measured by capillaroscopy, from baseline to the end of the study interventions.

Secondary endpoints

The secondary endpoints will assess differences between the study arms regarding (1) microvascular reactivity, (2) biochemical cardiometabolic risk markers, (3) anthropometric data, (4) quality of life, (5) functional capacity and (6) adherence to the study's interventions. The study's endpoints are detailed in box 2.

Box 2 Endpoints

Primary endpoint

- Capillary density.

Secondary endpoints

- Microvascular reactivity assessed by laser speckle imaging.
- Body mass index.
- Waist circumference.
- Body composition.
- Functional capacity.
- Biochemical cardiovascular risk markers: low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, fasting glucose, insulin, adiponectin, leptin, C reactive protein, total plasma nitric oxide.
- Quality of life.
- Adherence.

MONITORING AND SAFETY

Adverse or unintended events will be continuously monitored by one of the investigators (DVB) and reported to the other team members for adjudication; no independent data monitoring committee will be implemented due to the characteristics of the study (small, single centre). Safety analyses will include tabulation of type and frequency of all adverse events occurring during the study. Premature trial termination due to safety reasons is not expected, as there is little harm posed by the study's interventions due to the careful medical evaluation of the subjects before initiation of the protocols.

STATISTICAL CONSIDERATIONS

Based on previous investigations from our group,[77 78] 16 subjects are needed in each study arm to observe a difference of at least seven capillaries/mm^2 after PORH, with a SD of 7 capillaries/mm^2, an alpha of 5% and power of 80%. To increase the chance of attaining significant differences in secondary endpoints and to allow for IQdropout, 20 subjects will be included in each arm.

For descriptive analysis, categorical data will be presented as per cent frequencies and compared between groups by χ^2 or Fisher's exact test. Continuous variables will be tested for normality and presented as the mean±SD deviation and compared using analysis of variance or presented as the median and 25th to 75th IQR and compared using Mann-Whitney test. Logistic regression and linear regression analyses will be employed to model data with respect to the independent effects on the study's endpoints, without using missing outcome data imputation. Data will be analysed with SPSS V.20.0.

TRIAL STATUS

This study will be initiated in May 2018. Recruitment is expected to be complete by the end of 2018. We believe that the trial will be complete and results will be available in 2019. The current protocol is V.1.0 (08/10/2017).

ETHICS, PATIENT SAFETY AND DISSEMINATION

The trial complies with the Declaration of Helsinki and has been approved by the local ethics committee; any amendments to the study protocol will be submitted. Participants will be informed of the purpose of the study and of the study's procedures before beginning the clinical trial. All study subjects will provide written informed consent before enrolment and randomisation, which will be performed by research staff not taking part in the trial team; online supplementary appendix 3 contains the informed consent form. Patient discontinuation from the study may occur at any time, per patient's request or due to lack of adherence to

the study protocol or the occurrence of any significant condition which may limit or contraindicate the performance of exercise. Discontinued patients will not have their data analysed. If any adverse event occurs to one of the study's participants, access to healthcare will be provided by the National Institute of Cardiology and other institutions of the Brazilian public healthcare system. Unblinding of patient allocation may occur if any adverse event occurs and information regarding the patient's intervention is deemed necessary for medical management.

All study-related information will be stored securely at the study site. A staff of the institution's research team (outside the study's team of investigators) will feed data into datasheets in a computer with restricted access, so that data may be analysed while blinded to patients' intervention. The final dataset will be available to the study team (AL, DVB, RM and ET). All laboratory specimens, reports, data collection, process and administrative forms will be identified by a coded number to maintain participant confidentiality. The investigators take responsibility for the integrity of the data, the writing of the manuscript and the dissemination of the results. Authorship eligibility will follow standard recommendations, [79] and professional writers will not be hired.

The results of this study will be released to the healthcare community. Datasets or statistical codes will not be made public. The study's sponsors did not have any participation in study design and will not have participation in collection, management, analysis or interpretation of data or the writing of the report.

PATIENT AND PUBLIC INVOLVEMENT

Neither patients nor public were involved in the development of the research question, study design, outcome measures, recruitment to and conduct of the study or assessment of the burden of the intervention. The results of the study will be disseminated to study participants by means of lectures given by the investigators.

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Competing interests None declared.

Patient consent Not required.

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