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Study Protocol

Noninvasive imaging assessment of rehabilitation therapy in heart failure with preserved and reduced left ventricular ejection fraction (IMAGING-REHAB-HF): design and rationale

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Abstract

Background: Studies have shown significant benefits of exercise therapy in heart failure (HF) with a reduced ejection fraction (HFrEF) and HF with a preserved ejection fraction (HFpEF). The mechanisms responsible for the beneficial effect of exercise in HFrEF and HFpEF are still unclear. We hypothesized that the effect of exercise on myocardial remodeling may explain its beneficial effect.

Methods: IMAGING-REHAB-HF is a single-center, randomized, controlled clinical trial using cardiac magnetic resonance imaging, vasomotor endothelial function, cardiac sympathetic activity imaging and serum biomarkers to compare the effect of exercise therapy in HFpEF (LVEF $\geq$ 45%) and HFrEF (LVEF $<$ 45%). Subjects will be assessed at baseline and after 4 months. The exercise program will consist of three 60-min exercise sessions/week. The primary endpoints are the effect of exercise on myocardial extracellular volume (ECV), left ventricular (LV) systolic function, LV mass, LV mass-to-volume and LV cardiomyocyte volume. Secondary endpoints include the effect of exercise on vasomotor endothelial function, cardiac sympathetic activity and plasmatic biomarkers. Patients will be allocated in a 2:1 fashion to supervised exercise program or usual care. A total sample size of 90 patients, divided into two groups according to LVEF:HFpEF group (45 patients:30 in the intervention arm and 15 in the control arm) and HFrEF group (45 patients:30 in the intervention arm and 15 in the control arm) – will be necessary to achieve adequate power.

Conclusion: This will be the first study to evaluate the benefits of a rehabilitation program on cardiac remodeling in HF patients. The unique design of our study may provide unique data to further elucidate the mechanisms involved in reverse cardiac remodeling after exercise in HFpEF and HFrEF patients.

Keywords: biomarkers, exercise-training, fibrosis, left ventricular remodeling, magnetic resonance imaging, rehabilitation program, T1 mapping techniques

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Introduction

Heart failure (HF) is a clinical syndrome associated with substantial healthcare costs, high hospitalization rates and premature mortality.[1] Primarily due to the global rise in aging, obesity and other risk factors, the incidence and prevalence of HF will increase.[1,2] More recently, the syndrome has been categorized into three groups, according to the left ventricular (LV) ejection fraction (LVEF): HF with reduced LVEF or LVEF $\leqslant$ 40% (HFrEF); HF with preserved LVEF or LVEF $\geqslant$ 50% (HFpEF); and HF with mid-range LVEF of 40–49% (HFmrEF).[3] However, during the last decades, HFrEF was historically characterized by LVEF $<$ 45%, and HFpEF by LVEF $\geqslant$ 45%. In the present study, we will use 45% LVEF as a threshold to divide the HF groups.

Numerous studies have shown a similar and markedly elevated risk of mortality between HFpEF and HFrEF.[4,5] For example, Lee and colleagues showed similar long-term prognoses between HFpEF and HFrEF patients, adjusted for age and sex. The mortality was equally high in both groups (74% and 95% at 5 and 10 years, respectively).[6] In recent years, pharmacological treatment of HFrEF has significantly improved through control of neurohormonal cascade, including adrenergic and renin–angiotensin–aldosterone system blockade.[7] In contrast, many pharmacological treatment trials showed neutral or negative results in the prognosis of HFpEF patients.[7–12]

The HFpEF phenotype is characterized by concentric/restrictive LV remodeling, and HFrEF is commonly characterized by eccentric/dilated LV remodeling, whereas both phenotypes contribute to cardiac dysfunction and the progression of HF.[13,14] Different patterns of extracellular cardiac fibrosis[15] and cardiomyocyte adaptations[16] may help to explain the different phenotypes, and why responses to treatment might be different between these two conditions. Despite heterogeneity regarding therapeutic responses, nonpharmacological approaches may potentially affect outcomes across the full spectrum of HF. Exercise training programs improve functional capacity and quality of life,[17–19] improve endothelial and muscular functions[20,21] and may reduce neurohormonal activation in HFrEF.[17–19] The benefits of exercise training in HFpEF patients are less studied, but the available evidence suggests that exercise could also improve functional limitations and quality of life.[22,23] Some observations also showed that rehabilitation programs may improve cardiac function and reduced hospitalization in patients with both HFrEF and HFpEF (Table 1).[24–26] However, the potential effects of an exercise rehabilitation program on cardiac remodeling, a surrogate marker for clinical outcomes, remains poorly investigated in HFrEF and HFpEF patients.[27,28]

Our group has recently developed novel magnetic resonance imaging (MRI) markers of myocardial remodeling that may be useful in this scenario. Based on the MRI T1-mapping imaging, both the expansion of extracellular volume (ECV), a marker of interstitial fibrosis, and the intracellular lifetime of water (τ_{ic}), a marker of cardiomyocyte size and cellular hypertrophy,[32] can be accurately characterized. ECV is a strong independent predictor of adverse cardiovascular events and has excellent potential prognostic value.[33–35] In addition, we have validated this technique histologically in a mouse model of pressure overload, demonstrating that: (1) the MRI biomarker may capture the dynamic tissue physiology and treatment effects in HF models;[36] and (2) it differentiates cellular hypertrophy from interstitial fibrosis serially during the development of both phenotypes after pressure overload.[32] In the present study, we hypothesize that exercise training may have a favorable effect on myocardial tissue remodeling in HF patients. We further hypothesize that the effect of exercise on myocardial remodeling, systolic and diastolic function, cardiac sympathetic activity and plasmatic biomarkers may be different between HFrEF and HFpEF patients. In addition, we expect that exercise training may have a favorable effect attenuating cardiomyocyte hypertrophy and interstitial fibrosis, decreasing cell size and myocardial interstitial fibrosis. Changes are also expected in circulating microRNAs and in sympathetic cardiac activity. These novel and investigative changes may be able to differentiate the role of rehabilitation in myocardial remodeling of patients with HFpEF and HFrEF.

Methods

Study design

IMAGING-REHAB-HF is a single-center (UNICAMP, Campinas, SP, Brazil), randomized, open-label, placebo-controlled clinical trial to compare the effect of a standardized HF rehabilitation protocol with usual care, including guideline-driven optimized medical therapy for HF, on

Table 1. The effects of exercise training on systolic and diastolic function in HFpEF.

Study	Group (*n*)	Age (years)	Men (%)	LVEF (% mean) BNP (pg/ml) VO_2 peak (ml/kg/min) (mean)	Training program	Duration protocol	Main endpoints	Results
Kitzman *et al.*[29]	*n* = 63 32 EG 31 CG	70 ± 7	24	58 ± 6 64.8 ± 74.5 14.2 ± 2.8	Aerobic supervised exercise (track walking + cycling) three times per week, with gradually increasing duration and intensity. Intensity: 40–50% of peak VO_2 to 60–70%. Duration increased to 15–20 min.	16 weeks	1. Peak VO_2 2. Brachial artery FMD (flow-mediated arterial dilation) in response to cuff ischemia 3. Carotid artery distensibility 4. LV function 5. Quality of life (QoL)	Peak VO_2 ↑ QoL ↑ Brachial artery FMD and carotid arterial ↔ Resting LV systolic and diastolic function ↔
Edelmann *et al.*[30]	*n* = 64 44 EG 20 CG	65 ± 7	64	67 ± 7 – 16.1 ± 4.9	Supervised, endurance (cycling) + resistance training Aerobic training. Week 1–4: at 50–60% of baseline peak VO_2 and week 5–12: 70% + resistance training	24 weeks	1. Peak oxygen uptake. 2. Systolic, diastolic function by echo 3. LV dimensions 4. QoL	Exercise capacity ↑ QoL ↑ Atrial reverse remodeling ↑ Left ventricular diastolic function ↑
Smart *et al.*[31]	*n* = 25 12 EG 13 CG	64.4 ± 6.4	52	58.9 ± 11.9 – 12.2 ± 3.6	Supervised, outpatient cycle ergometer exercise training. Initial intensity of 60–70% peak VO_2 up-titrated by 2 to 5 watts/week as tolerated.	16 weeks	1. Peak oxygen uptake. 2. Systolic, diastolic function by echo 3. LV dimensions 4. QoL	Peak VO_2 in the exercise ↑ V_E/VCO_2 slope ↓ Diastolic or systolic function ↔ QoL and depression scores ↔
Alves *et al.*[28]	*n* = 31 20 EG 11 CG	62.9 ± 10.2	71	56.3 ± 2.5 – 13.65 ± 4.9	Supervised endurance training (treadmill/cycle). Week 1–4: training at 70–75% of peak VO_2 and week 5–24: training at 70–75% of peak VO_2	24 weeks	1. Peak oxygen uptake. 2. Systolic, diastolic function by echo 3. LV dimensions 4. QoL	Exercise tolerance ↑ LVEF ↑ Diastolic function ↑ Left ventricular dimensions ↔
Fu *et al.*[27]	*n* = 60 30 EG 30 CG	60.5 ± 2.7	66	57.6 ± 1.9 – –	30 min of cycling, three times/week 80% of peak VO_2	12 weeks	1. Peak oxygen 2. Systolic, diastolic function by echocardiogram 3. QoL	LVEF ↔ Diastolic function ↑ VO_2 peak ↑ V_E/VCO_2 slope ↓ QoL ↑

BNP, brain natriuretic peptide; EG, exercise group; CG, control group; LVEF, left ventricular election fraction; peak VO_2, oxygen uptake during peak exercise; V_E/VCO_2, ventilator efficiency; ↑, increase and/or improvement; ↓, decrease and/or worsen; ↔, maintenance/neutral effect.

myocardial tissue remodeling across four groups of HF patients: HFrEF (rehabilitation or usual care) and HFpEF (rehabilitation or usual care). During the screening process, therapy for HF will be carefully reviewed to identify individuals under maximal medical therapy, including, when clinically indicated by recent guidelines, the use of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers, beta blockers, mineralocorticoid receptor antagonists and more recently angiotensin receptor–neprilysin inhibitor.

Our research team will contact all cardiologists from our hospital network to refer HF patients. In addition to that, we will also contact and advertise the current protocol in other neighborhood health institutions. All recruited patients, in both HFpEF and HFrEF groups, will maintain optimal HF medical therapy after randomization according to the most recent guidelines.[37,38] Patients with HFrEF and HFpEF will be enrolled as indicated in the study design flow chart (Figure 1). Quickly after recruitment, HFpEF and HFrEF patients will be randomly allocated to rehabilitation or usual care in a 2:1 fashion, in blocks of 6 patients, to achieve a total of 30 individuals for each intervention arm and 15 in each control arm (randomization will be performed using the website https://www.sealedenvelope.com), giving a total of 90 patients: 45 HFpEF and 45 HFrEF patients. Regardless of allocation group, patients will be motivated to follow all clinical recommendations and to follow protocol steps. Our research staff will contact patients to remind them about their research appointments and visits. All participants will receive a unique random code as ID and all protected health information (PHI) will be protected using HIPAA-compatible encryption. All collected variables will be entered blinded to any PHI. Data will be stored in a.csv format using a home-developed database stored on a safe local network. Our team will search for duplicate entries and entries with abnormal value ranges every week in a fashion blinded to any patient-sensitive information. Data source files will be available for independent quality control assurance. Variables of interest will be obtained at baseline (before intervention initiation) and after 4 months of supervised rehabilitation or usual care.

The UNICAMP Research Ethics Committee under the registration number CAAE: 53967215.8.0000.5404 approved the study (ClinicalTrials.gov identifier: NCT03084679). All patients will provide written informed consent prior to participation in the study. Detailed research procedures, including all clinical visits as well as all blood tests and quality of life (QoL) questionnaires, are displayed in Table 2. Inclusion and exclusion criteria are displayed below. Additional design details according to the most recent SPIRIT recommendation for interventional trials are available (online supplemental file in the online-only data supplement).

Inclusion criteria for HFrEF:

- Age 18–75 years.
- Functional limitation (New York Heart Association class II or worse).
- Evidence of LVEF dysfunction (LVEF $< 45\%$ assessed by transthoracic echocardiogram using Simpson's method) within 3 months of recruitment.
- No contraindications to exercise (by ACC/AHA criteria[39]).
- Cardiac MRI eligible criteria (no metallic hazards, eGFR > 40 ml/min/1.73 m^2, etc.).
- A prior diagnosis of HF (by Framingham criteria).
- Stable diuretic therapy and euvolemic status (as assessed by an attending cardiologist within 1 month of cardiopulmonary exercise testing).
- Qualifying echocardiography.

Inclusion criteria for HFpEF:

- Age 18–75 years.
- Functional limitation (New York Heart Association class II or worse).
- Evidence of LVEF dysfunction (LVEF $\geqslant 45\%$ assessed by transthoracic echocardiogram using Simpson's method) within 3 months of recruitment.
- No contraindications to exercise (by ACC/AHA criteria[39]).
- Cardiac MRI eligible criteria (no metallic hazards, eGFR > 40 ml/min/1.73 m^2, etc.).
- A prior diagnosis of HF (by Framingham criteria).
- Stable diuretic therapy and euvolemic status (as assessed by an attending cardiologist within 1 month of cardiopulmonary exercise testing).
- Qualifying echocardiography.

Figure 1A

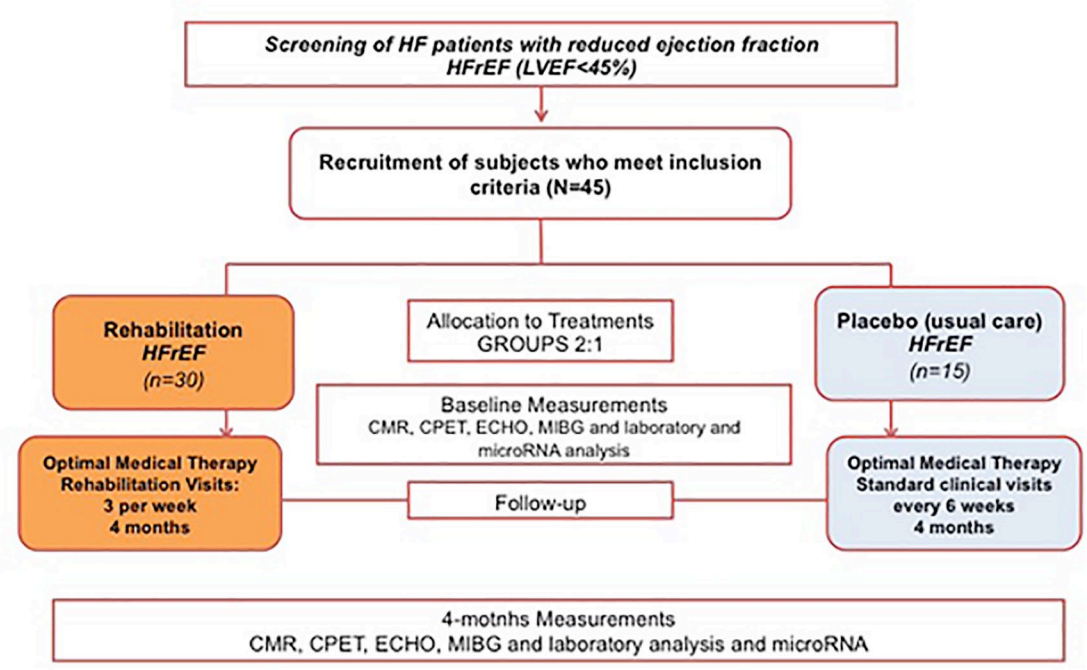

Figure 1B

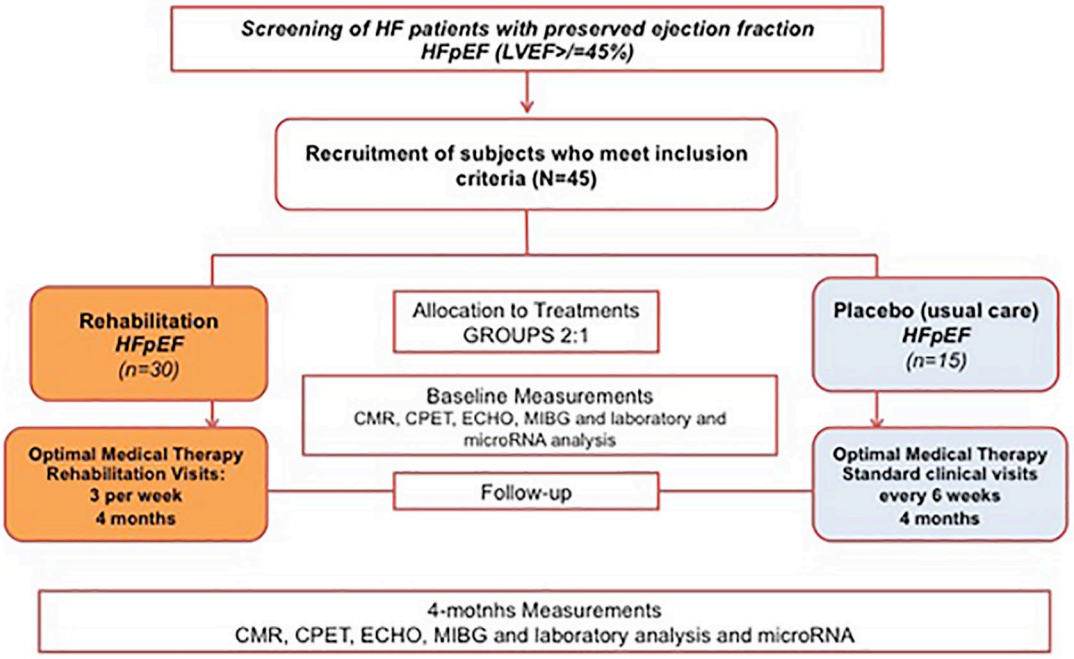

Figure 1. (a) Study flow chart for heart failure with reduced ejection fraction (HFrEF). (b) Study flow chart for heart failure with preserved ejection fraction (HFpEF).
CMR, cardiac magnetic resonance; CPET, cardiopulmonary exercise testing; ECHO, echocardiographic evaluation; LVEF, left ventricular ejection fraction; HF, heart failure; MIBG, [^{131}I]/[^{123}I] metaiodobenzylguanidine.

Table 2. Overview of all visits and tests scheduled for participants of the IMAGING-REHAB-HF trial.

	Screening	Visit 1 baseline/ randomization	Rehabilitation visits 3 × week for 4 months (only for patients allocated to rehabilitation)	Tele call every 4 weeks ± 3 d	Usual clinical visits ± 7 d every 2 months ± 7 d	Final visit
Procedures						
Informed consent	X					
Check inclusion/exclusion criteria	X					
Medical history and family history	X					
Clinical examination	X		X		X	
Demographics	X					
Vital signs	X	X	X		X	X
Safety blood tests and NT-Pro-BNP	X	X			X	X
Research blood		X				X
Genetic blood sample		X				X
MRI		X[a]				X[d]
MIBG		X[b]				X[e]
ECHO		X				X
FMD		X				X
CPET		X[c]				X[f]
QoL questionnaires		X				X
Supervised rehabilitation		X	X			X
Adverse event assessment		X	X	X	X	X
Record or review concomitant meds	X	X	X	X	X	X
Check adherence to optimal HF therapy	X	X	X	X	X	X

[a]Screening MRI to be done only if echo criteria fulfilled. Note MRI can be done up to 12 weeks before visit 1.
[b]Screening mIBG to be done only if echo criteria fulfilled. Note mIBG can be done up to 12 weeks before visit 1.
[c]Screening CPET to be done only if echo criteria fulfilled. Note MRI can be done up to 12 weeks before visit 1.
[d]Final MRI can be done up to 4 weeks after the final visit.
[e]Final mIBG can be done up to 4 weeks after the final visit.
[f]Final CPET can be done up to 4 weeks after the final visit.
CPET, cardiopulmonary exercise testing; ECHO, echocardiographic evaluation; FMD, flow-mediated arterial dilation; MIBG, [131I]/[123I] metaiodobenzylguanidine; MRI, magnetic resonance imaging; NT-Pro-BNP, N-terminal pro b-type natriuretic peptide; QoL, quality of life.

Exclusion criteria:

- Severe ischemia by stress testing in the prior 6 months of screening.
- Hypertrophy cardiomyopathy or infiltrative heart disease (e.g. amyloidosis).
- Severe COPD ($FEV_1 < 1$ L), severe pulmonary hypertension (PA systolic pressure > 60 mmHg) not due to left HF
- Severe right- or left-sided valvular heart disease (stenosis or regurgitation).
- Myocardial infarction (by serum troponin >99th percentile with clinical syndrome) or revascularization within 3 months of screening.
- Anemia (hemoglobin < 10 g/dl) within 1 month of CPET (cardiopulmonary exercise testing).
- Severe hepatic disease.
- Systolic BP < 95 mmHg at the screening visit.
- Unable to walk or to perform cardiopulmonary exercise testing.
- Malignancy (receiving active treatment) or other life-threatening diseases.
- Pregnant or lactating women.
- Participation in any other clinical trial within the previous 30 days.
- Atrial fibrillation.

Cardiac MRI

Cardiac MRI studies will be performed at 3 tesla (Achieva, Philips Medical Systems, Best, the Netherlands) with a six-element phased-array coil. The cardiac MRI protocol will include the following EKG gated sequences: precontrast T1 measurement; myocardial cine tagging with radial read-outs at 5° flip-angle excitations; cine myocardial function assessment with retrospectively gated SSFP technique (TR/TE/flip-angle = 3.2/1.8 ms/45°; slice thickness = 6 mm; 192 × 170 matrix; FOV = ~340 × 320 mm); LGE imaging (10–15 min after 0.2 mmol/kg gadolinium-DTPA); multiple (minimum of four) postcontrast T1 measurements. Standard SSFP sequences will be used for cine imaging of LV function. Myocardial tagging will be used to evaluate circumferential myocardial strains in short-axis slices, using HARP software.[40] T1 measurements will be performed with a breath-hold Look–Locker technique,[41,42] based on a spoiled gradient-echo cine sequence (without SSFP), with a temporal resolution of 80 ms (for precontrast T1 measurements) and 55 ms (for postcontrast T1 measurements), using a nonslice selective adiabatic inversion pulse applied after the detection of an R-wave, followed by a segmented gradient-echo acquisition (for 17 or more cardiac phases), (TR/TE/flip-angle = 5/2.2 ms/10°; slice thickness = 8 mm; 192 × 100 matrix; FOV = ~340 × 30–340 mm; NEX = 1 and a SENSE of 2 for parallel imaging acceleration), as used previously in studies by our group.[43–45] T1 measurements will be made at three slice locations (basal, mid, apical), with one slice imaged per 15–20 s breath-hold. We will acquire five T1 datasets in each patient. A two-site model of 1H exchange will be used to fit both τ_{ic} and ECV.[43]

Cardiac sympathetic imaging with ^{123}I-mIBG

To assess sympathetic hyperactivity in the heart, planar and SPECT/CT (single photon emission computed tomography/computed tomography) images of the myocardial innervation will be performed after the intravenous administration of 111–185 MBq (3–5 mCi) of ^{123}I-mIBG (IPEN, Rio de Janeiro, Brazil). The images will be obtained 15–30 min (early) and 3–4 h (delayed) after radiopharmaceutical injection using SPECT/CT equipment (Symbia T2, Siemens Healthcare, Erlangen, Germany). A heart-to-mediastinum uptake ratio (H/M) will be obtained by drawing regions of interest (ROIs) over the heart and mediastinum. SPECT/CT images of the heart will be used to evaluate the regional sympathetic activity. ^{123}I-mIBG SPECT images will also be compared with stress and rest ^{99m}Tc-sestamibi-SPECT myocardial perfusion images performed on a separate day within 1 week to account for differences between regional innervation and perfusion. Cardiac sympathetic hyperactivity is associated with HF progression, arrhythmias and cardiac death. ^{123}I-mIBG uptake and its distribution in the myocardium will be evaluated by a trained and certified nuclear physician using a semi-quantification method (H/M ratio) and polar maps obtained from the SPECT images. Early and late cardiac uptake of ^{123}I-mIBG will be used to calculate the cardiac washout rate of ^{123}I-mIBG.

Cardiopulmonary exercise testing

CPET will be performed using established standard clinical methods.[46–51] CPET provides several physiologic measurements indicative of acute cardiovascular adaptation to exercise and

cardiovascular reserve capacity. Measurement of these quantitative parameters will permit the determination of fundamental relationships between myocardial tissue and cardiovascular reserve capacity. Patients will undergo maximum ramp protocol with work rate increment of 5–15 watt/min every minute until exhaustion on cycle ergometry (MedGraphics, St. Paul, USA) as described earlier.[49] The respiratory gas exchange will be measured at rest and during exercise (Medical Graphics Corp, St. Paul, USA). Oxygen uptake (VO_2), carbon dioxide output (VCO_2), and respiratory exchange ratio will be calculated by the gas exchange on a breath-by-breath basis in a computerized system. Peak VO_2 will be defined as the highest O_2 uptake, averaged over 30 s, during the last minute of symptom-limited exercise.[49] We will also assess, as previously described:[49] (1) anaerobic threshold (AT) and respiratory compensation point (RCP); (2) oxygen pulse; (3) ventilatory efficiency (V_E/VCO_2 slope); (4) exercise oscillatory ventilation (EOV); (5) oxygen uptake efficiency slope (OUES); and (6) aerobic efficiency (VO_2/watt).

Echocardiographic data analysis

Two-dimensional echocardiographic evaluation (Vivid-S60, GE-Healthcare, Milwaukee, USA) will be undertaken with patients in a left lateral decubitus with a 3.5 MHz transducer in a standardized manner as approved by current American Society of Echocardiography (ASE) guidelines.[52] LV diastolic function, including tissue Doppler myocardial velocities (acquired in early diastole at the anterior, inferior, lateral and septal mitral annulus in the apical two-chamber and four-chamber) will be assessed in all patients also following recent ASE recommendations.[53] Peak systolic longitudinal strain and strain rate of the left ventricle contraction will be performed using speckle-tracking based imaging. All data will be stored in cine-loop format and will be analyzed blinded to patients' information.

Flow-mediated dilation

Flow-mediated dilation enables assessment of the vasomotor function of the endothelium and correlates with cardiovascular prognosis. The procedure happens as follows. After overnight fasting and avoidance of vasoactive medications for the previous 24 h, the patient is placed lying down in a quiet room with a controlled temperature (20–22°C). A cuff is placed around the forearm and baseline measurements of the brachial artery are obtained: diameter and flow parameters. The cuff is then inflated up to 50 mmHg above the systolic blood pressure for 5 min. After 5 min of ischemia of the limb, the cuff is deflated and the baseline parameters are reassessed. Brachial artery measurements will be performed using a high-resolution ultrasound (Vivid S-60). Percentage change in brachial diameter and flow parameters will be calculated. Flow parameters are the velocity–time integral of the antegrade and retrograde brachial flow, as determined by Doppler flow evaluation, and the derived parameters of blood flow (the product of cross-sectional area and Doppler velocity) and shear rate (four times velocity divided by the arterial diameter). An independent physician will analyze videos offline a posteriori. These are validated methods used for covariate adjustment and macro- and microvascular function. The same experienced physician blinded to patients' data will analyze brachial artery reactivity.

Biochemical and biomarkers analysis

Peripheral blood samples will be collected at baseline and after 16 weeks of the rehabilitation program or the usual care. The analysis will include: glucose (mg/dl), glycated hemoglobin (%), triglycerides (mg/dl), high- and low-density lipoprotein cholesterol (mg/dl), high-sensitivity C-reactive protein (CRP, mg/dl), creatinine kinase (CK, mg/dl), creatinine kinase muscle/brain fraction (CKMB, mg/dl), high-sensitivity troponin T (cTnT) and N-terminal pro-b-type natriuretic peptide (NT-pro-BNP) and interleukin-6 (IL-6). Hemoglobin (pg/dl), hematocrit (%), total white blood count (g/dl) and glomerular filtration rate (GFR, ml/min/1.73 m^2), as well as markers of collagen metabolism (synthesis and degradation), comprising C-terminal propeptide of type I procollagen (PICP, μg/L), matrix metalloproteinase 1 (MMP-1, ng/ml) and tissue inhibitor of metalloproteinase 1 (TIMP-1, ng/ml) will also be measured.

Detection and quantification of miRNAs by quantitative PCR

The expression of serum miRNAs will be performed at baseline and after the rehabilitation program in all HF groups. At baseline, the expression of serum miRNAs will be assessed using the

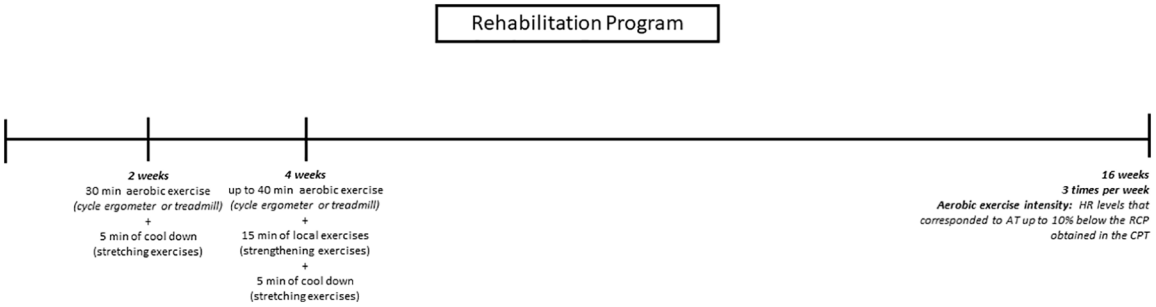

Figure 2. Rehabilitation program.
AT, anaerobic threshold; CPET, cardiopulmonary exercise testing; HR, heart rates; RCP, respiratory compensation point.

TaqMan OpenArray Human MicroRNA system, a quantitative polymerase chain reaction (qPCR)-based miRNA array platform that contains 754 microRNAs on a microfluidic platform across two sets of primer pools, panel A and B (Life Technologies, Carlsbad, Californina, USA), as previously reported.[54] The serum expression of miRNAs that show different expression between the HFpEF and HFrEF groups at baseline will be reanalyzed by real-time PCR after the rehabilitation program in HF patients. Recently, a series of circulating miRNA were shown to provide adequate differentiation between HFrEF and HFpEF,[55,56] enhancing the characterization of HF subtypes over traditional biochemical and imaging biomarkers. Based on this observation, we will evaluate whether any miRNA evaluated at baseline will predict changes in CPET, MRI and NT-proBNP analyses after the rehabilitation program.[54]

Rehabilitation intervention

The rehabilitation program was previously described to improve functional capacity in HFrEF patients.[57] The exercise program will be conducted for 16 weeks, three 60-min exercise sessions per week (Figure 2). Each exercise session will consist of 30 min of aerobic exercise on an ergometer bicycle or on a treadmill in the first 2 weeks and up to 40 min in the rest of the period, 15 min of local strengthening exercises and 5 min of cool-down with stretching exercises. The exercise intensity will be established by heart rate levels that correspond to the AT up to 10% below the RCP obtained in the CPET. When a training effect is observed, the bicycle work rate or the velocity will be increased to return to the target heart rate levels.[57] Patients will undergo exercise training under supervision at UNICAMP. The untrained patients will be instructed to avoid any regular exercise program but should retain their daily habits.

The rehabilitation exercise protocol has been shown to be not only safe but also to improve outcomes in HF patients. Although we don't expected an increased incidence of side-effects with the current intervention, any side-effects will be collected and graded according to the most recent Common Terminology Criteria for Adverse Events (NCI CTCAE). In addition, the current intervention will be discontinued if patients demonstrate any signs of significant clinical deterioration or decompensated HF.

Study outcomes

Primary outcomes. The primary outcome of the current study is to investigate whether the rehabilitation program compared to usual care is associated with significant favorable myocardial remodeling assessed by cardiac MRI determination of ECV in noninfarcted segments (i.e. myocardium without LGE).

Secondary outcomes. The secondary outcomes will assess whether the intervention will modify established cardiac MRI of myocardial remodeling and echocardiographic myocardial strain parameters, prognostic indicators derived from CPET, biochemical laboratory parameters, including conventional and novel HF biomarkers, cardiac sympathetic activity, and peripheral endothelial function as indicated in Table 3.

Sample size and power calculations and statistical methods. The primary outcome of the present study is ECV change after intervention or usual

Table 3. Primary and secondary outcomes.

MRI	ECHO	CPET	MIBG	Biomarkers	FMD
LVEDVi (ml/m^2)	GLS	Peak VO_2 AT	Early [^{123}I] mIBG H/M	NT-proBNP, IL-6, cTnT, PICP, MMP-1 TIMP–1.	Brachial dilatation
LVESVi (ml/m^2)	GCS	RCP	Late [^{123}I] mIBG H/M	Hb (pg/dl), Ht (%), WBC (10^3/mm^3)	Blood flow
LVMi (g/m^2)	Strain Rate	EOV		HDL (mg/dl), LDL (mg/dl), Trig (mg/dl)	Shear rate
LAVi (ml/m^2)		OUES		CRP (mg/dl)	
Intracellular lifetime of water (τ_{ic}) extracellular volume fraction		Oxygen pulse VO_2/watt		Glucose (mg/dl), glycated hemoglobin (%)	
				GFR, CK (mg/dl), CKMB (mg/dl)	

AT, anaerobic threshold; CK, creatinine kinase; CKMB, creatinine kinase muscle/brain fraction; CRP, high-sensitivity C-reactive protein levels; cTnT, high-sensitivity troponin T; EOV, exercise oscillatory ventilation; GCS, global circumferential strain; GFR, glomerular filtration rate; GLS, global longitudinal strain; Hb, hemoglobin; HDL, high-density lipoprotein; H/M, heart-to-mediastinum ratio; Ht, hematocrit; IL-6, interleukin 6; LAVi, left atrium volume (LAV); LDL, low-density lipoprotein; LVEDVi, left ventricle end diastolic volume; LVMi, left ventricle mass index; LVESVi, left ventricle end systolic volume; MMP-1, matrix metalloproteinase 1; NT-pro-BNP, N-terminal pro b-type natriuretic peptide; OUES, oxygen uptake efficiency slope; PICP, C-terminal propeptide of type I procollagen; RCP, respiratory compensation point; V_E/VCO_2, ventilatory efficiency; VO_2/watt, aerobic efficiency; TIMP-1, tissue inhibitor of metalloproteinase 1; Trig, triglycerides; WBC, white blood cells.

care. The sample size calculation for this study was based on a previous estimate of a clinically meaningful change of histological fibrosis (i.e. collagen volume fraction) amounting to 3%, which corresponds to an approximately 0.038 change of ECV. (This estimate of the ECV change for a 3% increase of histological fibrosis was derived from the average observed correlation between histological fibrosis and ECV in various diseases such as hypertension and aortic stenosis.) To detect an ECV change of this magnitude in myocardial regions without LGE (i.e. noninfarcted) with 80% power and an alpha error of 0.05 requires >27 individuals for each HF group receiving the intervention under investigation.[58]

Numeric data will be presented as means ± standard deviation, proportions, or medians (interquartile range) according to its distribution. *t* tests, for continuous data, and Fisher's test or χ^2 test, for dichotomous data, will be used to compare baseline variables. Changes after rehabilitation (usual care *versus* rehabilitation) among groups (HFpEF and HFrEF) will be assessed by analysis of covariance and/or liner mixed effect (LME) regression models (package *lme4* in R) when appropriate. Post-hoc comparisons adjusted for multiple testing will be applied for secondary analyses. Pearson's method or Spearman rank correlation will be used to assess statistical dependence between variables. Analyses will be performed with SAS 9.4 (SAS Institute, Inc., Cary, USA) and using R (version 3.3.2, R Foundation; http://www.R-project.org).

Discussion

The present proposed study is a prospective and randomized trial, designed to determine whether the rehabilitation program causes different effects on the reverse remodeling of myocardial tissue and on the cardiac function in patients with HFpEF and HFrEF, respectively. This is the first study that evaluates the benefits of moderate aerobic exercise training on interstitial fibrosis, cardiomyocyte size and cellular hypertrophy in patients with HF. In addition, this trial will provide novel evidence to investigate the effects of exercise training on reverse myocardial tissue remodeling. The design of our study may provide

unique data to further elucidate the mechanisms involved in reverse myocardial tissue remodeling, by comparing it with changes in cardiac sympathetic activity, plasmatic biomarkers, and miRNA after the rehabilitation program in HF patients.

Exercise training is an important, effective and safe strategy, class I recommendation, for treatment of patients with stable class II to III HF.[59] The rehabilitation program is associated with important reductions in adverse events and hospitalization of patients with HFrEF or HFpEF.[60] Moreover, poor and intermediate physical activity levels were associated with a twofold increased risk of HF hospitalization and mortality in patients with HFpEF.[61]

Despite the consolidated positive systemic effects of regular exercise training in HF patients, the benefits of exercise training on myocardial tissue remodeling and cardiac function remain controversial. Evidence suggests that exercise training could improve functional and structural changes in the LV. In HF animal models, exercise training increases fractional shortening and reduces cross-sectional fiber diameter and cardiac collagen volume.[62] Several reports have investigated the effects of different cardiac rehabilitation programs on myocardial remodeling in CAD patients with HF with preserved[63–65] and reduced[66–68] EF. In patients with HFrEF, some studies showed that exercise training could improve cardiac output, stroke volume, and LVEF, while other studies did not show benefits of exercise training on cardiac function.[69] Erbs and colleagues showed that moderate aerobic exercise training has a beneficial effect on stroke volume, cardiac output and LVEF in advanced chronic HFrEF patients.[26] Exercise training also significantly improves the ratio E/E′, suggesting an improvement in diastolic function in patients with either HFrEF or HFpEF.[24] Although the beneficial effects of exercise training have been seen in some of these observations, partially improving various markers of LV remodeling, none of these studies specifically assessed myocardial tissue phenotyping using recently developed noninvasive imaging modalities.

The potential for reverse remodeling of myocardial tissue could be associated with the level of cardiomyocyte hypertrophy and interstitial collagen content. Unfortunately, standard, clinically available imaging methods are limited in evaluating myocardial tissue properties. The current imaging methods detect large and advanced tissue pathologies in patients, like necrosis by late gadolinium enhancement with cardiac MRI, but cannot detect small and earlier tissue changes. Novel cardiac MRI techniques to quantify the cardiomyocyte hypertrophy and interstitial expansion are noninvasive and applicable in patients.[32,70–72] Using these innovative cardiac MRI techniques, the present study will show the effects of a regular program of exercise on interstitial fibrosis, cardiomyocyte diameter and cellular hypertrophy in patients with HF. It is well known that pharmacological treatment has different effects on reverse remodeling in HFpEF and HFrEF, so it is possible that exercise training also has different effects on myocardial tissue in patients with HFpEF and HFrEF. The present study is the first to compare the effects of exercise training on cardiac function and phenotype in both HFpEF and HFrEF. In addition, we explore whether cardiac sympathetic activity, endothelial dysfunction and/or miRNAs explain the differences between responses to intervention regarding reverse remodeling and improvement in plasmatic biomarkers.

Conclusion

HF is a burdensome condition associated with high healthcare costs. Although major clinical trials have shown benefits of pharmacological treatment, particularly in HFrEF, morbidity and mortality do not differ significantly between the two spectra of the disease (HFrEF and HFpEF). In parallel, evidence suggests exercise training programs may be beneficial for both HFrEF and HFpEF patients, but little is known about their effect on cardiac remodeling. In this study, we aim to evaluate the possible effects of moderate aerobic exercise training on interstitial fibrosis, cardiomyocyte size and cellular hypertrophy in HF patients. The unique design of our study may provide data to further elucidate the mechanisms involved in the reverse myocardial tissue remodeling, by assessment of cardiac sympathetic activity, plasmatic biomarkers and miRNA changes after the rehabilitation.

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Conflict of interest statement

The author(s) declared following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Dr. Neilan reports consultancy for Parexel, Intrinsic Imaging, BMS and Aprea Therapeutics unrelated to the contents of this article. The other authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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