

## Effects of short-term high-intensity interval and continuous exercise training on body composition and cardiac function in obese sarcopenic rats

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### ABSTRACT

**Aim:** We investigated the effects of high-intensity interval and continuous short-term exercise on body composition and cardiac function after myocardial ischemia-reperfusion injury (IRI) in obese rats.

**Methods:** Rats fed with a standard chow diet (SC) or high-fat diet (HFD) for 20 weeks underwent systolic blood pressure (SBP), glycemia and dual-energy X-ray absorptiometry analyses. Then, animals fed with HFD were subdivided into three groups: sedentary (HFD-SED); moderate-intensity continuous training (HFD-MICT); and high-intensity interval training (HFD-HIIT). Exercised groups underwent four isocaloric aerobic exercise sessions, in which HFD-MICT maintained the intensity continuously and HFD-HIIT alternated it. After exercise sessions, all groups underwent global IRI and myocardial infarct size (IS) was determined histologically. Fat and muscle mass were weighted, and protein levels involved in muscle metabolism were assessed in skeletal muscle.

**Results:** HFD-fed versus SC-fed rats reduced lean body mass by 31% ( $P < 0.001$ ), while SBP, glycemia and body fat percentage were increased by 10% ( $P = 0.04$ ), 30% ( $P = 0.006$ ) and 54% ( $P < 0.001$ ); respectively. HFD-induced muscle atrophy was restored in exercised groups, as only HFD-SED presented lower gastrocnemius (32%;  $P = 0.001$ ) and quadriceps mass (62%;  $P < 0.001$ ) than SC. PGC1- $\alpha$  expression was 2.7-fold higher in HFD-HIIT versus HFD-SED ( $P = 0.04$ ), whereas HFD-HIIT and HFD-MICT exhibited 1.7-fold increase in p-mTOR<sup>Ser2481</sup> levels compared to HFD-SED ( $P = 0.04$ ). Although no difference was detected among groups for IS ( $P = 0.30$ ), only HFD-HIIT preserved left-ventricle developed pressure after IRI (+0.7 mmHg;  $P = 0.9$ ).

**Significance:** Short-term exercise, continuous or HIIT, restored HFD-induced muscle atrophy and increased mTOR expression, but only HIIT maintained myocardial contractility following IRI in obese animals.

### 1. Introduction

Obesity, known as a multifactorial chronic disease resulting in excessive body fat accumulation, increases the risk of cardiovascular diseases, including hypertension, coronary artery disease and myocardial infarction [1–3]. The loss of muscle mass has also been associated with cardiovascular risk factors and adverse health outcomes, such as physical disability and mortality [4–6]. Therefore, obesity associated with muscle mass loss, known as sarcopenic obesity, may exert a greater impact in cardiovascular diseases and mortality compared to obesity or sarcopenia alone [4].

Aerobic exercise training contributes to clinically significant fat loss, muscle mass gain and cardiovascular risk reduction [7,8]. Exercise protects the heart against cardiac insults by preconditioning the myocardium [9,10]. Studies using animal models provide evidence that both long-term [11,12] (weeks to months) and short-term [13–15] (1–5 days) aerobic exercise improves myocardial tolerance to ischemia-reperfusion injury (IRI) [16]. Although it is clear that moderate-to-high continuous aerobic exercise induces cardioprotection, the potential role of high-intensity interval short-term exercise in providing increased protection against IRI is poorly understood [17,18], especially in

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association with pre-existing comorbidities [19]. Evidence in this sense would be important as high-intensity interval training (HIIT), which consists of alternating periods of greater and lower intensity within an exercise session, has been increasingly prescribed to subjects with and without cardiovascular diseases [20] to overload the cardiovascular system and stimulate greater adaptations in a shorter exercise session [20]. Well-controlled clinical trials involving patients with cardiovascular diseases have demonstrated that HIIT is more beneficial than moderate-intensity continuous training (MICT) for improvements in aerobic capacity, insulin sensitivity and endothelial and mitochondrial function [21,22].

However, the impact of HIIT on body mass and composition is controversial [23]. Recently, a meta-analysis including 36 human trials indicated that HIIT provided 28.5% greater reduction in total fat mass than MICT [24]. On the other hand, Martins et al. [23] indicated that isocaloric training protocols of HIIT and MICT exert similar improvements on body composition in sedentary obese patients. In addition, the effects of few sessions of HIIT on body composition or key proteins related to muscle mass metabolism have been neglected. Data in this sense would be interesting as there seems to exist an inverse association between the incidence of cardiovascular disease and preservation of skeletal muscle mass [4,6]. Therefore, we aimed to investigate the effects of short-term HIIT and MICT with equal volume on body mass composition, as well as cardiac function following IRI in diet-induced obese rats.

## 2. Materials and methods

### 2.1. Animals and ethics statement

Animal experiments complied with the ARRIVE guidelines and were carried out in accordance with the Guide for the Care and Use of Laboratory Animals according to the conventional guidelines for experimentation with animals (National Institute of Health Guide, 8th edition, 2011) and were approved by the Ethics Committee on Animal Use of the Instituto Oswaldo Cruz (Protocol number 039/2016).

Male Wistar rats provided by the animal facilities at the Instituto Oswaldo Cruz (Rio de Janeiro, RJ, Brazil) were kept under a 12:12-hour light-dark cycle in a temperature-controlled environment (22 °C) with food and water provided *ad libitum*.

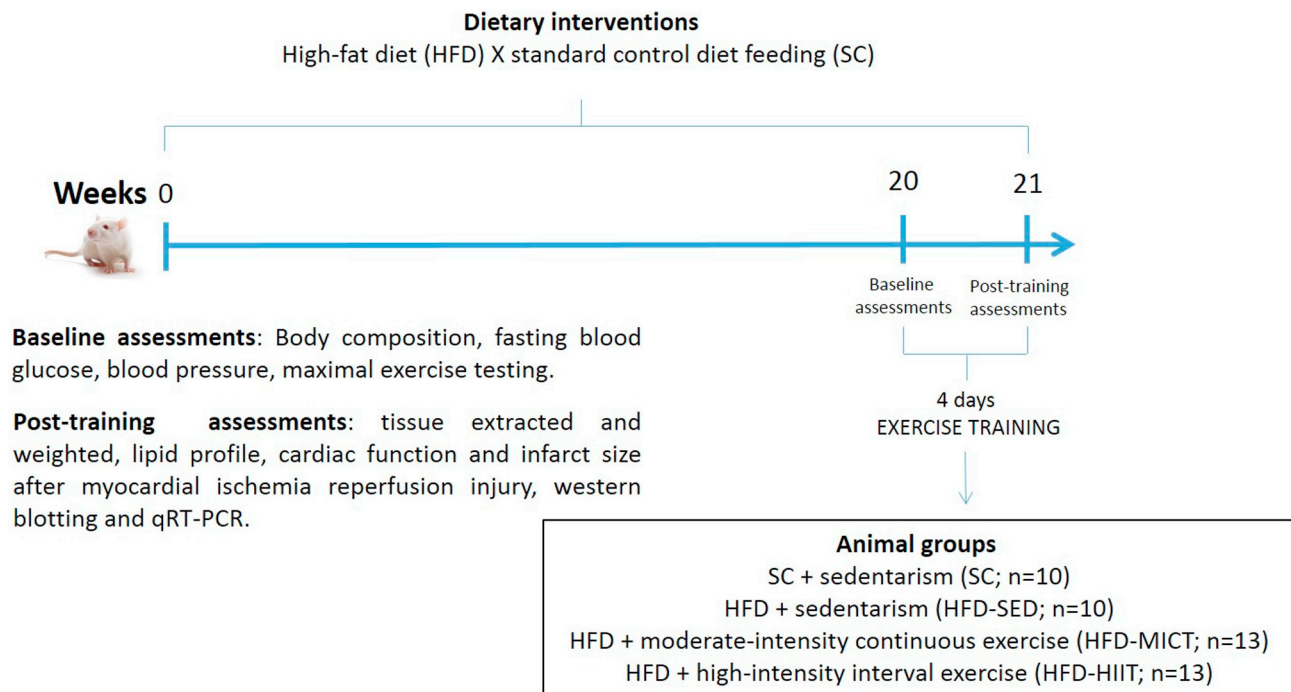
### 2.2. Experimental design

At six weeks of age, animals were randomly allocated into a standard chow diet (SC, n = 10) or a high-fat diet (HFD, n = 36) group. The SC diet contained 23% of energy from protein, 71% from carbohydrates, 6% from lipids and 1.3% of NaCl (17.9 kJ/kg); while the HFD contained 14% of energy from protein, 56% from carbohydrates, 30% from lipids and salt supplementation (standard chow + corn starch + condensed milk + animal fat + 0.5% NaCl) (23 kJ/kg). The main fat source in the HFD was saturated fat (lard) [25]. At the end of the 20th week of diet, blood pressure, fasting blood glucose and dual-energy X-ray absorptiometry (DXA) analysis were performed in SC and HFD groups.

Following those assessments, animals that received HFD were randomly subdivided into three experimental groups: 1) sedentary (HFD-SED, n = 10); 2) moderate-intensity continuous exercise training (HFD-MICT, n = 13); and 3) high-intensity interval exercise training (HFD-HIIT, n = 13). Then, HFD-MICT and HFD-HIIT underwent 4 isocaloric aerobic exercise sessions, in which HFD-MICT maintained the intensity at 50% of oxygen uptake reserve ( $VO_{2R}$ ) while HFD-HIIT alternated 3 min of 60%  $VO_{2R}$  and 4 min of 80%  $VO_{2R}$ .

After 24 h of the end of exercise sessions, the blood of all animals was collected and their hearts were excised and submitted to IRI in the Langendorff system. Following reperfusion, the left ventricles were excised to histologically quantify the infarct size area and assess its morphology, as presented below.

After the death of the animals, gastrocnemius and quadriceps femoris muscles, and epididymal and inguinal fat pads were dissected and weighted. Skeletal muscle was stored at -80 °C until molecular measurements. The experimental design is shown in Fig. 1.



**Fig. 1.** Experimental design of the study. Wistar rats fed for 20 weeks with a standard control (SC) or a high-fat diet (HFD) were submitted to baseline assessments. Then, animals that received HFD were randomly subdivided into a sedentary (HFD-SED), moderate-intensity continuous exercise training (HFD-MICT) and high-intensity interval exercise training (HFD-HIIT) group. Following four days of isocaloric aerobic exercise sessions (HFD-MICT and HFD-HIIT) or sedentarism (SC and HFD-SED), all groups underwent post-training assessments.

### 2.3. Resting blood pressure and fasting blood glucose

Before performing the exercise protocols, resting blood pressure and fasting glucose were assessed in SC and HFD groups. Resting blood pressure was measured with a computerized tail-cuff plethysmography system (Visitech Blood Pressure Analysis System, model BP 2000, Apex, NC, USA) in conscious animals in the morning (8 am to 12 pm). At least one week before recording the blood pressure, rats were acclimated for three consecutive days using the pre-warmed (37 °C) tail cuff device.

Blood glucose was measured in animals after 8 h of fasting. A blood sample was obtained through a little incision of the tail tip to measure the blood glucose levels by means of a portable glucose monitor (One Touch Ultra 2®, LifeScan Inc. Johnson & Johnson, Milpitas, CA, USA).

### 2.4. Body Composition assessment via dual-energy X-ray absorptiometry (DXA)

To verify the effect of diet on body fat and lean mass, before performing the exercise protocols, SC and HFD groups underwent DXA analysis (Lunar IDXA ME, GE Health Care, Little Chalfont, United Kingdom). Data were quantified through Encore 2008 software (GE Health Care Version 12.20). For the analysis, the animals were anesthetized through intraperitoneal injection of ketamine and xylazine (50 and 5 mg·kg<sup>-1</sup>; Sigma Chemical Co, St. Louis, USA) and then positioned under the scanning area of the equipment in ventral decubitus. The scanning was performed by following a sagittal line, which passed under the center of anatomical points, such as the skull, spine and pelvis.

### 2.5. Exercise protocol

Initially, all animals were adapted to a motor-driven rodent treadmill (HT 2.0; Hectron Fitness Equipment, Rio de Janeiro, Brazil) at a low velocity (10 m/min, 0% inclination, 10 min/day, 3 consecutive days). To determine maximal exercise capacity and individual exercise session duration, a maximal exercise testing was carried out in HFD-MICT and HFD-HIIT groups. The test began at an initial velocity of 10 m/min, which was increased by increments of 3 m/min every 3 min. The test was terminated when the animals were exhausted and remained at the end of the mat on the shock grid for 5 s. The bars of the shock grid deliver low electrical currents of ~2 mA that cause minor discomfort and no harm to the animals. For each animal, peak oxygen consumption (VO<sub>2peak</sub>) was measured by assessing the total airflow through the treadmill chamber and assessing the oxygen content of the expired gas using an electronic oxygen analyzer (AVS Projetos, SP, Brazil). The target workload during submaximal exercise bouts was calculated using the oxygen uptake reserve (VO<sub>2R</sub>) method, as previously described [26,27]:  $VO_{2R} = (\text{fraction intensity}) \times (VO_{2\text{peak}} - VO_{2\text{rest}}) + VO_{2\text{rest}}$ .

Between 48 h and 72 h after the maximal exercise testing, HFD-MICT and HFD-HIIT underwent four consecutive isocaloric aerobic exercise sessions, in which HFD-MICT maintained the intensity at 50% of VO<sub>2R</sub> while HFD-HIIT alternated 3 min of 60% VO<sub>2R</sub> and 4 min of 80% VO<sub>2R</sub>. To equalize exercise volume, the duration of exercise sessions of each animal was calculated to elicit 5 kcal of energy expenditure.

### 2.6. Isolated heart preparation

Following 24 h of the last exercise session, all rats, previously heparinized (500 i.u. kg<sup>-1</sup>, i.p.), were anesthetized via intraperitoneal injection of ketamine and xylazine (100 and 10 mg·kg<sup>-1</sup>; Sigma Chemical Co., St. Louis, USA) and the excised hearts were immediately cannulated through the aorta according to the method of Langendorff [28] and perfused via the coronary circulation at a constant flow rate of 10 ml·min<sup>-1</sup> with modified Krebs–Henseleit solution (mM: 118 NaCl,

4.7 KCl, 1.2 MgSO<sub>4</sub>, 1.2 KH<sub>2</sub>PO<sub>4</sub>, 25 NaHCO<sub>3</sub>, 10 glucose and 1.8 CaCl<sub>2</sub>, pH 7.4; gassed with 95% O<sub>2</sub>–5% CO<sub>2</sub>, 37 °C). A latex balloon was inserted in the left ventricle through the left atrium and adjusted to an end-diastolic pressure of 5–10 mmHg at baseline. After 30 min of baseline perfusion, all hearts underwent a period of 30 min of sustained global ischemia followed by 1 h of reperfusion. Left ventricle pressure (monitored via the latex balloon) was recorded at baseline and at the end of the reperfusion period for the calculation of the following variables (ANCAD software, AVS Projetos, Sao Paulo, Brazil): left ventricle developed pressure (LVDP) and maximal and minimum rate of ventricular pressure change (+dP/dt and –dP/dt; respectively) [9,28,29].

### 2.7. Lipids assessments

Immediately after the excision of the heart, 8 h-fasting blood was collected and plasma was separated by centrifugation (120 g for 15 min at 4 °C) and stored at –20 °C until lipids measurements. Total cholesterol and triglycerides were assessed through colorimetric enzymatic assays with endpoint reaction (87–2/100 and 133–1/500, respectively; Labtest, MG, Brazil) and measured with an automated spectrophotometer (BioTek, Winooski, VT, USA).

### 2.8. Infarct size area measurement and heart morphology

After reperfusion, hearts were removed from Langendorff and the infarct size area or morphology were analyzed. Infarct size area was determined in left ventricle samples through the triphenyl tetrazolium chloride (TTC) staining method, which distinguishes necrotic from viable myocardium [13,14]. The myocardial infarct area was assessed in each heart by a blinded observer using planimetry (Image J, NIH Image, USA), and the infarct area was expressed as a percentage of the total area of the left ventricle.

To qualitative assess heart morphology after IRI, fixed left ventricle samples were embedded in paraffin and 5-µm-thick sections were stained with hematoxylin and eosin. Digital images were obtained by light microscope (Leica Microsystems GmbH, Wetzlar, Germany) and an Infinity 1-5c camera (Lumenera Co., Ottawa, ON, Canada).

### 2.9. Western blot

The total skeletal muscle proteins were extracted in a homogenizing buffer with protease and phosphatase inhibitors (MS-SAFE, Sigma-Aldrich). Protein samples were used for electrophoresis and were transferred to a polyvinylidene difluoride membrane. The blot membrane was incubated overnight at 4 °C with the following primary antibodies: PGC1-α (SC-13067, Santa Cruz Biotechnology), total mTOR (SC-8319, Santa Cruz Biotechnology), phosphorylated mTOR<sup>Ser2481</sup> (SC-293132, Santa Cruz Biotechnology) and AMPKα1/2 (SC-25792, Santa Cruz Biotechnology). Binding of the primary antibodies were detected with the use of secondary antibodies, and ECL Western blot reagents were used to visualize images of the blots in a ChemiDoc-System (BioRad, Hercules, CA, USA). β-actin (SC-81178, Santa Cruz Biotechnology) was used as a loading control for proteins. The intensity of the chemiluminescent bands was quantified using ImageJ software, version 1.44 (NIH, [imagej.nih.gov/ij](http://imagej.nih.gov/ij), USA).

### 2.10. Real-time reverse-transcriptase polymerase chain reaction (RT-qPCR)

Total RNA was isolated from skeletal muscle using the RNeasy Fibrous Mini Kit (Qiagen, Hilden, Germany). The cDNA was prepared using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, CA, USA). All amplifications were performed using a 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA) with each forward and reverse primer (Thermo Fisher Scientific, MA, USA) (Supplementary Table 1) and the SYBR Green (Applied Biosystems, Foster City, CA, USA), according to the instructions of the

manufacturer. The expression of the target genes was normalized with the expression of  $\beta$ -actin, and the  $\Delta\Delta Ct$  method was used to determine gene expression.

### 2.11. Statistical analysis

All data normality was confirmed by the Shapiro-Wilk test, except for triglycerides and infarct size area. Differences between groups for the diet effects on blood pressure, fasting glucose and body composition, as well as maximal exercise capacity and duration, were tested by unpaired Student's *t*-test. Comparisons across groups for exercise effects on cholesterol, body composition, gene and protein expression were performed by one-way analysis of variance (ANOVA), followed by Dunnett's or Tukey's multiple comparisons test, while triglycerides and infarct size area were tested by Kruskal-Wallis test followed by Dunn's multiple comparisons test. Differences for cardiac function parameters were tested by two-way ANOVA, followed by Bonferroni as *post hoc* test. The results are expressed as the mean  $\pm$  standard deviation (SD), except for triglycerides and infarct size area that are expressed as the median (range). P-values of  $< 0.05$  were considered statistically significant. All calculations were made by computer-assisted analysis using a commercially available statistical package (Graphpad Prism, Graphpad Software, San Diego, CA, USA).

## 3. Results

### 3.1. Diet effects on blood pressure, fasting glucose and body composition

As demonstrated in Table 1, HFD group presented higher systolic blood pressure, fasting blood glucose and body mass gain than SC group. No difference was detected for food intake between groups. DXA assessment revealed that HFD group exhibited higher total body mass, body fat and lower lean body mass than SC group (Table 1).

### 3.2. Maximal exercise capacity and exercise sessions

Table 2 demonstrates maximal exercise capacity, oxygen uptake, duration and velocity of isocaloric exercise sessions for each group. Oxygen uptake at maximal capacity ( $P = 0.58$ ) and during isocaloric exercise sessions ( $P = 0.34$ ) were similar across groups, while duration of exercise was longer in HFD-MICT than HFD-HIIT ( $P < 0.0001$ ). Additionally, velocity corresponding to 80%  $VO_{2R}$  was significantly higher than 60%  $VO_{2R}$  ( $P < 0.01$ ) and 50%  $VO_{2R}$  ( $P < 0.001$ ), but no difference was detected between 50% and 60%  $VO_{2R}$  ( $P = 0.16$ ).

### 3.3. Cardiac function following myocardial insult

Fig. 2 presents the cardiac function parameters obtained before and after IRI of experimental groups. At baseline, no difference was detected among groups. As shown in Fig. 2A and B, the myocardial insult

**Table 1**

Hemodynamic, fasting blood glucose and body composition data for Standard Chow (SC) and High-Fat Diet (HFD) groups at the 20th week.

	SC (n = 10)	HFD (n = 10)	$P^a$
Systolic blood pressure (mmHg)	145.4 $\pm$ 10.7	160.1 $\pm$ 16.7	0.04
Fasting blood glucose (mg/dL)	111.9 $\pm$ 20.9	157.8 $\pm$ 45.3	0.006
Food intake (g/animal/day)	15.3 $\pm$ 0.7	17.2 $\pm$ 4.7	0.58
Body mass gain (g)	245.5 $\pm$ 19.7	343.3 $\pm$ 66.6	0.01
Total body mass (g)	427.5 $\pm$ 19.7	530.1 $\pm$ 71.6	0.002
Lean mass (g)	316.8 $\pm$ 16.6	241.1 $\pm$ 29.1	$< 0.001$
Body fat (g)	99.1 $\pm$ 13.4	272.6 $\pm$ 74.9	$< 0.001$
Body fat (%)	23.9 $\pm$ 2.8	51.9 $\pm$ 9.4	$< 0.001$

Results expressed as the mean  $\pm$  standard deviation (SD).

<sup>a</sup> Unpaired Student's *t*-test.

**Table 2**

Maximal exercise capacity and exercise sessions characteristics.

	HFD-MICT (n = 13)	HFD-HIIT (n = 13)
$VO_{2peak}$ (mL/kg-min)	62.2 $\pm$ 19.5	66.9 $\pm$ 21.0
Training $VO_2$ (mL/kg-min)	31.9 $\pm$ 18.0	46.3 $\pm$ 21.1
Exercise duration (min)	34.5 $\pm$ 5.7	21.7 $\pm$ 3.4*
Exercise velocity (m/min)		
50% $VO_{2R}$	13.2 $\pm$ 2.0	–
60% $VO_{2R}$	–	14.6 $\pm$ 2.8
80% $VO_{2R}$	–	18.1 $\pm$ 3.2#

Results expressed as the mean  $\pm$  standard deviation (SD). HFD-MICT, high-fat diet and moderate-intensity continuous exercise training group; HFD-HIIT, high-fat diet and high-intensity interval training group,  $VO_2$ , oxygen uptake;  $VO_{2R}$ , oxygen uptake reserve.

\*  $P < 0.05$  vs HFD-MICT.

#  $P < 0.05$  vs 50%  $VO_{2R}$  and 60%  $VO_{2R}$ .

significantly reduced the LVDP and  $+dP/dt$  in SC ( $-44.9$  mmHg and  $-1.253$  mmHg/s; respectively), HFD-SED ( $-31.6$  mmHg and  $-1.104$  mmHg/s; respectively), and HFD-MICT ( $-46.5$  mmHg and  $-836$  mmHg/s).  $-dP/dt$  was significantly increased in SC ( $+565$  mmHg/s) and HFD-SED ( $+470$  mmHg/s) after the myocardial insult, while no difference was detected in HFD-MICT ( $+321$  mmHg/s). No significant difference was observed in HFD-HIIT for LVDP ( $+0.7$  mmHg),  $+dP/dt$  ( $-549$  mmHg/s) and  $-dP/dt$  ( $+198$  mmHg/s).

### 3.4. Lipid profile

As demonstrated in Table 3, HFD-SED, HFD-MICT and HFD-HIIT groups presented higher total plasma cholesterol than SC group ( $P < 0.05$ ). Additionally, plasma triglycerides were higher in HFD-MICT and HFD-SED when compared to SC ( $P = 0.01$ ), but HFD-HIIT presented similar results to SC ( $P = 0.07$ ).

### 3.5. Infarct size and heart morphology

As presented in Fig. 3, infarct size was similar among groups (SC:  $42.9 \pm 54.0$  vs HFD-SED:  $47.6 \pm 45.3$  vs HFD-MICT:  $29.2 \pm 57.9$  vs HFD-HIIT:  $35.9 \pm 49.5\%$ ; Fig. 3;  $P = 0.30$ ). Histological analysis revealed that experimental groups exhibited similar pattern of connective tissue between cardiomyocytes (Supplementary Fig. 1), as expected after a cardiac insult [30].

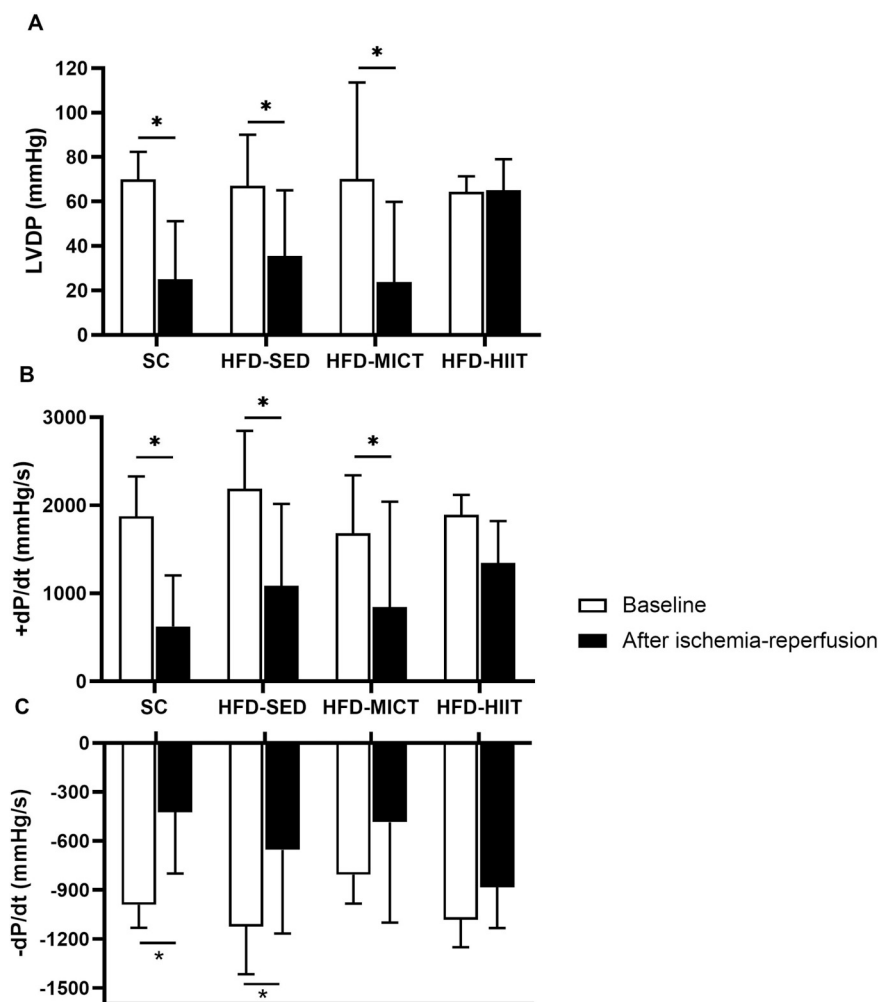
### 3.6. Muscle and adipose tissue mass after exercise protocols

After the death of the animals, muscle and adipose tissue were extracted and weighted. As presented in Fig. 4, HFD-SED exhibited lower gastrocnemius ( $1.48 \pm 0.39$  g; Fig. 4A;  $P = 0.0001$ ) and quadriceps femoris ( $0.48 \pm 0.05$  g; Fig. 4B;  $P = 0.0003$ ) mass than the other groups. However, no difference was detected between SC, HFD-MICT and HFD-HIIT for gastrocnemius (SC:  $2.21 \pm 0.19$  vs HFD-MICT:  $2.1 \pm 0.41$  vs HFD-HIIT:  $2.35 \pm 0.32$  g; Fig. 4A;  $P > 0.05$ ) and quadriceps femoris mass (SC:  $1.27 \pm 0.41$  vs HFD-MICT:  $0.92 \pm 0.21$  vs HFD-HIIT:  $1.03 \pm 0.19$  g; Fig. 4B;  $P > 0.05$ ).

As for the adipose tissue, high-fat diet-fed rats in comparison to SC rats presented higher epididymal (SC:  $5.31 \pm 1.02$  vs HFD-SED:  $21.45 \pm 3.62$  vs HFD-MICT:  $18.82 \pm 7.09$  vs HFD-HIIT:  $15.86 \pm 4.44$  g; Fig. 4C;  $P < 0.0001$ ) and inguinal fat mass (SC:  $1.65 \pm 0.71$  vs HFD-SED:  $5.67 \pm 1.21$  vs HFD-MICT:  $5.20 \pm 1.81$  vs HFD-HIIT:  $4.65 \pm 1.14$  g; Fig. 4D;  $P < 0.0001$ ). No differences were detected among high-fat diet-fed groups ( $P > 0.05$ ).

### 3.7. Protein and gene expression in skeletal muscle

PGC1- $\alpha$  expression was higher in HFD-HIIT versus HFD-SED



**Fig. 2.** Cardiac function parameters before and after myocardial ischemia-reperfusion injury. Panel A demonstrates the left ventricle developed pressure (LVDP), while panel B and C demonstrates the maximal (+dP/dt) and minimum (-dP/dt) rate of left ventricular pressure change; respectively. SC (standard chow, n = 8), HFD-SED (high-fat diet and sedentary, n = 7), HFD-MICT (high-fat diet and moderate-intensity continuous exercise training, n = 6), and HFD-HIIT (high-fat diet and high-intensity interval exercise training, n = 5) groups. Values expressed as means  $\pm$  SDs. Differences across groups tested by two-way ANOVA, followed by Bonferroni test. \*P < 0.05.

(127.4  $\pm$  47.3 vs. 45.7  $\pm$  20.6%; P = 0.04; Fig. 5B), whereas HFD-HIIT and HFD-MICT exhibited higher phosphorylated mTOR protein levels than HFD-SED (HFD-HIIT: 123.0  $\pm$  32.3 and HFD-MICT: 119.8  $\pm$  25.5 vs. HFD-SED: 70.9  $\pm$  20.9%; P = 0.04; Fig. 5C). SC and high-fat fed groups presented similar values for PGC1- $\alpha$  (Fig. 5B), p-mTOR<sup>Ser2481</sup> (Fig. 5C) and AMPK (Fig. 5D). No difference was detected across groups for AMPK protein expression levels (SC: 100.0  $\pm$  31.7, HFD-SED: 91.7  $\pm$  25.9, HFD-MICT: 101.9  $\pm$  21.6 and HFD-HIIT: 101.6  $\pm$  25.6%; P > 0.05; Fig. 5D).

No difference in *Pgc1- $\alpha$* , *Akt*, *Gsk3- $\beta$* , *Hsp70* and *Nrf2* gene expression was detected among group (Supplementary Fig. 2).

#### 4. Discussion

This study investigated the effects of isocaloric high-intensity interval and moderate-intensity continuous short-term exercise on body composition and cardiac function in obese rats following IRI. We

originally demonstrated that only four bouts of high-intensity interval or moderate-intensity continuous exercise prevented obesity-related muscle mass loss in rats. Additionally, molecular analyses in skeletal muscle showed an increase in PGC1- $\alpha$  and p-mTOR<sup>Ser2481</sup> protein expression in exercised animals. However, only high-intensity interval exercise maintained cardiac contractile function following IRI in obese rats.

We observed that rats fed with high-fat diet during 20 weeks presented higher systolic blood pressure, fasting glucose, body fat mass and lower total lean body mass. These results are aligned with previous data from our group [25,31], which demonstrated that long-term administration of the same high-fat diet induced central fat deposition, insulin resistance, glucose intolerance, hypertriglyceridemia, hypercholesterolemia, arterial hypertension, cardiac remodeling, and microcirculatory disturbances. Obesity is a state of low-grade chronic inflammation [32] and overexpression of classical arm of the renin-angiotensin system [33], thus it is possible that mediators that are systemically increased in

**Table 3**

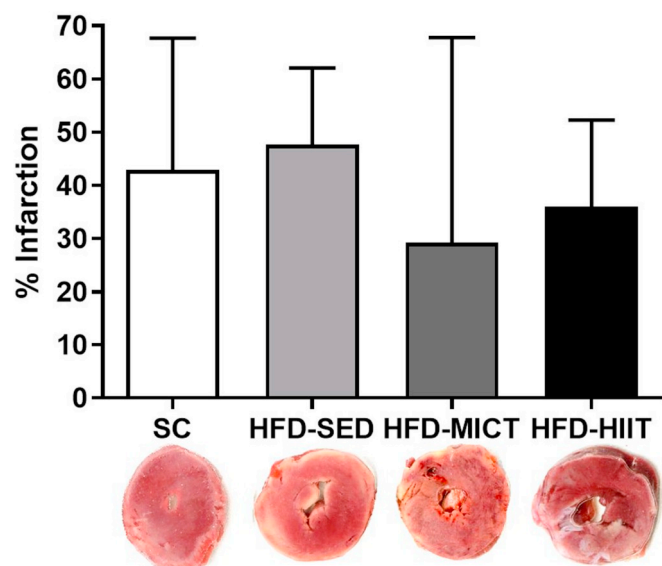
Lipid profile of experimental groups after exercise protocols.

	SC (n = 5)	HFD-SED (n = 4)	HFD-MICT (n = 5)	HFD-HIIT (n = 5)
Cholesterol (mg/dL)	153.5 $\pm$ 22.3	310.4 $\pm$ 29.3*	318.6 $\pm$ 98.8*	263.3 $\pm$ 53.8*
Triglycerides (mg/dL)	149.4 (55.3)	254.1 (92.9)*	243.1 (118.8)*	197.2 (150.8)

Cholesterol expressed as the mean  $\pm$  standard deviation, while triglycerides as median (range).

SC, standard chow group; HFD-SED, high-fat diet and sedentary group, HFD-MICT, high-fat diet and moderate-intensity continuous exercise training group; and HFD-HIIT, high-fat diet and high-intensity interval exercise training group.

\* P > 0.05 vs SC.



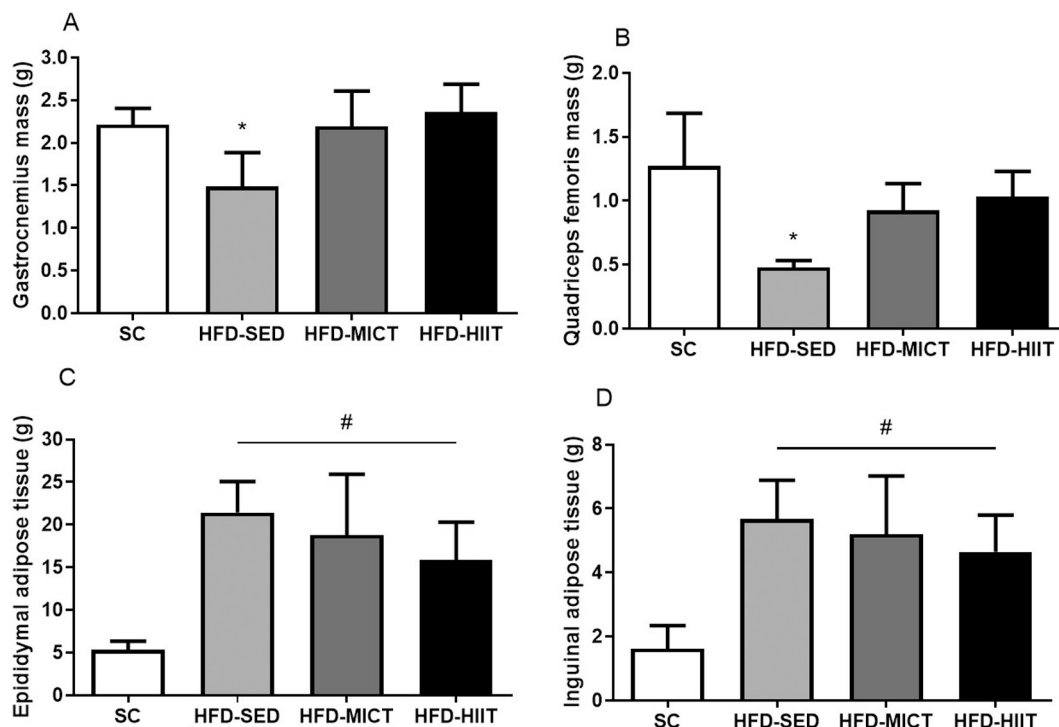
**Fig. 3.** Infarct size area after myocardial ischemia reperfusion injury in experimental groups. SC (standard chow,  $n = 5$ ), HFD-SED (high-fat diet and sedentary,  $n = 5$ ), HFD-MICT (high-fat diet and moderate-intensity continuous exercise training,  $n = 5$ ), and HFD-HIIT (high-fat diet and high-intensity interval exercise training,  $n = 5$ ) groups. Values expressed as median  $\pm$  range. Differences across groups tested by Kruskal-Wallis test, followed by Dunn's multiple comparisons test.

obesity may concomitantly affect the myocardium and skeletal muscle [34]. Accordingly, emerging evidence indicates that obesity may lead to cardiac remodeling [35] and impaired physical mobility, weakness, frailty and muscle loss and dysfunction in middle-aged and older adults [36]. Sarcopenic obesity has a stronger negative prognostic impact on cardiovascular health than obesity and sarcopenia alone [4,34,36,37].

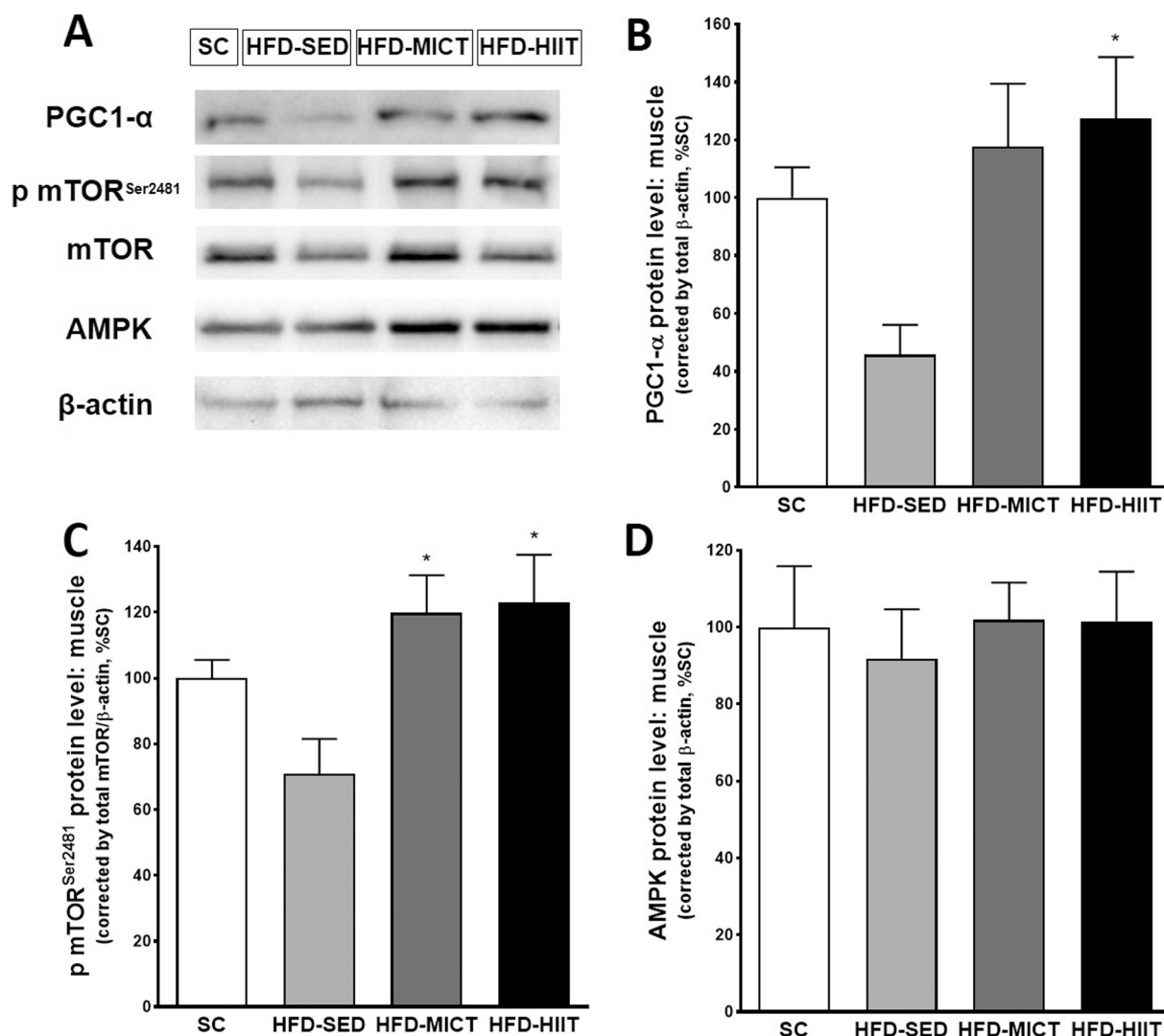
Therefore, developing cardioprotective management strategies that not only reduce body fat, but also revert muscle mass loss are paramount [38].

Clinical and experimental studies demonstrated that exercise training is an efficient strategy to counteract sarcopenic obesity [33,38–42]. For instance, Kim and Lee [39] investigated the role of low-intensity aerobic exercise training for 8 weeks on skeletal muscle protein degradation or synthesis in the plantaris muscle of high-fat-fed ovariectomized rats and demonstrated that exercise inhibited skeletal muscle protein degradation induced by obesity and menopause. One of the several exercise-induced benefits mediated by the protein kinase B (Akt)/mammalian target of rapamycin (mTOR) pathway [43] is the regulation of skeletal muscle size [43]. The phosphorylation of mTOR at Ser2481 has been described as a marker of mTOR complex 2 (mTORC2) integrity, leading to translocation of GLUT4 to the plasma membrane and increased glucose uptake [44–46]. Kleinert et al. [47] showed that mTORC2 is activated during exercise, through mTOR<sup>Ser2481</sup> phosphorylation, in mice skeletal muscle and also suggested mTORC2 as a partial regulator of skeletal muscle glucose metabolism. Herein, we found an increase in p-mTOR<sup>Ser2481</sup> in both exercised groups, which may be involved in reverting muscle mass loss due to improved glucose uptake by skeletal muscle [47].

Peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1 $\alpha$  regulates mitochondrial biogenesis and plays a central role in skeletal muscle adaptations to exercise [48,49]. Hasan et al. [50] demonstrated that rats fed with a high-fat diet for 16 weeks had increased fat mass and lower soleus muscle weight and PGC-1 $\alpha$  expression, and that the treatment with metformin for four weeks restored PGC-1 $\alpha$  expression and morphometric measures. In our study, albeit *Pgc1- $\alpha$*  mRNA levels were unchanged, four sessions of HIIT increased PGC-1 $\alpha$  protein expression in skeletal muscle of obese rats. Gene expressions of *Akt*, *Gsk3- $\beta$* , *Hsp70* and *Nrf2* were also not altered by the exercise protocols. However we cannot rule out the participation of those proteins in muscle mass remodeling, as their protein levels were not



**Fig. 4.** Skeletal muscle and fat pad mass in experimental groups. Gastrocnemius (A), quadriceps femoris (B) skeletal muscle mass, and epididymal (C), inguinal (D) adipose tissue mass in standard chow (SC;  $n = 6$ ), high-fat diet and sedentary (HFD-SED;  $n = 6$ ), high-fat diet and moderate-intensity continuous exercise training (HFD-MICT;  $n = 6$ ), and high-fat diet and high-intensity interval exercise training (HFD-HIIT;  $n = 6$ ) groups. Values expressed as means  $\pm$  SDs. Differences across groups tested by one-way ANOVA, followed by Tukey's test. \* $P < 0.05$  vs all groups and #  $P < 0.05$  vs SC group.



**Fig. 5.** Western blot analysis. (A) Representative bands of Western blot of skeletal muscle tissue. (B) Protein expression of PGC1- $\alpha$ . (C) Protein expression of phosphorylated mTOR<sup>Ser2481</sup> corrected by total mTOR. (D) Protein expression of total AMPK. SC (standard chow, n = 5), HFD-SED (high-fat diet and sedentary, n = 5), HFD-MICT (high-fat diet and moderate-intensity continuous exercise training, n = 5), and HFD-HIIT (high-fat diet and high-intensity interval exercise training, n = 5) groups. Data reported as a percentage of the SC. Values expressed as means  $\pm$  SDs. Differences across groups tested by one-way ANOVA, followed by Tukey's test. \*P < 0.05 vs HFD-SED.

assessed and post-transcriptional modifications and/or increased protein activities might be affecting their function [51,52]. Thus, although increases in PGC1- $\alpha$  and mTOR<sup>Ser2481</sup> protein expression are suggestive of their involvement as mechanisms counteracting obesity-related muscle mass loss, this premise warrants further analysis, such as other downstream targets of mTOR, mRNA levels and protein activity in skeletal muscle.

Interestingly, we have demonstrated that few moderate-intensity continuous and high-intensity interval isocaloric exercise sessions produced similar effects on quadriceps and gastrocnemius muscle mass of obese rats, which suggest that exercise volume, not intensity, might be crucial in this response. By contrast, although our results indicate that more exercise sessions are needed to induce a more significant effect of exercise on body fat mass and metabolic profile, our data concerning triglycerides suggest that exercise seems to improve metabolic profile in an intensity-dependent manner.

As for the exercise impact on the heart, our LVDP and +dP/dt data suggest that no cardioprotection was afforded against IRI at 50% of VO<sub>2R</sub>. In contrast, myocardial relaxation, measured by -dP/dt, seems to be preserved after MICT protocol, which warrants further research. It is still unclear the required exercise intensity to achieve cardioprotection,

as related data are limited and controversial. Prior investigations into the role of intensity suggested that exercise below 55–60% of VO<sub>2max</sub> do not achieve IRI resistance [11,53], whereas another study concluded that both moderate- (55% VO<sub>2max</sub>) and relatively high-intensity (75% VO<sub>2max</sub>) exercise equally protected against ischemia reperfusion-induced myocardial stunning [54]. Therefore, our results are aligned with previous data demonstrating that no cardioprotection is achieved at this intensity level [11,53], reinforcing the premise that there may be a threshold in exercise intensity to achieve cardioprotection [18,19].

On the other hand, HIIT protocol maintained both myocardial contractility and relaxation after the cardiac insult. Among the few studies investigating the effect of HIIT on cardioprotection against IRI, Rahimi et al. [55] demonstrated that 5 days of high-intensity interval exercise protected the heart of healthy rats, while Fallahi et al. [56] demonstrated that healthy rats submitted to eight weeks of HIIT significantly increased nitric oxide metabolites and reduced myocardial infarct size after IRI when compared to control rats. Regarding randomized clinical trials, Wisloff et al. [21] have also provided evidence that HIIT, but not MICT, increased the left ventricular ejection fraction in patients with heart failure.

Taken together, these results suggest that intensity or alternating

intensity within a session may play a key role in cardioprotection. However, the relative contribution of exercise intensity and intermittent nature of the stimulus in exercise-induced effects on cardioprotection and muscle mass remodeling remains undefined. Further studies, especially those with three-arm designs comparing the effects of exercise intensity with modality (continuous or interval), are warranted to elucidate this issue.

Curiously, we found that cardiac function but not myocardial infarct size was favored in HIIT vs. other groups. The quite large but non-significant differences of infarct size data between exercised and non-exercised groups suggest that the study may be underpowered to detect this endpoint due to the relatively small sample size and/or large inter-individual variability of such markers, which must be acknowledged as a major limitation of the study. In addition, molecular analyses to support findings of cardioprotective phenotype were not carried out in the cardiac tissue. Therefore, further studies investigating the mechanisms by which HIIT provides cardiac functioning recovery after IRI in obese rats are necessary. The lack of a healthy control group submitted to exercise should also be mentioned, as it precluded further analysis on exercise-induced cardioprotection and body composition changes in obese *versus* normal-weight rats. Finally, marked physiological differences are notably observed between different species; thus, direct extrapolation of these findings from rats to humans should be approached with caution. Despite these limitations, our findings regarding the effects of HIIT on the heart and skeletal muscle mass are relevant as putative mechanisms triggered by obesity, such as the proinflammatory profile, may concomitantly affect them both [34]. Future studies on the role of inflammatory markers and angiotensin-II on HIIT-induced effects upon the heart and skeletal muscle mass would be interesting.

## 5. Conclusions

In conclusion, short-term continuous or high-intensity interval exercise reversed the high-fat diet-induced muscle atrophy of obese rats, at least in part, due to modulation of mTOR signaling pathway. However, only high-intensity interval exercise afforded cardiac functional recovery of obese rats after IRI. Taken together, our findings provide new insights with clinical relevance, since high-intensity interval exercise not only afforded greater advantage over conventional continuous protocols, but also required a shorter session to achieve such benefits, which may favor adherence of those patients with limited time to regular exercise.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lfs.2020.117920>.

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## Declaration of competing interest

The authors declare that there are no conflicts of interest.

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