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High-intensity interval training increase GATA4, CITED4 and c-Kit and decreases C/EBPβ in rats after myocardial infarction

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ABSTRACT

Aim: Myocardial infarction (MI), an important cause of morbidity and mortality, can be followed by left ventricular dysfunction and cardiomyocyte loss. Cardiac repair mechanisms may subsequently improve left ventricular function. Exercise training has been suggested to have cardioprotective effects against MI damage, but detailed knowledge is lacking on the effects of different types and intensities of exercise training on molecular targets of cardiomyocyte regeneration.

Main methods: MI was induced in male Wistar rats by ligating the left anterior descending coronary artery. After MI induction, the rats were randomly assigned to one of five groups: sham operated, and experimental MI followed by no exercise, or low, moderate or high intensity exercise Cardiac function and infarct size were assessed by echocardiography and Evans blue/TTC staining, respectively. The expression of mRNA markers and proteins associated with myocardial regeneration was measured with RT-PCR and western blotting.

Key findings: Exercise training at different intensities improved cardiac function and levels of stem cell and cardiomyocyte markers, and reduced infarct size. mRNA levels of GATA4, Nkx2.5 and c-Kit and protein expression of Nkx2.5 and c-Kit were significantly increased in all MI-exercise groups. The high-intensity exercise group had greater increases than the low and moderate intensity exercise groups. In the high-intensity exercise group, Sca-1 and CITED4 increased more than in the low-intensity exercise group. C/EBPβ mRNA and protein levels decreased after exercise training, with greater reductions in the high-intensity exercise group than the low- or moderate-intensity groups.

Significance: The findings suggest that by targeting cardiogenesis, high-intensity training can exert cardioprotective effects against cardiac dysfunction in an experimental model of MI.

Abbreviations: MI, myocardial infarction; CSCs, cardiac stem cells; c-Kit, type III receptor tyrosine kinase that encodes stem cell factor; GATA4, GATA-binding protein 4; Nkx2.5, NK2 homeobox 5; C/EBPβ, CCAAT/enhancer-binding protein β; Tbx5, T-box transcription factor 5; CITED4, CREB-binding protein [CBP]/p300-interacting transactivator with ED-rich carboxy-terminal domain-4; Sca-1, stem cell antigen-1; Mef-2c, myocyte-specific enhancer factor 2c; LAD, left anterior descending coronary artery; FS, fractional shortening; MI-Sed, MI sedentary; MI-LIT, MI-low-intensity interval training; MI-MIT, MI-moderate-intensity interval training; MI-HIT, MI-high-intensity interval training; 2D, two dimensional; LVIDd, left ventricular internal diameter in diastole; LVIDs, left ventricular internal diameter in systole; LVPWd, left ventricular posterior wall thickness in diastole; LVPWs, left ventricular posterior wall thickness in systole; LVEF, left ventricular ejection fraction; VO_{2max}, maximal oxygen uptake; IS, infarct size; AAR, area at risk; TTC, 2.3.5-triphenyl-2H-tetrazolium chloride; RT-PCR, reverse transcription polymerase chain reaction; qRT-PCR, quantitative real-time polymerase chain reaction; PVDF, polyvinylidene difluoride; EF, ejection fraction

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1. Introduction

Myocardial infarction (MI) is one of the primary causes of morbidity and mortality worldwide [1,2]. Although early reperfusion by surgical procedures is currently the most effective therapeutic strategy for MI, it can itself result in additional damage that increases post-MI mortality because of cardiomyocyte loss with subsequent remodeling and heart failure (cardiac dysfunction) [2,3]. Inadequate oxygen is a first step toward an inflammatory response, myocardial cell death (necrosis, apoptosis and necroptosis), and subsequent scar formation at the site of ischemia–reperfusion injury [4]. Post-MI loss of cardiomyocyte loss plays a key role in heart failure, and increases mortality [5]. Although MI symptoms are mitigated by conventional interventions, these treatments cannot repair the infarcted tissue; thus cardiac dysfunction remains an important issue [6].

In recent decades many studies have searched for alternative therapies for MI, with a focus on myocardial regeneration [6–9]. The findings demonstrated that cardiac regeneration by stem cell induction (e.g. mesenchymal stem cells, embryonic stem cells, bone marrow-derived stem cells, cardiac progenitor cells and endothelial progenitor cells) in the damaged heart is a potentially effective new mechanism for cardiac repair after myocardial injury [6,9,10]. However, regenerative capacity was shown to be limited in the adult mammalian heart [11], and a regenerative capacity sufficient enough to repair injuries has been reported only in the heart of newborn mice [12]. New cardiomyocytes can be generated from resident cardiac stem cells (CSCs) or existing cardiomyocytes, and in fact nearly half of the cardiomyocytes are replaced during a normal human lifespan [7,8], but cardiomyocyte renewal gradually decreases with age [13]. In addition, DNA synthesis in myocyte nuclei and mitosis in residual cardiomyocytes are reportedly increased after MI compared to the healthy human heart [14]. Although cardiac tissue is a post-mitotic tissue and cardiomyocyte renewal has been observed in adult hearts, cardiomyocyte loss after MI was found to be increased, and the regenerative capacity was inadequate to replenish of them [15]. Previous studies have investigated different techniques to increase regenerative capacity, such as cardiac tissue stimulation by activating cardiomyocyte division, and the induction of mesenchymal stem cells, embryonic stem cells, and cardiac progenitor cells in the damaged heart [6,10].

Previous studies showed that exercise training led to physiological cardiac hypertrophy through activation of the insulin-like growth factor-1/PI3K/Akt signaling pathway. Physiological hypertrophy was reflected as increases in cardiomyocyte size and proliferation, and as the differentiation of c-Kit cells to cardiomyocytes [9,16,17]. However, little data are available on the molecular mechanisms and signaling transduction pathways involved in adult cardiomyocyte proliferation, and the factors responsible for the control of cardiomyocyte proliferation in the adult heart are unclear.

GATA-binding protein 4 (GATA4) and NK2 homeobox 5 (Nkx2.5) are transcription factors that act as adaptor molecules that detect regulatory sequences in DNA and control gene expression by targeting protein complex assembly [18]. In the adult heart, GATA4 and Nkx2.5 are important regulators of cardiomyocyte proliferation (cardiogenesis) and differentiation, and are known markers of cell proliferation during cell cycling growth [19]. CCAAT/enhancer-binding protein β (C/EBPβ) is a member of the family of basic leucine zipper transcription factors which affects cell growth and differentiation. Overexpression of C/EBPβ downregulates the expression of GATA4, Nkx2.5, T-box transcription factor 5 (Tbx5), and all genes related to physiological hypertrophy [9]. CITED4 (CREB-binding protein [CBP]/p300-interacting transactivator with ED-rich carboxy-terminal domain-4) is another transcription factor implicated as a downstream effector in exercise-related transcription, and induces hypertrophy and hyperplasia in cardiomyocytes [20]. Neuregulin-1, a potent mitogen for regeneration that stimulates cardiomyocyte proliferation after myocardial injury, has been linked to increased CITED4 after exercise training in a model of MI [21]. In addition, C/EBPβ and CITED4 are central to the cardiac response to exercise and cardioprotection against adverse remodeling [9,21]. Currently, the intensity of exercise training is accepted as an important factor in physiological hypertrophy in the adult heart [22,23]. Moreover, high-intensity interval training has been found to be more effective than moderate intensity training in reducing infarct size and increasing cardiorespiratory fitness and left ventricular function in patients with cardiovascular disease and in animal models of MI [24–27].

Several studies have shown that GATA4, Nkx2.5, Tbx5, c-Kit, C/EBPβ and CITED4 expression are regulated by exercise training after MI [9,20,21,28,29]. However, the effects of optimal dosage, intensity, frequency and duration of exercise training on the expression of GATA4, Nkx2.5, Tbx5, c-Kit, stem cell antigen-1 (Sca-l), myocyte-specific enhancer factor 2c (Mef-2c), C/EBPβ and CITED4 after MI remain unclear. At present there is little evidence that the intensity or frequency of interval training affects GATA4, Nkx2.5, Tbx5, c-Kit, Sca-l, Mef-2c, C/EBPβ or CITED4 expression after MI. For the present study, we hypothesized that high-intensity interval training may improve cardiac function by targeting regeneration markers in post-MI rats. To test our hypothesis, we investigated cardiac function, infarct size and markers of myocardial regeneration with western blotting and RT-PCR in a rat model of MI in which animals performed physical exercises at different levels of intensity.

2. Materials and methods

2.1. Animals and ethical statement

Fifty-five 10-week-old male Wistar rats weighing 250–300 g were used for this study. The animals were kept in temperature-controlled room with a 12 h:12 h light/dark cycle at 60 ± 5% humidity. Food and sterile water were available ad libitum. All experimental procedures and protocols were approved by the Animal Ethics Committee of Rajaie Cardiovascular, Medical, and Research Center (approval code: RHC.AC.IR.REC.1393.28). The study conformed to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH Publication, 8th Edition, 2011).

2.2. Experimental design

The animals were randomly divided into 5 groups of 11 rats each: sham-operated control animals (sham, $n = 11$) underwent the same surgical procedure as the four MI groups (see below) but without ligation of the left anterior descending artery (LAD). Fractional shortening (FS) ≤35% was the inclusion criteria for the MI groups in this study. These animals underwent acute LAD ligation and were randomly divided into sedentary MI (MI-Sed, $n = 11$), MI with 6 weeks of low-intensity interval training (MI-LIT, n = 11), MI with 6 weeks of moderate-intensity interval training (MI-MIT, n = 11) and MI with 6 weeks of high-intensity interval training (MI-HIT, n = 11).

2.3. Myocardial infarction model

The rats were anaesthetized with an intraperitoneal injection of sodium thiopental (50 mg/kg), and placed supine on a thermal pad under a heating lamp to maintain body temperature at 37 °C. Ventilation was controlled with room air and oxygen using a Harvard rodent ventilator (VentElite Small Animal, Harvard model 683, Holliston, USA) (tidal volume 2–3 mL, respiratory rate 65–70 per min). After the chest was opened at the fourth intercostal space via left thoracotomy, the heart was exposed by an incision through the pericardium and the LAD was permanently ligated about 2 mm distal from the tip of the left atrial appendix with a 6–0 silk suture slip knot (Ethicon Inc., Lubbock, USA). Successful ligation was verified by regional cyanosis of the myocardial surface. After LAD ligation, the chest

wall was closed in layers; the lungs were inflated by increasing positive end expiratory pressure. Then the animals were allowed to recover by withdrawing the ventilator. The animals received cefazolin (25 mg/kg, intramuscularly), flunixin (1–2 mg/kg, intramuscularly), and warm sterile saline (0.5–1 mL, subcutaneously).

Sham-operated rats underwent the same operation without LAD ligation and served as the control group.

2.4. Evaluation of cardiac function

Transthoracic two-dimensional (2D) echocardiography was performed using a 10-MHz linear array transducer connected to a Vivid 7 expert ultrasound system at a speed of 100 mm/s (General Electric-Vingmed Ultrasound, Horten, Norway), under sodium thiopental anesthesia (50 mg/kg, ip) at 4 and 10 weeks after surgery. Parasternal 2D and M-mode short-axis views at the level of the papillary muscle were used to measure left ventricular internal diameter in diastole (LVIDd), left ventricular internal diameter in systole (LVIDs), posterior wall thickness in diastole (LVPWd), and posterior wall thickness in systole (LVPWs). Left ventricular ejection fraction (LVEF, %) was calculated with the following formula: $(LVIDd^2 - LVIDs^2)/LVIDd^2$. To calculate percentage FS we used the formula [(LVIDd-LVIDs)/LVIDd] × 100 [30,31].

All echocardiographic examinations were done by a cardiologist with expertise in small animal echocardiography who was blinded to the study group allocations.

2.5. Training protocols

Four weeks after surgery and echocardiographic evaluation, rats in the MI groups were randomly divided into MI-Sed, MI-LIT, MI-MIT and MI-HIT groups. Exercise training lasted for 6 weeks. Animals in the MI groups were placed on a motorized treadmill (Iranian Danesh Salar, Tehran, Iran) and walked at a slow pace (5 m/min) for 5 min per day, 5 days per week in the 3rd and 4th weeks after surgery. (The animals in MI groups in the 1st and 2nd week of recovery period only having rest and in the 3rd and 4th weeks were walking at a slow place). Maximal oxygen uptake (VO_{2max}) was measured during maximal exercise as previously described [32].

The sham and MI-sedentary animals received no exercise training. In the other three groups, each session consisted of 10 min of warm-up at 40%–50% VO_{2max}. Each low-intensity interval training (LIT) session consisted of 10 intervals of 4 min at 55%–60% VO_{2max} separated by 2 min at 45%–50% VO_{2max}. Sessions in the moderate-intensity interval training (MIT) group consisted of 10 intervals of 4 min at 65%–70% VO_{2max} separated by 2 min at 50%–60% VO_{2max}. High-intensity interval training (HIT) sessions consisted of 10 intervals of 4 min at 85%–90% separated by 2 min at 50%–60% VO_{2max} [23,32]. The exercise intensity for each week of training was based on a previous report of the relationship between running speed and VO_{2max} in a similar post-MI rat model [32]. The running speed was increased gradually during 6 weeks of training by 0.02 m/s per week [33]. Treadmill inclination during training and testing was 0 degrees.

2.6. Infarct size

Myocardial infarct (IS) and area at risk (AAR) were evaluated by Evans blue/TTC staining. At the end of the exercise training intervention, i.e. 10 weeks after surgery, the animals were placed under deep anesthesia, the LAD was ligated again and 1 mL 2% Evans blue dye was injected into the femoral vein. Then the heart and aorta were rapidly removed. The heart tissues were kept at −20 °C for 24 h; then the heart was cut transversally into 1-mm slices. The slices were incubated at 37 °C for 10 min in 1% TTC, and fixed in 10% formalin overnight to improve the contrast between infarcted and noninfarcted regions. The percentage of the left ventricle stained with red formazan derivative was used to calculate AAR (AAR/LV) and IS was expressed with reference to the AAR (IS/AAR). A digital camera was used to photograph slices of interest, and Image J software (NIH, Bethesda, MD, USA) was used to evaluate AAS and IS.

3. RT-PCR analysis

3.1. Quantitative real-time polymerase chain reaction

Total RNA isolated from the cardiac tissue was used for reverse transcription polymerase chain reaction (RT-PCR) or quantitative real-time polymerase chain reaction (qRT-PCR). Total RNA was extracted with the Easy Pure RNA Kit (TransGen Biotech, Beijing, China) in accordance with the manufacturer's instructions. For RT-PCR, the cDNAs were synthesized with TransScript First-Strand cDNA Synthesis SuperMix (TransGen Biotech) from total RNA as templates.

For qRT-PCR, TransStart Top Green qPCR SuperMix (TransGen Biotech) was used in the Rotor-Gene 6000 system (Corbett, Concorde, NSW, Australia). The PCR–reaction mixtures contained 5 μL TransStart Top Green qPCR SuperMix, 1 μL template cDNA, 1 μL forward and reverse primers, and 3 μL sterile distilled water. The thermal cycling conditions consisted of an initial denaturation step at 95 °C for 2 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 30 s. β-actin was used for normalization, and the $2^{-\Delta\Delta Ct}$ method was applied to evaluate target gene mRNA expression [34]. All reactions were done in triplicate. All analyses were performed by investigators who were blinded to the group assignments. The forward and reverse primer sequences are listed in Table 1.

3.2. Western blot

Immunoblots were obtained from samples of protein lysates from left ventricular cardiomyocytes isolated from MI-induced and sham-operated rats. The samples were homogenized at 4 °C in 50 mM Tris-HCl buffer (pH 7.4) (1 mM EDTA, 1% Triton X-100, 1 mM phenyl methyl sulfonyl fluoride, 1 μg/mL aprotinin, 1 μg/mL pepstatin, and 1 μg/mL leupeptin). Protein concentration in the homogenate was determined by the Bradford test. Equal amounts of protein (25 μg) in each sample were loaded for 12.5% standard SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride (PVDF) membranes (Merck Millipore, Darmstadt, Germany) with a wet system (Bio-Rad, Marnes-la-coquette, France). The PVDF membranes were blocked overnight in TBS-T buffer (100 mM Tris-HCl 0.9% NaCl and 0.1% Tween 20 [pH 7.4]) containing 5% non-fat dry milk and then incubated for 3 h at room temperature with rabbit polyclonal anti-C/EBPβ, rabbit polyclonal anti-c-Kit, rabbit polyclonal anti-βactin (1/1000, Santa Cruz, California, USA), and rabbit polyclonal anti-Nkx2.5 (1/1000, Biorbyt, California, USA). The membranes were washed with TBS-T buffer 3 times (15 min) and then incubated with secondary antibodies (goat anti-rabbit IgG-HRP; 1:5000; Santa Cruz) for 90 min at room temperature. The blots were exposed to X-ray film by Super Signal West Pico Chemiluminescent Substrate (Thermo Fisher Scientific, Waltham, USA).

Table 1
Forward and reverse primer sequences.

Gene name	Forward primer sequences	Reverse primer sequences
GATA4	*ATGTTCAGGCTGGAGAGCAAG*	*TGGGTGCAGTGCAGATGTTAC*
Tbx5	*CACAGCGATAAAAGCCGTCTC*	TACCAGCCCCGATTACACATC
Nkx2.5	*CGCCCTTCTCAGTCAAAGAC*	GAAAGCAGGAGAGCACTTGG
CITED4	*ACGAGGGTGGTTTTGCAGTCT*	CAACTCAGCCAGACAGAGGAA
C/EBPβ	*CAAGCTGAGCGACGAGTACAA*	ACAGCTGCTCCACCTTCTTCT
c-Kit	*GAAAGGGAGGCCCTAATGTC*	CGTTTGAGCTGTCACAGGAA
Sca-1	CATCTTTCTCCTGGCCCTACT	GAGGACTGAGCCCAGGATGAA
Mef-2c	AAGGGAATGGATACGGCAAC	TCCTAGATTCATAGGGGGAGGA
β-actin	CGGTCAGGTCATCACTATCGG	ATGCCACAGGATTCCATACCCA

Table 2
Body, heart and left ventricle weights, heart weight/body weight ratio, and left ventricle weight/body weight ratio after experimental exercise training.

	Sham	MI-Sed	MI-LIT	MI-MIT	MI-HIT
BW beginning (g)	265 ± 28.5	274 ± 7.3	285 ± 13.6	262 ± 26.2	283. ± 12.9
BW final (g)	335 ± 22.3	340 ± 25.1	348 ± 19.4	338 ± 24.2	375 ± 27.3$^{\#,*,\&}$
HW (mg)	924 ± 21.6	968 ± 24.8	1090 ± 34.2$^{*,\#}$	1086 ± 40.7$^{*,\#}$	1312 ± 49.5$^{\dagger,\#\#,\&}$
HW/BW ratio (mg/g)	2.7 ± 0.2	2.8 ± 0.1	3.1 ± 0.2$^{*,\#}$	3.2 ± 0.2$^{*,\#}$	3.5 ± 0.3 $^{\dagger,\#\#,\&}$
LV weight (mg)	695 ± 27.9	720 ± 32	878 ± 29.1$^{*,\#}$	881 ± 32.6$^{*,\#}$	1130 ± 46.3$^{\dagger,\#\#,\&}$
LV weight/ BW (mg/g)	2.1 ± 0.1	2.1 ± 0.1	2.5 ± 0.2$^{*,\#}$	2.6 ± 0.1$^{*,\#}$	3.1 ± 0.2$^{\dagger,\#\#,\&}$

Values are presented as means ± SD. BW; body weight, HW; heart weight, LV; left ventricular.
* $P < 0.05$ vs. Sham.
† $P < 0.01$ vs. Sham.
$^{\#}$ $P < 0.05$ vs. MI-Sed.
$^{\#\#}$ $P < 0.01$ vs. MI-Sed.
$^{\&}$ $P < 0.05$ vs. MI-LIT and MI-MIT.

Quantitative analyses of the monomeric band data were done with Image J software [35]. All analyses were performed by investigators who were blinded to the group assignments.

3.3. Statistical analysis

The data are expressed as the mean ± standard deviation. All statistical analyses were done with IBM SPSS software (version 19.0; IBM Corp, Armonk, NY, USA). One-way ANOVA was used to identify differences between groups. When a significant difference was detected by ANOVA, the Bonferroni post-hoc test was used. Statistical significance was set at $P \leq 0.05$.

4. Results

4.1. Body weight, heart weight, heart weight/body weight ratio and left ventricular/body weight ratio

Table 2 shows that there was no significant difference in initial body weight among experimental groups. At the end of study final body weight, heart weight, heart weight/body weight ratio, left ventricular weight and left ventricular weight/body weight ratio were significantly greater in groups MI-LIT and MI-MIT than the sham and MI-Sed groups ($P < 0.05$). However, in group MI-HIIT, final body weight, heart weight/body weight ratio, left ventricular weight and left ventricular weight/body weight ratio were markedly greater than in group MI-Sed and the sham group ($P < 0.01$), MI-LIT and MI-MIT ($P < 0.05$).

4.2. Effect of low-, moderate- and high-intensity interval exercise on cardiac function after MI

Left ventricular function was assessed with echocardiography 4 and 10 weeks after surgery (Fig. 1). LVIDd and LVIDs were greater in group MI-Sed at 10 weeks than in the sham group ($P < 0.01$). LVIDd and LVIDs were significantly lower in groups MI-MIT and MI-HIT than group MI-Sed ($P < 0.05$). However, LVIDs (but not LVIDd) was significantly lower only in group MI-LIT compared to group MI-Sed. High-intensity interval training had a greater effect on LVIDs than MI-LIT ($P < 0.05$). There were no significant differences in LVPWd or LVPWs in groups MI-LIT and MI-MIT compared to group MI-Sed or the sham group. Left ventricular posterior wall thickness was significantly greater in group MI-HIT than in the sham group and groups MI-Sed, MI-LIT and MI-MIT ($P < 0.05$). Fractional shortening was significantly less in group MI-Sed than in the sham group ($P < 0.01$). After the 6-week exercise intervention, FS was greater in groups MI-LIT and MI-MIT than in group MI-Sed ($P < 0.05$). Also, FS was significantly greater in group MI-HIT than in group MI-Sed ($P < 0.01$). The increase in FS was greater in group MI-HIT than in groups MI-LIT and MI-MIT ($P < 0.05$). Ten weeks after surgery, ejection fraction (EF) was significantly lower in group in MI-Sed than in the sham group ($P < 0.01$), and was significantly greater in groups MI-LIT and MI-MIT compared to group MI-Sed ($P < 0.05$). The increase in EF was greater in group MI-HIT than group MI-LIT ($P < 0.05$).

4.3. Effect of low-, moderate- and high-intensity interval exercise on infarct size

There were no significant differences in AAR among the sham, MI-Sed, MI-LIT, MI-MIT or MI-HIT groups. Representative TTC-stained cardiac tissue sections are shown in Fig. 2. The IS in group MI-LIT was smaller than in group MI-Sed ($P < 0.05$), and was significantly smaller in groups MI-MIT and MI-HIT compared to group MI-Sed ($P < 0.01$). In addition, IS was significantly smaller in group MI-HIT than in group MI-LIT ($P < 0.05$).

4.4. Effect of exercise training on gene expression

The data for mRNA expression are shown in Fig. 3. GATA4 expression was lower in group MI-Sed than in the sham group ($P < 0.05$). GATA4 mRNA levels were significantly higher in groups MI-LIT and MI-MIT than in group MI-Sed ($P < 0.05$). GATA4 mRNA expression was also greater in group MI-HIT than in groups MI-LIT and MI-MIT ($P < 0.01$). Tbx5 mRNA expression was lower in all MI groups compared to the sham group, and among the former groups it was greatest in group MI-HIT ($P < 0.05$). Sca-1 mRNA expression was significantly lower in group MI-Sed compared to the sham group ($P < 0.05$). Sca-1 mRNA in groups MI-LIT ($P < 0.05$), MI-MIT and MI-HIT ($P < 0.01$) was significantly increased compared to group MI-Sed. However, Sca-1 mRNA levels in group MI-HIT were significantly greater than in group MI-LIT ($P < 0.05$). High-intensity interval training increased Nkx2.5 mRNA levels more than moderate- or low-intensity interval training ($P < 0.05$). The levels of Nkx2.5 mRNA in groups MI-LIT, MI-MIT ($P < 0.05$) and MI-HIT ($P < 0.01$) were higher than in the sham group. CEBP/β mRNA levels were greater in the MI-Sed group than in the sham group, and were significantly greater in groups MI-MIT ($P < 0.05$) and MI-HIT ($P < 0.01$) compared to group MI-Sed. Moreover, group MI-HIT showed a greater decrease in CEBP/β mRNA than group MI-LIT ($P < 0.05$). The levels of CITED4 mRNA in groups MI-MIT ($P < 0.05$) and MI-HIT ($P < 0.01$) were significantly greater than in groups MI-Sed and MI-LIT. In contrast, there were no significant differences between the sham group and groups MI-Sed and MI-LIT. Mef-2c mRNA levels were significantly greater in groups MI-MIT and MI-HIT compared to group MI-Sed ($P < 0.05$). After 6 weeks of exercise training, c-Kit mRNA levels were higher in groups MI-LIT, MI-MIT ($P < 0.05$) and MI-HIT ($P < 0.01$) than in group MI-Sed, and the increase in group MI-HIT was greater than in groups MI-LIT and MI-MIT ($P < 0.05$).

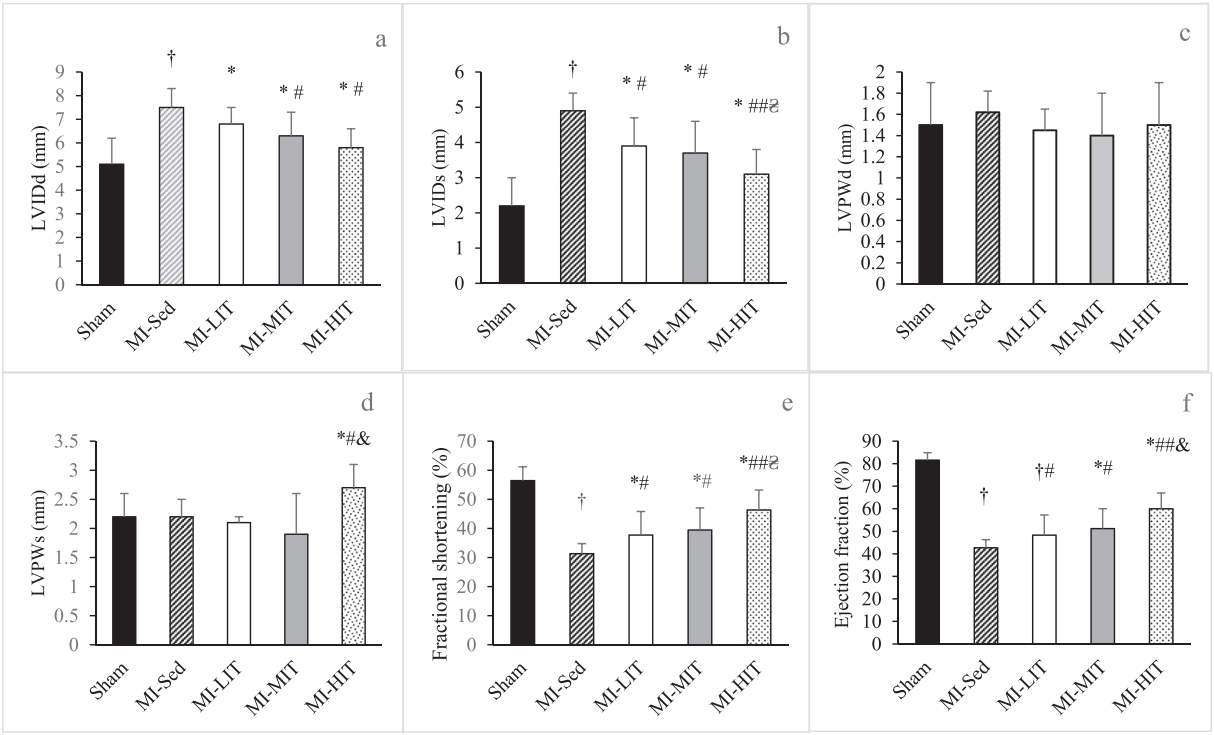


Fig. 1. 6 weeks of interval training at different intensities improved heart function after MI. a, b) LVIDd and LVIDs increased significantly after LAD ligation (*P < 0.05, †P < 0.01 compared to the sham group). LVIDd decreased markedly after exercise training (#P < 0.05 compared to MI-Sed). LVIDs decreased significantly after exercise training (#P < 0.05, ##P < 0.01 compared to MI-Sed). LVIDs in group MI-HIT decreased significantly compared to group MI-LIT (ƨP < 0.05). c, d) The magnitude of LVPWd and LVPWs did not vary in different groups, except for LVPWs in group MI-HIT, which was greater than in the sham group (* P < 0.05), group MI-Sed (#P < 0.05) and groups MI-LIT and MI-MIT (&P < 0.05). e, f) Significantly lower FS and EF were observed after LAD ligation (* P < 0.05, †P < 0.01 compared to the sham group). Significantly greater FS and EF were seen in groups MI-LIT, MI-MIT (#P < 0.05 compared to group MI-Sed) and MI-HIT (##P < 0.01 compared to group MI-Sed). Values are shown as means ± SD.

4.5. Effect of exercise training on protein expression

Ten weeks after surgery, the expression of c-Kit protein in heart tissue was significantly greater in group MI-HIT than in the sham group and group MI-Sed (Fig. 4). Nkx2.5 protein expression in groups MI-MIT and MI-HIIT was significantly greater than in the sham group (P < 0.01), group MI-Sed (P < 0.01) and group MI-LIT (P < 0.05). However, C/EBPβ protein expression increased more in group MI-Sed

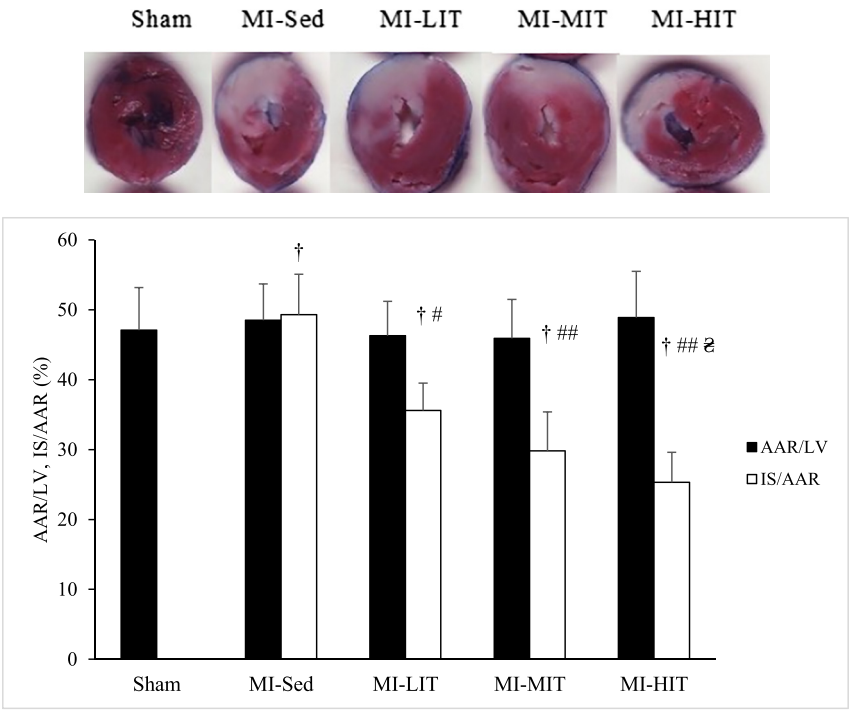


Fig. 2. 6 weeks of of interval training at different intensiteis reduced infarct size after MI. There were no significant differnces among groups in AAR/LV ratio. Myocardial infarct volume was significantly greater in all MI groups compared to the sham group (†P < 0.01). TTC staining showed that 6 weeks of MIT, HIT (## < 0.01) and LIT (#P < 0.05) significantly reduced infarct volume compared to group MI-Sed. Infarct volume in group MI-HIT was significantly smaller than in group MI-LIT (ƨP < 0.05). Values are shown as means ± SD.

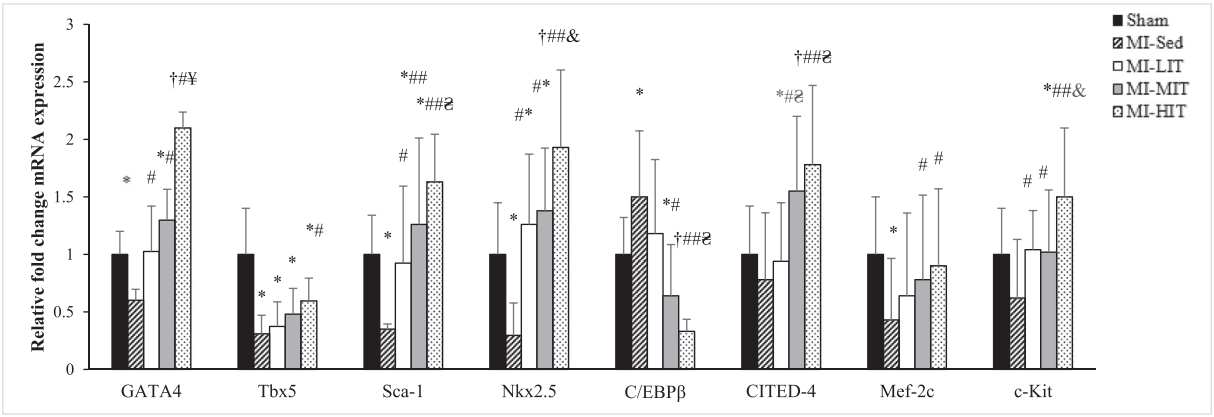


Fig. 3. MI-LIT, MI-MIT and MI-HIT increased factors positively associated with myocyte renewal, and decreased factors negatively associated with myocyte renewal. GATA4 gene expression after 6 weeks of exercise was significantly lower in all MI groups (* $P < 0.05$, $^{\dagger}P < 0.01$) compared to the sham group. GATA4 gene expression was markeely increased ($^{\#}P < 0.05$) compared to group MI-Sed. In group MI-HIIT, GATA4 gene expression was significantly greater (¥ $P < 0.01$) than in groups MI-LIT and MI-MIT. Tbx5 gene expression was significantly lower in group MI-Sed (* $P < 0.05$) than in the sham group. Tbx5 expression in group MI-HIT was significantly greater than in group MI-Sed ($^{\#}P < 0.05$). Sca-1 expression was decreased by LAD ligation ($^{\#}P < 0.05$), and was significantly greater in all exercise groups ($^{\#}P < 0.05$, $^{\#\#}P < 0.01$) than in group MI-Sed. Sca-1 expression in group MI-HIT was significantly greater than in group MI-LIT ($^{\$}P < 0.05$). Nkx2.5 gene expression after LAD ligation was decreased (* $P < 0.05$, $^{\dagger}P < 0.01$ compared to the sham group). Exercise training was associated with increased Nkx2.5 expression ($^{\#}P < 0.05$, $^{\#\#}P < 0.01$) compared to group MI-Sed. Nkx2.5 expression in group MI-HIT was also significantly greater compared to groups MI-LIT and MI-MIT ($^{\&}P < 0.05$). After LAD ligation, CEBP/β expression was decreased (*$P < 0.05$, $^{\dagger}P < 0.01$) compared to the sham group. CEBP/β expression in groups MI-MIT and MI-HIT was lower ($^{\#}P < 0.05$, $^{\#\#}P < 0.01$) then in group MI-Sed. CITED4 gene expression in groups MI-MIT and MI-HIT was increased compared to the sham group (* $P < 0.05$, $^{\dagger}P < 0.01$), and compared to group MI-Sed ($^{\#}P < 0.05$, $^{\#\#}P < 0.01$). The magnitude of CITED4 expression in groups MI-MIT and MI-HIT was significantly greater than in group MI-LIT ($^{\$}P < 0.05$). Mef-2c mRNA expression was lower in group MI-Sed (* $P < 0.05$) compared to the sham group, and was increased after 6 weeks of exercise training in groups MI-MIT and MI-HIT ($^{\#}P < 0.05$) compared to group MI-Sed. c-Kit mRNA expression was greater in groups MI-LIT, MI-MIT and MI-HIT ($^{\#}P < 0.05$, $^{\#\#}P < 0.01$) compared to group MI-Sed. Values are shown as means ± SD.

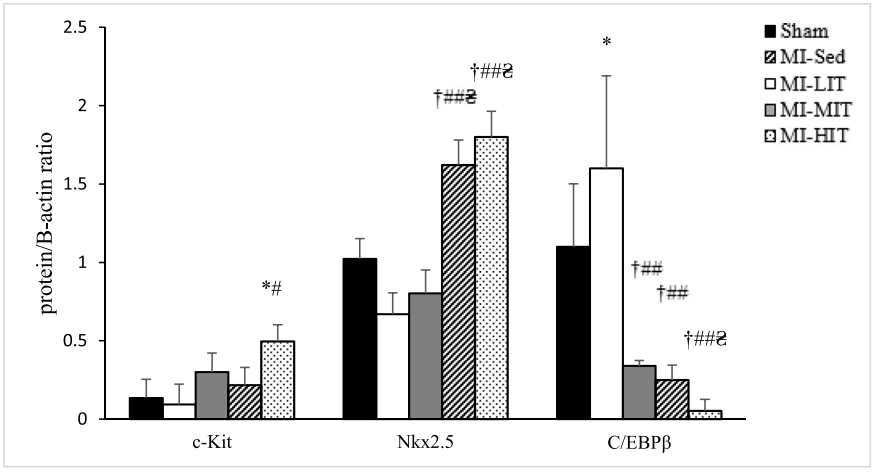
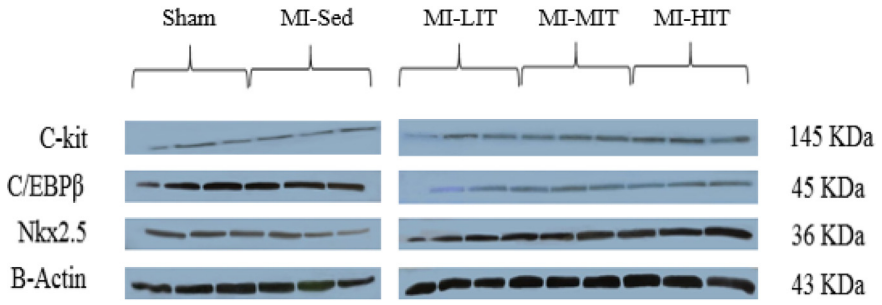


Fig. 4. Westrn blot assays of cardiomycyte regeneration markers showed that different intensities of exercise training improved cardiac function. c-Kit protein levels were significantly greater in group MI-HIT (* $P < 0.05$ compared to the sham group, $^{\#}P < 0.05$ compared to group MI-Sed). After 6 weeks of exercise training, Nkx2.5 protein levels were greaer in groups MI-MIT and MI-HIT ($^{\dagger}P < 0.01$ compared to the sham group, $^{\#\#}P < 0.01$ compared to group MI-Sed, $^{\$}P < 0.05$ compared to group MI-LIT). C/EBPβ protein levels were markedly higher after MI (* $P < 0.05$ compared to the sham group). C/EBPβ protein levels in groups MI-LIT, MI-MIT and MI-HIT were significantly higher ($^{\#\#}P < 0.01$) compared to group MI-Sed, and significantly higher in group MI-HIT ($^{\$}P < 0.05$) compared to group MI-LIT. Values are shown as means ± SD.



than in the sham group. Exercise training decreased C/EBP-β in groups MI-LIT, MI-MIT and MI-HIT. C/EBPβ protein expression in group MI-HIT showed a significantly greater reduction than in group MI-LIT ($P < 0.05$).

5. Discussion

The present results show that 6 weeks of HIT significantly decreased IS and improved cardiac function in a post-MI rat model. High-intensity interval training had beneficial effects by increasing GATA4, Nkx2.5,

CITED4, c-Kit, Sca-1, Tbx5, and Mef-c2 mRNA expression along with c-Kit and Nkx2.5 protein expression, and by decreasing C/EBP-β mRNA and protein expression.

In comparison to group MI-Sed, LVIDd was decreased by approximately 9% in group MI-LIT, 16% in group MI-MIT and 22% in group MI-HIT. After 6 weeks of training, LVIDs was decreased by approximately 20% in group MI-LIT, 24% in group MI-MIT and 36% in group MI-HIT. Although MIT, LIT and HIT all improved cardiac function, the magnitude of improvement was significantly greater in group MI-HIT (40% for EF and 47% for FS) than in group MI-LIT (13% and 20% respectively) or group MI-MIT (19% and 25% respectively). In post-MI patients, EF and FS are lower than in healthy counterparts [23,26,27].

Apparently, the type and intensity of exercise training and the activation of different metabolic pathways have different effects on contractile function. Wisloff et al. reported that HIT (8 min at 85%–90% VO_{2max} with 2 min at 50%–60% VO_{2max}) increased contractility, VO_{2max}, EF and FS in post-MI animals [36]. Other studies found that resistance training and aerobic exercise training did not improve systolic dysfunction in post-MI animals [37,38]. Exercise training in the post-MI period improves cardiac function via several mechanisms and signaling pathways. The cardioprotective effects of exercise training have been documented as decreased pathological remodeling, upregulation of endothelial nitric oxide synthesis, decreased reactive oxygen species levels, and improved mechanical energy efficiency [4,33,39].

The intensity of exercise training appears to have a direct relationship with cardiac function. Wisloff et al. reported that HIT (4 min at 90%–95% peak heart rate and 3 min at 50%–70% peak heart rate) had a greater effect on left ventricular function (EF, end-diastolic and systolic volume) and VO_{2peak} compared to continuous MIT in patients with heart failure [39].

Reduced IS after exercise training is an important mechanism in improved cardiac function. In the present study, IS was decreased in all training groups. However, the decrease in group MI-HIT was greater than in groups MI-LIT and MI-MIT, although the differences were not significant. Infarct size is associated with necrosis and necroptotic cell death, and earlier work by our group showed that HIT can attenuate necroptosis markers post-MI [4]. In addition, IS was reportedly decreased after exercise training via the activation of signaling pathway and cardiomyocyte renewal in the infarcted heart [20,28,41].

After exercise training in the present study, the levels of GATA4, Sca-1, Nkx2.5 and CITED4 mRNA were increased, and C/EBPβ mRNA was decreased. These results show that HIT was associated with greater increases in GATA4, Nkx2.5 and c-Kit mRNA levels than LIT or MIT in post-MI rats. In contrast, C/EBPβ mRNA levels decreased more after HIT than after LIT. In addition, c-Kit and Nkx2.5 protein expression in group MI-HIT decreased more than in groups MI-LIT and MI-HIT, and the decrease in C/EBPβ protein expression was also greater in group MI-HIT compared to the other exercise groups.

Previous studies have shown that exercise training may stimulate the proliferation of preexisting cardiomyocytes and resident CSCs in vivo [7–9,17,23]. Cai et al. reported that 4 weeks of exercise training (50 min at 16 m/min for 5 day per week) improved cardiac function via the upregulation of neuregulin-1, activated ErbB2, ErbB4 and PI3K/Akt signaling transduction, and increased the number of GATA4, Nkx2.5 and c-Kit cells in post-MI rats [28]. Leite et al. reported that 4 weeks of swimming exercise training (2 daily sessions, 90 min, 5- days per weeks) slightly increased c-Kit expression in stem cells from healthy hearts in trained animals. Additionally, they found no significant differences in Sca-1 or colony forming efficiency between trained and sedentary healthy male C57BL/6 mice [8]. Another study found that myocyte formation and Ki67+ cardiomyocyte nuclei correlated directly with the intensity and duration of training. Cardiogenesis was significantly lower after LIT for 2 weeks than after HIT for 4 weeks [23]. The response of mature myocytes to increased workloads during high-intensity exercise training upregulates the secretion of growth factors such as IGF−I, TGF-β and neuregulin-1. The response of CSCs from the adult heart to growth factors may activate cardiac progenitors and trigger lineage commitment. As a result, increased c-Kit expression may be effective in enhancing cardiac regeneration and repair after MI [23]. In the present study, we found that different intensities of interval training led to increased GATA4, Nkx2.5, c-Kit, Sca-1 and CITED4 mRNA expression in post-MI rats. Notably, however, the expression of mRNA was significantly greater in group MI-HIT than groups MI-MIT or MI-LIT.

Cardiac regeneration is a complex process which is regulated by multiple mechanisms and signaling pathways [28] . In this regard, Boström et al. reported that C/EBPβ is a transcription factor that was downregulated in swim exercise-trained mice [9]. C/EBPβ thus appears to produce signaling that is central to the cardiac response to exercise and the development of cardioprotection against adverse remodeling [9,21]. Our results show that the downregulation of C/EBPβ mRNA and decrease in protein expression were greater in group MI-HIT than group MI-LIT. The effect of exercise on cardiomyocyte hypertrophy and proliferation involves a reduction in the expression of transcription factor C/EBPβ and a linked increase in the expression of CITED4. The downregulation of C/EBPβ may activate a serum response factor, which in turn may contribute to activate the exercise gene set (i.e., GATA4, Tbx5, Nkx2.5, and Mef-2c) [9].

Resident CSCs, especially c-Kit CSCs, have been identified as an important source for myocardial regeneration after injury [40,41]. One study documented that when c-Kit CSCs were delivered intracoronary after ischemia–reperfusion injury, they promoted myocardial repair, reduced IS, attenuated ventricular remodeling, and improved cardiac function [10]. In this context, Cai et al. demonstrated that exercise training augmented the number of c-Kit cells and upregulated the expression of GATA4 and Nkx2.5 transcription factors [28]. Our results showed that c-Kit mRNA and protein levels were significantly increased in trained animals, and that the increases in mRNA and protein expression were significantly greater in group MI-HIT than in groups MI-LIT and MI-MIT.

This study has a several limitations. First, bromodeoxyuridine (BrdU) induction and flow cytometry or immunohistochemical staining with DAPI are widely used to evaluate cardiomyocyte renewal and the number of cardiomyocyte nuclei. We studied mRNA and protein expression, and used western blotting to investigate the effects of exercise training intensity on markers of cardiogenesis. The lack of financial resources precluded us from using BrdU induction and DAPI staining. Second, the stem cell makers c-Kit, Sca-1, Nkx2.5, and GATA4 are expressed in myocytes and nonmyocyte progenitors such as endothelial cells, fibroblasts and mast cells. Because we studied mRNA and protein expression of stem cells markers in homogenized left ventricular tissue after the interventions, our data did not allow us to differentiate between cardiomyocytes and other types of cells potentially involved in cardiac regeneration.

6. Conclusions

We investigated the effect of 6 weeks of low-, medium- and high-intensity interval training on GATA4, Nkx2.5, Tbx5, c-Kit, Sca-l, Mef-2c, C/EBPβ and CITED4 mRNA and c-Kit, Nkx2.5and C/EBPβ protein expression in a rat model of MI. After 6 weeks of HIT, left ventricular function (EF, FS) improved, GATA4, Nkx2.5, Tbx5, c-Kit, Sca-l, Mef-2c and CITED4 expression increased, and C/EBPβ expression decreased. The improved left ventricular function in post-MI rats with exercise training was mediated in part by increased GATA4, Nkx2.5, Tbx5, c-Kit, Sca-l, Mef-2c and CITED4 levels, and decreased C/EBPβ levels.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

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