Effects of two different exercise paradigms on cardiac function, BDNF-TrkB expression, and myocardial protection in the presence and absence of Western diet

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ABSTRACT

Background: Brain-derived neurotrophic factor (BDNF) - tropomyosin-related kinase receptor B (TrkB) signaling is a vital regulator of myocardial performance. Here, we tested the impact of high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT) on heart function, metabolic parameters, and serum/cardiac BDNF (with its TrkB receptor) in animals fed a Western (WD) or regular diet (ND). Further, myocardial expression of pro-inflammatory cytokine interleukin-18 (IL-18) and cardioprotective molecule heme oxygens-1 (HO-1) were monitored.

Methods: Wistar rats were divided into HIIT, MICT, and sedentary (SED), all fed a WD or ND, for 12 weeks. Heart function, protein expression, and serum factors were assessed via echocardiography, western blotting, and ELISA, respectively.

Results: WD plus SED caused insulin resistance, dyslipidemia, visceral fat deposition, serum BDNF depletion as well as cardiac upregulation of IL-18 and downregulation of HO-1, without affecting, heart function and BDNF-TrkB expression. The cardiometabolic risk factors, serum BDNF losses, and IL-18 overexpression were similarly obviated by HIIT and MICT, although HO-1 expression was boosted by HIIT exclusively (even in ND). HIIT enhanced heart function, regardless of the diet. HIIT augmented cardiac BDNF expression, with a significant difference between ND and WD. Likewise, HIIT instigated TrkB expression only in ND.

Conclusions: HIIT and MICT can cope with myocardial inflammation and cardiometabolic risk factors in WD consumers and, exclusively, HIIT may grant further protection by increasing heart function, BDNF-TrkB expression, and HO-1 expression. Thus, the HIIT paradigm should be considered as a preference for subjects who require heart function to be preserved or enhanced.

1. Background

Exercise training is an integral part of the global health strategies to counter cardiovascular diseases outbreak [1], with its proven ubiquitous beneficial effects on the heart [2] and other physiological systems [3]. Exercise-induced adaptations are accompanied by increased aerobic capacity and cardiac function, lowering the risk of cardiovascular mortality [2]. Exercise training not only acts as a proxy for optimization of myocardial performance in normal heart function [4] but also weakens myocardial dysfunction caused by significant cardiac events [5] or diet/obesity [6]. To exert its protective effects, exercise training may inhibit or activate a variety and complex molecular mechanisms in the heart and non-cardiac organs [2,7,8]. For instance, following myocardial infarction, exercise training limits cardiac dysfunction by secreting or enriching a wide array of protective factors in the heart [9]. Likewise, in a disturbed cardiometabolic milieu, similar to what occurs during long-term consumption of a diet enriched with fat and sucrose (Western diet, WD) or a high-fat diet (HFD), exercise training improves cardiac function by repressing inflammatory mediators in the myocardium [10,11]. However, the molecular mechanisms underpinning exercise-induced cardiac protection remain to be explored further, particularly after consolidated modes of aerobic training, i.e., high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT), and with respect to the different cardiometabolic...
Interestingly, genetic BDNF overexpression during development promotes murine heart growth, both vascularization, and capillarization to the optimization of myocardial performance. These actions span from the regulation of heart development impart cardioprotection, but may display diverse effects by targeting distinct mechanisms.

By their direct role in neuronal cell development and function, brain-derived neurotrophic factor (BDNF) confers pleiotropic beneficial (vital) effects on the non-neuronal tissues, especially cardiovascular cells. These actions span from the regulation of heart development to the optimization of myocardial performance. Interestingly, genetically BDNF overexpression during development shortens and calcium cycling. In these animals, deleting sarcolemmal TrkB dampens basal cardiac contraction and relaxation. The cardiac BDNF-TrkB actions occur independently of β-adrenergic receptor (β-AR) stimulation; in fact, they run in parallel with β-AR signaling to modulate myocardial mechanical responses, and they unfold, at least in part, via Ca²⁺/calmodulin-dependent protein kinase II (CaMKII)-mediated signaling. Later on, this initial evidence was validated by Fulgenzi and colleagues (2015) who suggested, however, that the truncated isoform instead of the full-length TrkB receptor, i.e., TrkB-T1 and not TrkB-FL, mediates BDNF-evoked positive inotropy and lusitropy in isolated/perfused mouse hearts.

Circulating and brain BDNF has been shown to be responsible for the improvement of cognitive performance by exercise training. Likewise, higher circulating BDNF concentrations due to exercise training may be associated with protection against cardiovascular disease. However, it remains largely unclear whether exercise training-induced modulation of cardiac function is coupled with BDNF-TrkB augmentation in the heart. From this concept, only a few studies, in fact, investigated the impact of exercise training on cardiac BDNF levels. In a study, Lee et al. (2018) showed that exercise training positively affects cardiac BDNF expression, but their evaluations were purely focused on ischemic hearts. Still, more research should be performed to validate the capacity of BDNF-TrkB as a significant mediator in cardiac benefits of exercise training, importantly in the presence and absence of WD. This dietary pattern is spreading across the world and has enough capacity for jeopardizing the proper function of the vital organ of the body, particularly in the presence of a sedentary lifestyle. Indeed, WD is able to, directly and/or indirectly, and even independent of obesity phenotype, target the heart system and weaken myocardial performance by several mechanisms. Uncovering the impact of exercise in the presence of daily WD consumption can provide valuable insights into the protective capacity of physical activity on the heart. To our knowledge, no study so far has portrayed the influence of exercise training on heart function and BDNF-TrkB expression under different cardiometabolic conditions, WD vs. regular diet (ND). This investigation aimed to fill this gap. We speculated that a potent stimulus is required for enhancing cardiac BDNF-TrkB expression, more than its initial (physiological) levels. Therefore, it is essential to compare different intensities of aerobic training in the guise of HIIT and MICT protocols.

In sum, the present study was designed to provide an initial experimental groundwork apt to compare the influences of HIIT and MICT paradigms on cardiac function, aerobic capacity, metabolic parameters, serum/cardiac BDNF, with its associated TrkB receptor, between rats fed a WD and ND. Beyond that, since inflammation has been proven to mediate the deleterious cardiac effects of WD, the myocardial expression of pro-inflammatory cytokine interleukin-18 (IL-18) and a pivotal cardioprotective molecule heme oxygenase-1 (HO-1) were also monitored.

2. Methods

2.1. Ethical approval

This study was approved by the National Research Ethics Committee of Guilan University of Medical Science (IR.GUMS.REC.1397.264 and IR.GUMS.REC.1397.267) and rigidly performed in accordance with the guidelines for animal research adopted by the American Physiological Society.

2.2. Animals and experimental design

Healthy male Wistar rats (n = 60, 8 weeks old, 248.9 ± 2.5 g initial mean body weight), as well as experimental ND and WD, were obtained from the Razi Vaccine and Serum Research Institute (Iran). After 1-week of the acclimation period, the animals were randomly assigned to six equal groups (n = 10/group): no-exercise sedentary (SED) controls, fed either a ND (ND+SED) or WD (WD+SED); and exercise of HIIT and MICT, fed either a ND (ND+HIIT/ND+MICT) or WD (WD+HIIT/WD+MICT). The rodents ND and WD were prepared according to the formulation of the formula (TD.08485-Envigo (fat: 13 % kcal; carbohydrate: 67.9 % kcal; protein: 19.1 % kcal) and TD.88137-Envigo (fat: 42 % kcal; sucrose: 42.7 % kcal; protein: 15.2 % kcal; cholesterol: 0.2 % kcal), respectively [21]. All rat groups were housed in a standard laboratory animal room (12/12 h light/dark cycle, 22 ± 2 °C, relative humidity: 55% ± 5%) with ad libitum feeding of ND or WD and water, until the completion of the experiments, which lasted 12 weeks. Three of the rats were excluded from the study due to their incapacity to perform the exercise training interventions. The total body weight and food intake levels were recorded weekly. Forty-eight hours after the last exercise session, echocardiography evaluations were performed, then the animals were sacrificed under deep anesthesia. Tissues from the left ventricular (LV) were snap-frozen and then stored at −80 °C. Western blotting and enzyme-linked immunosorbent assay (ELISA) techniques were performed for the detection of cardiac protein expression and serum factors, respectively. A flowchart illustrating the steps of the methodology is presented in Fig. 1.

2.3. Exercise training protocol

To improve running skills and reduce equipment-related stress, all rats were allowed to practice on the 0° inclined motorized treadmill at a speed of 10 m/min, for 10–15 min per day, for five consecutive days. Before the beginning of either HIIT or MICT, and also at the 4th and 8th weeks of experiments, the maximal physical capacity (MPC) of each rat was estimated by the maximal incremental running test (MIRT), in order to adjust the relative intensity for exercise protocols. In this test, the animals ran on a constant uphill treadmill (15° inclination) until voluntary exhaustion; the initial speed (10 m/min) was gradually increased (3 m/min every 3 min) until the rats were incapable of continuing even with mild stimulation by a flexible plastic cane. The speed at the point of exhaustion was considered as 100% of MPC and used for adjusting the accurate intensity/speed of HIIT and MICT protocols across the study. The rats in either HIIT or MICT protocol engaged in 12 weeks of running on the uphill treadmill at 15° inclination, five consecutive days/week, in conformity with those of Hafstad et al. [6]. Each HIIT session comprised of 10 bouts of 4 min high intensity running at 85–90% of MPC, followed by 2 min of lower intensity running at 50–60% MPC. In contrast, MICT comprised the continuous endurance running at 65–70% MPC. The rats subjected to HIIT, ran 60 min in each exercise session, whereas the total running time in the MICT sessions lasted close to 100 min. Exercise of HIIT and MICT were matched for volume, denoting that the total covered distances during the HIIT sessions were identical to that of MICT; accordingly, the MICT duration in all exercise sessions was adjusted to the running distance of HIIT. Both exercise protocols were executed after an initial warm-up.
period, for 10 min, at 50–60% MPC. The running pace was gradually increased — from 19 to 33 m/min for HIIT and 15–20 m/min for MICT over eight weeks — and sustained at these intensities for the last four weeks. These running paces were obtained by regular MIRT every four weeks. The SED controls were placed on the inactive treadmill for 20 min a day to ensure that regular transporting of animals between housing and the exercise room does not affect the results. These groups also ran on the treadmill, at a speed of 10 m/min, for 5 min, once a week, to maintain running skills. All experiments were performed at similar times of day to prevent potential diurnal effects. After 12 weeks, a final MIRT was performed and the running time to exhaustion was recorded as aerobic capacity.

2.4. Echocardiography

The conventional parameters of LV systolic and diastolic function were assessed at rest, by a Vivid 7 echocardiography system (GEU, Horten, Norway) with a 10–12 MHz probe. All the evaluations were performed at the animal lab of Rajaie Cardiovascular Medical and Research Center (Iran) by an experienced echocardiography operator blinded to the experimental groups. We adhered to the comprehensive guidelines of the American Society of Echocardiography to attain reliable assessments [26]. The LV parameters of chamber dimensions and wall thickness were obtained from all parasternal standard axis views by M-mode two-dimensional imaging. The LV end-systolic (ESV) and end-diastolic (EDV) volumes as well as LV internal-systolic (LVIDd) and internal-diastolic (LVIDd) diameters were used to calculate the ejection fraction (EF %) and fractional shortening (FS %), respectively, as the following formulas: EF = (EDV – ESV) / EDV * 100 and FS = ((LVIDd – LVEDd) / LVEDd * 100). The LV diastolic function was determined by the pulsed wave Doppler imaging ratio of transmitial flow velocities filling over the peak early and late diastolic phases (E/A ratio).

2.5. Western blotting

Western blot analysis was performed according to a previously described procedure [27]. Briefly, myocardial tissue samples were homogenized in ice-cold RIPA lysis buffer covered with a protease inhibitor cocktail (Sigma-Aldrich). The supernatants obtained after 30 min centrifugation at 12,000g (4 °C), were kept for total protein quantifying (Bradford assay) and Western blotting. An equal amount of protein (typically 20 μg) was loaded on SDS-PAGE (12.5% acrylamide gels) and subsequently blotted onto a polyvinylidene difluoride membrane (Roche, UK), using electroblotting. Blocking of the blots was performed (60 min at 37 °C) with 5% non-fat dry milk in Tris-buffered saline containing 0.05%Tween-20 (TBS-T). The blots were then incubated at 4 °C overnight with diluted (1:1000) primary antibodies (Santa Cruz Biotechnology, USA) against anti-BDNF (Cat. #: sc-546), anti-TrkB (Cat. #: sc-377218), anti-IL-18 (Cat. #: sc-7954), anti-HO-1 (Cat. #: sc-136960), and anti-β-Actin (Cat. #: sc-130657). After washing steps (4 × 10 min), incubation of the blots (120 min at 37 °C) was performed with mouse anti-rabbit IgG-HRP (Cat. #: sc-2357) and m-IgGk BP-HRP (Cat. #: sc-516102) secondary antibodies (1:5000 dilution; Santa Cruz Biotechnology, USA). The target protein bands were visualized using a chemiluminescence substrate (Pierce Biotechnology, Rockford, IL). Quantitative data were obtained from western blot bands using ImageJ software (NIH-1.62, USA) and normalized to the values of β-actin loading control.

2.6. Serum analysis

Blood samples were collected through cardiac puncture in fasting rats. Separated sera in a 10-min centrifuge at 3,000 rpm were used to measure all the target factors. Rat ELISA kits (Thermo Fisher Scientific, USA) were supplied to determine BDNF (Sensitivity: 12 pg/mL) and insulin (Sensitivity: 5 μU/ml) concentrations. Photometric determination of glucose, triglyceride (TG), and total cholesterol (TC) levels were performed using the quantitative detection kits (Pars Azmoon, Iran). The homeostasis model assessment of insulin resistance index (HOMA-IR) was obtained from the product of fasting values of insulin (mmol/l) and glucose (mmol/l) divided into 22.5 as a constant [28].

2.7. Visceral fat mass

The visceral adipose tissue, including mesenteric (surrounding the small bowel and the colon), bilateral perirenal retroperitoneal, and epididymal (perigonadal fat), were carefully dissected from the abdominal cavity [29]. To prevent weight changes due to evaporation, the purified white fat pads were weighed together immediately, using a laboratory balance with the resolution of 0.1 mg (A&D, Japan).
2.8. Statistical analysis

The sample size was estimated using the G*Power Software (version: 3.1.9.7), based on previous animal studies [4,20]. A power of 0.8 (α error = 0.05) was obtained. All data are presented as means ± SEM. The normal distribution of data was verified by a Shapiro-Wilk test. A repeated-measures ANOVA was performed to assess weekly changes in mean total body weight and food intake levels. All data were compared with one-way ANOVA and Tukey’s post hoc test. In all cases, data analyses were performed using the IBM SPSS Statistics (version: 22.0) at P < 0.05.

3. Results

3.1. Total body weight and food intake levels

As shown in Table 1, there were no significant differences in mean total body weight and food intake levels among groups at the baseline. Total body weight was significantly increased in all groups compared with their initial values after the 12 weeks (P < 0.05). The final body weight of rats was not significantly different between WD + SED and ND + SED. In exercise groups, total body weight was significantly lower only in WD + HIIT compared with WD + SED (P < 0.05). The food intake levels were significantly reduced in WD compared with ND + SED throughout the study (P < 0.05). Neither HIIT nor MICT was able to alter the food intake levels in rats.

3.2. Effects of HIIT and MICT on metabolic parameters in either ND or WD feeding

As shown in Fig. 2 (A, B, C, D, respectively), HOMA-IR, visceral fat mass, and serum levels of TG and TC were significantly increased in WD compared with ND + SED (P < 0.05). In exercise groups, WD + HIIT and WD + MICT showed a significant decrease in all metabolic parameters compared with WD + SED (P < 0.05), with no significant differences between the exercises modes. The metabolic parameters were not affected by either HIIT or MICT in the ND rats.

3.3. Effects of HIIT and MICT on serum BDNF levels and cardiac protein expression of BDNF-TrkB-T1 in either ND or WD feeding

As shown in Fig. 3 (A and B), the coexistence of WD and SED had no significant effect on the protein expression of cardiac BDNF and its TrkB-T1 receptor compared with ND + SED. In exercise groups, HIIT significantly increased cardiac BDNF protein expression in rats fed a ND or WD compared with their SED counterparts (P < 0.05). This effect was significantly higher in ND + HIIT compared with WD + HIIT (P < 0.05). Likewise, HIIT significantly increased cardiac TrkB-T1 protein expression (P < 0.05), but only in the rats fed a ND. There were no significant changes in cardiac BDNF-TrkB-T1 protein expression in the MICT groups.

Fig. 3 (C) also shows that serum BDNF levels were significantly reduced in WD + SED compared with ND + SED (P < 0.05). In exercise groups, WD + HIIT and WD + MICT showed a significant improvement in serum BDNF levels compared with SED + WD (P < 0.05), with no significant differences between the exercises modes. Likewise, ND + HIIT was associated with a significant increase in serum BDNF levels more than ND + MICT, as compared with ND + SED (P < 0.05).

3.4. Effects of HIIT and MICT on cardiac function in either ND or WD feeding

As shown in Fig. 4 (A, B, C, respectively), there were no significant differences in EF, FS, and E/A ratio between WD + SED and ND + SED. In exercise groups, ND + HIIT and WD + HIIT showed a significant increase in EF and FS compared with their SED counterparts (P < 0.05), without regard to the dietary patterns. No significant differences were observed in the E/A ratio among groups.

3.5. Effects of HIIT and MICT on aerobic capacity in either ND or WD feeding

In this study, running time-to-exhaustion was considered as aerobic capacity. We found no significant differences in the baseline aerobic capacity among groups (data not shown). As shown in Fig. 5, aerobic capacity was not affected by the coexistence of WD and SED compared with ND + SED. In exercise groups, HIIT and MICT significantly increased aerobic capacity in rats fed a ND or WD compared with their SED counterparts (P < 0.05). There were no significant changes in aerobic capacity between the exercise modes and diets.

3.6. Effects of HIIT and MICT on cardiac protein expression of IL-18 and HO-1 in either ND or WD feeding

As shown in Fig. 6 (A), cardiac IL-18 protein expression was significantly increased in WD + SED compared with ND + SED (P < 0.05). In exercise groups, cardiac IL-18 protein expression was significantly lower in both WD + HIIT and WD + MICT groups compared with WD + SED (P < 0.05), with no significant differences between the exercise modes. Likewise, WD + MICT showed a significant decrease in cardiac IL-18 compared with WD + SED (P < 0.05). In exercise groups, cardiac IL-18 protein expression was significantly reduced in WD + SED compared with ND + SED. There were no significant changes in cardiac HO-1 protein expression in the MICT groups.

4. Discussion

As a central finding of the present study, we uncovered that the HIIT paradigm confers higher cardiac benefits than MICT in either ND or WD feeding. Indeed, although both HIIT and MICT were able to blunt WD-related modifications, such as metabolic disturbances, serum BDNF depletion, and myocardial inflammation (IL-18 overexpression), only...
the exercise of HIIT led to improvement in the cardioprotective molecule HO-1 expression, augmented myocardial BDNF-TrkB-T1 expression, and enhancement of resting cardiac function.

4.1. Metabolic parameters after HIIT or MICT in the presence and absence of WD

In this study, animals receiving WD for 12 weeks exhibited significant dyslipidemia, insulin resistance (HOMA-IR), and visceral fat deposition, indicating a metabolic syndrome condition. This finding is in agreement with previous evidence, where HFD or WD in rats caused metabolic perturbations [30]. Our observation that WD consumption leads to adverse metabolic changes and an obvious increase in visceral fat mass without inducing overall obesity is in accordance with several animal studies pointing out that WD can promote visceral fat deposition and adiposity index, but does not cause a significant increase in final body weight [31,32]. These findings can be justified through the prevalence of metabolically obese but normal-weight subjects who have markedly a higher amount of visceral adipose tissue coupled with various metabolic abnormalities, particularly insulin resistance [33]. In analogy with previous studies [34], our findings showed that WD, despite being palatable, is associated with a significant reduction in food intake levels. This observation may be explained by the fact that WD provides long-term satisfaction for the studied rats since the energy density of this type-food is approximately 50% higher than in ND [34].

Our findings indicated that HIIT and MICT, with analogous effects, blunt the WD-induced metabolic syndrome manifestations. This observation is in accordance with several, but not all past research studies assessing metabolic parameters after HIIT and MICT. In good agreement, Batacan et al. (2018) [35] and Haram et al. (2009) [36] showed that HIIT and MICT paradigms are similarly effective in obviating visceral fat deposition in rats with metabolic syndrome or those fed a WD. The observation that only the HIIT exercise is associated with lower final body weight (at the end of 12 weeks) in rats fed a WD is in accordance with those of Maillard et al. (2019) [37], where obese animals exhibited a significant reduction in total body weight after HIIT and not that of MICT. Also, in another study, MICT failed to overcome body weight gain in HFD rats [11]. The increase of the HIIT-induced α/β adrenergic receptor ratio in subcutaneous adipose tissue may contribute to exercise intensity-dependent weight loss or prevention [37]. In accord with previous studies [38,39], no significant changes in metabolic parameters in our ND rats after HIIT or MICT hints at the possibility that, in normal metabolic situations, exercise acts as a means to preserve metabolic parameters almost in a physiological range.

4.2. Cardiac function after HIIT or MICT in the presence and absence of WD

In this study, animals receiving WD for 12 weeks exhibited no significant changes in echocardiographic parameters of systolic (EF and FS) and diastolic (E/A ratio) function at rest, compared with the ND group. This observation is in accordance with several, but not all past research studies assessing cardiac function after HFD or WD feeding. In earlier studies, subjecting animals to HFD or hyperglycemia, for 15–28 weeks, was not sufficient enough to ultimately cause or exacerbate cardiac dysfunction even in the presence of a previous significant cardiac event.
such as myocardial infarction [40,41]. These findings were substantiated by Agrimi et al. (2019) [27] most reliable study, where HFD (obesity) for 18 weeks caused no cardiac dysfunction per se. There is no obvious explanation for contradictory results that exist [10,20]. Still, it is likely that the type, strain, and age of the rodents employed, the differences in diet and duration of exposure, methods and criteria for cardiac function assessments, anesthetic procedures during echocardiography, and unknown environmental factors are all relevant parameters. In this context, WD has been shown to impair myocardial function in vitro (papillary muscle) but not in vivo (echocardiogram) [42].

In the current study, animals receiving HIIT but not MICT showed a significant increase in systolic cardiac function at rest, compared with the SED controls, both in the WD and ND feeding. This observation shares many similarities with the recent study by Verboven et al. (2019) [4], where resting cardiac function in healthy rats was markedly enhanced by either HIIT or MICT. Our evidence that MICT did not increase resting systolic cardiac function in healthy rats is not unexpected, due to the moderate intensity of the exercise bouts (70% MPC). In fact, functional changes in the cardiovascular system require a wide array of adaptations that occur by high-intensity exercise (i.e., >70% MPC) in the long run [43-45]. Accordingly, HIIT has been reported as the only exercise mode to enhance the capillary density of rat hearts [4]. Still, the discrepancy between MICT results may be attributable to a higher baseline (initial) value of EF in our study compared with that of others (EF = 74 vs. 63 %, respectively). Likewise, MICT has been reported not to enhance EF in healthy mice with a high initial value of EF (~84%) [10]. In this study, animals receiving HIIT and MICT for 12 weeks displayed no significant changes in diastolic cardiac function (E/A ratio) at rest, compared with the SED controls, both in the WD and ND feeding, challenging the benefits of exercise training to diastolic cardiac function. This finding, however, is in accordance with the evidence that exercise training for 8–10 weeks does not change the E/A ratio in young healthy rats [46] and patients with hypertension [47]. It may be noted that the E/A ratio parameter is less sensitive than those of tissue Doppler imaging, probably due to the influence of hemodynamic conditions [47]. Accordingly, exercise training in 116 cardiac patients has been shown to increase Doppler imaging parameters without altering the E/A ratio [47].

Aerobic capacity is labeled as a single best parameter linked to cardiac function [48]. High aerobic capacity levels have been shown to

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Fig. 3. (A-B) Cardiac protein expression of brain-derived neurotrophic factor (BDNF) - truncated isoform of tropomyosin-related Kinase B (TrkB-T1) and (C) serum brain-derived neurotrophic factor (BDNF) levels after 12 weeks of high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT) in rats fed a Western (WD) or regular (ND) diet. Values are expressed as mean ± SEM (n = 6). *denotes P < 0.05 between groups; # P < 0.05 vs. WD + SED.
substantially reduce the risk of cardiovascular diseases [49]. In the current study, animals receiving HIIT and MICT showed an analogous increase in aerobic capacity compared with the SED controls, both in the WD and ND feeding. This observation is in accordance with studies that HIIT and MICT similarly increase aerobic capacity in mice [6] and healthy SED men [50]. The comparable effects between the two exercise modes are an unsurprising finding, considering that HIIT and MICT have been shown to be equally effective in fostering aerobic metabolism elements such as citrate synthase [51]. However, as opposed to MICT, the exercise of HIIT not only saves time but has also emerged (in this study) as the only exercise mode to enhance cardiac function.

In the current study, the increase of HIIT-induced systolic cardiac function in healthy rats was coupled with an augmentation in BDNF-TrkB-T1 expression in the myocardium. Similarly, exercise training in rats with myocardial infarction has been shown to improve systolic cardiac function by restoring myocardial BDNF content [19]. It can therefore be noted that part of the exercise-imparted cardiac function benefits occurs via the enhancement of BDNF-TrkB-T1 expression, both in healthy and ischemic myocardium. Of note, in parallel with the direct effect on the heart, BDNF might also modulate myocardial mechanical responses by stimulating \( \beta \)-AR signaling. This can be explained, at least in part, by an overt increase in sympathetic nervous system activity after the direct application of BDNF into the paraventricular nucleus of the hypothalamus [52,53].

4.3. BDNF-TrkB-T1 axis after HIIT or MICT in the presence and absence of WD

In the present study, animals receiving WD for 12 weeks displayed no significant changes in cardiac BDNF-TrkB-T1 expression, compared with

![Fig. 4. (A) Ejection fraction (EF), (B) fractional shortening (FS), and (C) the ratio of peak early to late atrial Doppler flow velocity (E/A ratio) after 12 weeks of high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT) in rats fed a Western (WD) or regular (ND) diet. Values are expressed as mean ± SEM (n = 8–10). \(^*\)P < 0.05 vs. ND + SED; \(^\#\)P < 0.05 vs. WD + SED.](image)

![Fig. 5. Running time-to-exhaustion (aerobic capacity) after 12 weeks of high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT) in rats fed a Western (WD) or regular (ND) diet. Values are expressed as mean ± SEM (n = 8–10). \(^*\)P < 0.05 vs. ND + SED; \(^\#\)P < 0.05 vs. WD + SED.](image)
induced BDNF and TrkB-T1 expression blunted or disappeared, respectively, in rats subjected to WD. Altogether, this study reveals that the HIIT paradigm exclusively leads to a significant augmentation in cardiac BDNF-TrkB content.

This is the first evidence, to our knowledge, of animals receiving HIIT, but not MICT, exhibited a marked increase in cardiac BDNF-TrkB-T1 expression, compared with the SED controls, while different effects were present between the ND and WD feeding. This adaptation, in fact, was partially jeopardized by WD feeding. Thus, the increase of HIIT-expression in rat myocardium is different from those of Lee et al. (2018) [19], who found that aerobic training for 4 weeks increased cardiac BDNF expression in rats. This disparity may be, in part, due to differences in the animals employed. In the study conducted by Lee et al. [19], rats had cardiac dysfunction, whereas those enrolled in our study were healthy (normal heart function). Importantly, in rats with ischemic myocardium, exercise necessarily strives for restoring BDNF expression almost to initial levels; however, in intact hearts, exercise acts as a trigger for increasing BDNF expression, more than its initial (physiological) levels. In the second one, unlike MICT, a more powerful stimulation such as found in HIIT, may be required to activate some signaling pathways in cardiovascular systems. In accordance, in the study by Lee et al. [19], where the TrkB-T1 receptor remained (unchanged) at high levels after myocardial infarction, MICT failed to increase it markedly; instead, the exercise of HIIT in our study not only augmented BDNF but also triggered TrkB-T1 expression in rat myocardium, suggesting that cardiac BDNF-TrkB-T1 expression is sensitive to training intensity. At present, nor is it clear how exercise intensity or HIIT impacts cardiac BDNF-TrkB content; however, before the leap to that, the central mechanism(s) of cardiac BDNF secretion by exercise need to be defined in-depth. Still, in the wake of evidence that skeletal muscle contraction is followed by BDNF release [54,55], we speculate, temporarily, that repeated increases in myocardial contraction or efferent cardiac nerve activity mediate part of the effect of exercise on cardiac BDNF augmentation. Some likelihood is that cardiomyocytes uptake peripheral BDNF via the TrkB-T1 receptor and, by doing so, activate the CaMKII-mediated signaling, eventually increasing cardiac contractility [19]. In this study, serum BDNF levels in rats fed a ND were higher after HIIT than MICT. This evidence may explain, tentatively, our observation that only HIIT effectively increases cardiac BDNF levels.

This study appears to indicate a causal relationship between the WD feeding and little or no changes in cardiac BDNF expression and TrkB-T1, respectively, after exercise intervention, meaning that WD negatively impacts the modulation of BDNF-TrkB-T1 expression by HIIT. Our measurements are few to describe how this occurs, but if circulating BDNF influences the behavior of its own cardiac isoform, it may be justified in part by lower serum BDNF levels in HIIT + WD compared with HIIT + ND. Likewise, the detrimental effects of WD, such as metabolic disturbances and cardiac inflammation (IL-18 overexpression) may be relevant factors, considering that these conditions can potentially alter some critical signaling pathways in the heart and brain. Importantly, inflammation and metabolic syndrome have been suggested to impair BDNF-TrkB signaling in the brain [56] and Notch angiogenesis signaling in the heart [57], respectively.

In this study, animals receiving WD for 12 weeks displayed a significant reduction in serum BDNF levels, compared with the ND group. This finding is in agreement with those of Hoseindoost et al. (2019) [58], who found that HFD (obesity) for 8 weeks leads to a marked decline in serum BDNF levels in mice per se. Interestingly, HFD and psychosocial stress synergistically reduced BDNF-TrkB expression in the heart [27]. However, it may be implied that the present conditions of isolated WD or HFD are not sufficient, at least for 12–18 weeks, to jeopardize cardiac BDNF-TrkB content.
essential to know that serum BDNF is represented by that stored in mostly platelets [61-63], plasma BDNF suggests to release from the central nervous system, such as hippocampal and endothelial cells [61], and BDNF expresses within contracting muscles but it cannot leave the muscle fibers [64].

Higher circulating BDNF levels have been extremely suggested to correlate with cardiovascular protection [18,65]. Here, we found that either HIIT or MICT exert positive effects on serum BDNF levels, both in ND and WD. Indeed, HIIT and MICT not only increased serum levels of BDNF in rats fed a ND but also similarly ameliorated the WD-induced serum BDNF losses. This evidence is in accordance with studies that regular exercise increases resting serum BDNF levels [66-68] and, with those of Sabaghi et al. (2019) [69], in which, HIIT was able to counter serum BDNF depletion in a Parkinson’s model. The observation that HIIT in the ND group augments serum BDNF levels more than MICT is in agreement with the study by Hsu et al. (2020) [67], where resting serum BDNF levels in stroke patients were higher after HIIT than MICT. In general, exercise contributes to the addition of BDNF to circulation through multiple sources or tissues, such as the brain, skeletal muscle, vascular endothelial cells, and spleen (platelets) [61]. However, there is a strong belief that exercise can increase serum BDNF levels mainly via inducing thrombocytosis and thereby the addition of splenic platelets to the circulation [61,70]. This concept appears to be well substantiated by Walsh et al. (2017) [71], where acute handgrip exercise leads to the addition of BDNF and platelets to the serum through the increased sympathetic outflow-induced splenic constriction. Further, exercise may also trigger the release of BDNF by enhancing circulating catecholamines and subsequently platelet activation [55,72].

4.4. Myocardial IL-18 and HO-1 expression after HIIT or MICT in the presence and absence of WD

Our observation that regular intake of WD markedly increases cardiac IL-18 expression in relation to the ND group is in agreement with studies that either HFD or WD leads to overexpression of pro-inflammatory mediators, such as tumor necrosis factor-alpha (TNF-α) and IL-18 in mice myocardium [10,21]. Saturated fat and sugars in WD can directly activate the NLRP3 inflammasome [22,73], thereby producing IL-18, triggering the critical inflammatory cascades, and compromising cardiac function [21]. In accordance with the exclusive study conducted by Bostick et al. (2017) [74], our present investigation also showed that WD markedly reduces the HO-1 expression in the heart. The HO-1 molecule is a pivotal mediator of cardioprotection, attenuating inflammation, oxidative stress, and apoptosis [75]. Indeed, HO-1 confers exquisitely defense against different pathophysiological stress such as myocardial infarction [76]. Our data, together with previous findings [21,74], could imply that WD can jeopardize the cardiac system by increasing the expression of IL-18 while reducing HO-1.

Aerobic training acts effectively to enhance myocardial protection, preserving heart function, notably in the presence of significant co-factors for cardiometabolic abnormalities, such as HFD and WD [6,10,74]. To this end, exercise targets various molecules, whether destructive or protective. In vivo studies have attested that MICT protects mice myocardium against HFD by attenuating TNF-α expression [10]. There is also evidence showing that MICT dampens the serum concentration of interleukin-1β (IL-1β) in rats fed a HFD [11]. Likewise, our present findings show, for the first time, that HIIT and MICT, with similar effects, are able to cope with WD-induced IL-18 overexpression, labeled as a central pro-inflammatory cytokine mediating cardiac dysfunction linked to WD [21]. This protection may be related to an increase and decrease in the cardiac expression of IL-10 and P2X7 purinergic receptors [10], respectively [10,11]. What is more, since HO-1 overexpression is a significant inhibitor of IL-18 signaling [77], it is plausible that activation of HO-1 in the heart, as observed here, mediates the anti-IL-18 effects of exercise.

It is worth noting that BDNF may also have some anti-inflammatory properties. In this context, exploratory studies have shown that exogenously applied BDNF abrogates the formation of NLRP3 inflammasome [78] and multiple pro-inflammatory mediators [79,80]. Interestingly, BDNF also is able to augment IL-10 levels [79]. Therefore, exercise might rescue the heart from inflammation partially via activation of BDNF-TrkB signaling, although our present study does not directly cover this concept.

So far, only a few studies, assessed the cardiac expression of HO-1 after exercise training in the setting of WD. Specifically, Bostick et al. (2017) [74] found that subjecting mice to wheel-running exercise, for 16 weeks, can mitigate the WD-induced myocardial dysfunction by maintaining HO-1 levels. In addition, this study reveals that regular exercise, exclusively HIIT, for 12 weeks, not only prevented the cardiac HO-1 losses linked to WD but also led to a marked increase in HO-1 expression in rats fed a ND. Our observation of augmented HO-1 in the healthy (ND feeding) myocardium by HIIT is relatively novel evidence. The finding that MICT (treadmill running) is not powerful enough to significantly counteract the HO-1 downregulation due to WD is distinct from those of Bostick et al. (2017) [74]. This contradiction may be explained, in part, by differences in study duration, exercise protocol, type, and age of the animals used.

In the induction of HO-1 expression, several mechanisms play a role, but insulin signaling [81] is a possible pathway by which exercise may contribute to the upregulation of HO-1. In the current study, the increase of HIIT-induced HO-1 expression was coupled with cardiac BDNF augmentation. Therefore, it might be an open question, whether overexpression of HO-1 by physiological stress (e.g., exercise) potentiates the BDNF-TrkB signaling in the heart. It is important to note that bilirubin and carbon monoxide, as the downstream product of HO-1, have the capability to drive the BDNF expression in neuronal cells [82].

5. Conclusions

The present study allows us to highlight the changes induced by WD and daily exercise in factors affecting cardiac function. Our data reveal that, similar to HIIT, MICT can cope with WD repercussions, including metabolic disturbances, serum BDNF depletion, and cardiac IL-18 overexpression. However, only HIIT was capable of improving cardiac HO-1 expression, both in WD and ND, demonstrating that regular high-intensity exercise is required for further myocardial protection. These findings, therefore, advocate the trend of aerobic training, notably HIIT, for those who are unable or unwilling to avoid a WD pattern. This study, in addition, confirms that exercise, exclusively HIIT, can foster myocardial BDNF-TrkB-T1 protein expression, a pathway that plays a vital role in governing heart function. Thus, HIIT should be considered as a preferred exercise paradigm for subjects who require cardiac function to be preserved or enhanced, particularly athletes and heart failure patients. Further research is required for clinical validation.

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Author contributions

Abdulbaset Maroofi: conception and design of study, performed exercise intervention and animal data collection, contributed to echocardiographic data collection, analyzed data, prepared figures, and drafted the original manuscript. Ahmadreza Bagheri Rouch: contributed to animal data collection. Nasim Naderi: performed echocardiography. Arsalan Damirchi: design of study, supervision, and review of the manuscript.
Declaring of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcha.2022.101022.

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