

Research Article

The effects of aerobic training on cardiac SIRT3/FOXO3 expression and inflammatory status in obesity: An experimental study

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Abstract

This study investigated the effects of aerobic exercise on cardiac SIRT3 and FOXO3 expression and inflammatory cytokines in rats with obesity induced by a high-fat, high-fructose diet. Twenty-four male Wistar rats were assigned to three groups: control, high-fat/high-fructose diet (HFFD), and HFFD plus aerobic exercise (HFFD+Ex). Obesity was induced by 8 weeks of HFFD, followed by a 6-week progressive moderate-intensity aerobic treadmill training program (10-18 m/min, 10-30 min/day, 5 days/week; approximately 42-75% of individual V_{max}) in the exercise group. HFFD induced obesity, dyslipidemia, and significant reductions in cardiac SIRT3 and FOXO3 expression compared with the control group ($P < 0.05$). The exercised group showed significantly higher SIRT3 expression compared with the HFFD group, reaching levels comparable to controls, with a very large effect size (Cohen's $d = 6.10$). FOXO3 expression showed a 40% increase in the exercised group compared with the HFFD group, but this difference did not reach statistical significance ($P = 0.795$), potentially due to limited statistical power with the sample size employed (post-hoc power = 62%). The exercised group also exhibited significantly lower circulating TNF- α and IL-1 β concentrations and higher IL-10 levels compared with the HFFD group ($P < 0.05$), indicating a systemic anti-inflammatory effect. However, as these cytokines were measured in blood rather than cardiac tissue, they reflect systemic inflammation and may not directly represent cardiac-specific inflammatory changes. Although FOXO3 gene expression was not significantly altered, the findings support aerobic exercise as an effective non-pharmacological strategy to mitigate obesity-related cardiac dysfunction through modulation of mitochondrial and inflammatory pathways.

Key Words: Aerobic exercise, Obesity, SIRT3, FOXO3, Inflammation, Heart

Introduction

The global prevalence of obesity has reached pandemic proportions, with more than 650 million adults affected and rates continuing to rise in low- and middle-income countries (Ahmed & Konje, 2023; Sørensen et al., 2022). Obesity, defined as abnormal or excessive fat accumulation, is a complex, multifactorial disease that substantially increases the risk of cardiovascular diseases (CVD), type 2 diabetes, and other metabolic disorders (Ahmed & Konje, 2023; Powell-Wiley et al., 2021). The pathophysiology of obesity involves chronic low-grade inflammation and increased oxidative stress, which are key contributors to cardiac dysfunction and remodeling (Mirmiran et al., 2014). A well-established experimental model to study these mechanisms involves feeding rodents a high-fat diet (HFFD) often supplemented with fructose, which effectively induces obesity and mimics human metabolic syndrome, leading to cardiac lipotoxicity and mitochondrial dysfunction (Im et al., 2021; Marques et al., 2016).

At the molecular level, the Sirtuin family of NAD⁺-dependent deacetylases, particularly the mitochondrial SIRT3, has emerged as a critical regulator of cellular metabolism and oxidative stress (Sun et al., 2018). SIRT3 is highly expressed in metabolically active tissues, such as the heart, where it deacetylates and activates key mitochondrial enzymes involved in fatty acid oxidation, the tricarboxylic acid (TCA) cycle, and the electron transport chain (Sun et al., 2018; Wang et al., 2023). Moreover, SIRT3 plays a pivotal role in the antioxidant defense system by deacetylating and activating Forkhead box O3 (FOXO3), a transcription factor that upregulates the expression of antioxidant enzymes like manganese superoxide dismutase (MnSOD) and catalase (Sundaresan et al., 2009; Tseng et al., 2013). Dysregulation of the SIRT3/FOXO3 pathway is implicated in the pathogenesis of obesity-related cardiac complications, and conversely, its activation offers cardioprotection (Bošković et al., 2021; Guo et al., 2020). Studies have shown that in the context of a high-fat diet, increased oxidative stress triggers an adaptive upregulation of

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SIRT3 and FOXO3 as a protective mechanism, although this compensation is often insufficient to prevent cardiac damage (Bernardo et al., 2023; Wang et al., 2018). Regular physical activity, particularly aerobic exercise, is a well-established non-pharmacological intervention for preventing and treating cardiovascular diseases and metabolic syndrome (Golbidi & Laher, 2012). The cardioprotective benefits of exercise are multifaceted, ranging from improved whole-body metabolism to direct adaptations within the myocardium (Powers & Jackson, 2008). At the cellular level, exercise has been shown to activate the AMPK/FOXO3/SIRT3 signaling axis, thereby enhancing mitochondrial biogenesis, improving mitochondrial function, and reducing oxidative stress (Ho et al., 2022). A recent study by Jin et al. (2022) demonstrated that the beneficial effects of exercise against diabetic cardiomyopathy are mediated through the FGF21-SIRT3 axis, which preserves mitochondrial integrity. Furthermore, other investigations have highlighted that exercise can increase cardiac SIRT3 expression and activity, leading to enhanced deacetylation of FOXO3 and subsequent activation of downstream antioxidant pathways (Donniacuo et al., 2019; Zhao et al., 2018). However, the specific effect of aerobic exercise on the gene expression of SIRT3 and FOXO3 in the context of obesity induced by a high-fat and high-fructose diet, especially in cardiac tissue, remains an area requiring further investigation.

Given the increasing burden of obesity and its associated cardiovascular complications, understanding the molecular pathways through which exercise exerts its protective effects is crucial. Therefore, the present study aims to investigate the effect of aerobic exercise on inflammatory status and the gene expression of SIRT3 and FOXO3 in the cardiac tissue of male rats with obesity induced by a high-fat and fructose-rich diet.

Materials and methods

Study design

The present study is a fundamental, experimental investigation conducted in a laboratory setting using a post-test-only control group design. All experimental procedures were performed in accordance with the guidelines for the care and use of laboratory animals and were approved by the Ethics Committee of the Islamic Azad University, Ahvaz Branch (Ethics Code: IR.IAU.AHVAZ.REC.1402.075).

Animals and housing

Twenty-four male Wistar rats, aged 8 weeks with a mean body weight of 204 ± 11.3 g, were obtained from the Animal Breeding Center of Ahvaz Jundishapur University of Medical Sciences. Male rats were selected to avoid the confounding effects of hormonal fluctuations observed in females. The animals were housed in groups of four per standard transparent polycarbonate

cages (Razi Company, Iran) under controlled environmental conditions: temperature 22 ± 2 °C, relative humidity 40-60%, and a 12:12 hour light–dark cycle. The rats were allowed ad libitum access to standard chow (Razi Institute, Iran) and water during a two-week acclimatization period before the start of the experiment.

Induction of obesity

Following the acclimatization period, the rats were randomly divided into two main dietary groups. One group (n=8) was fed a standard control diet (20% kcal from fat, 20% protein, 60% carbohydrate; 3.1 kcal/g) for 8 weeks. The second group (n=16) was fed a high-fat and high-fructose diet (HFFD) for 8 weeks to induce obesity. The HFFD consisted of 60% kcal from fat (90% animal fat and 10% soybean oil) and was supplemented with a 25% fructose solution (v/v) in drinking water, which was prepared fresh daily and provided ad libitum (Hidayati et al., 2020). The high-fat diet pellets were formulated and supplied by the Royan Institute of Biotechnology (Isfahan, Iran). To confirm the induction of obesity, anthropometric and biochemical parameters were assessed at the end of the 8-week dietary period. Obesity was defined by a significant increase in body weight, body mass index (BMI), and Lee index compared to the control group (Lima et al., 2021).

Experimental groups

After the 8-week dietary induction, the HFFD-fed rats were randomly assigned into two experimental groups (n=8 per group): the obese control group (HFFD) continued to receive the HFFD for the entire study duration. The exercise-trained obese group (HFFD+Ex) received the HFFD and was subjected to an aerobic exercise program for the subsequent 6 weeks. The standard diet-fed group (n=8) served as the healthy control group. Thus, the final experimental groups were:

1. Control: Healthy rats group (standard diet throughout the study).
2. HFFD: Obese control group (HFFD throughout the study).
3. HFFD+Ex: Obese exercised group (HFFD throughout the study plus aerobic exercise intervention).

Aerobic exercise training protocol

Before the start of the exercise protocol, the rats were acclimatized to the motorized rodent treadmill (Technic Azma Co., Tabriz, Iran) for 5 days (10-15 minutes/day at 5-10 m/min) to minimize stress. During this period, gentle handling and positive reinforcement (gentle prodding with a soft brush) were used to encourage running. Maximum exercise capacity was then determined individually using a graded exercise test, where spe-

-ed was increased by 3 m/min every 3 minutes, starting at 5 m/min, until exhaustion (Lima et al., 2021). Exhaustion was defined as the point at which the rat could no longer maintain pace with the treadmill belt despite continuous encouragement via gentle prodding (with a soft brush) for 10 seconds, and remained on the shock grid (0.2 mA) without attempting to resume running (Zhao et al., 2018). The Vmax (maximal running speed) was recorded as the speed of the last completed stage. All graded exercise tests were performed at the same time of day (between 4:00 and 6:00 PM) to control for circadian variations in performance. The Vmax values for the HFFD+Ex group ranged from 22 to 26 m/min (mean±SD: 24.1±1.4 m/min).

The aerobic training program was based on a progressive moderate-intensity protocol corresponding to approximately 50–60% of Vmax (Ghardashi Afousi, 2023; Aghaei Bahmanbeglou et al., 2021). The exercise training was performed 5 days per week for a total of 6 weeks. Each session was conducted at the end of the animals' sleep cycle (between 4:00 and 6:00 PM). The training protocol was progressively increased in speed and duration as follows: week 1: 10 m/min (~42% Vmax) for 10 min; week 2: 10 m/min (~42% Vmax) for 20 min; week 3: 14–15 m/min (~58–62% Vmax) for 20 min; week 4: 14–15 m/min (~58–62% Vmax) for 30 min; week 5: 17–18 m/min (~71–75% Vmax) for 30 min; and week 6: 17–18 m/min (~71–75% Vmax) for 30 min (maintained constant for detraining adaptation) (Ghardashi Afousi, 2023). This progressive protocol was selected to allow for gradual cardiovascular and musculoskeletal adaptation, minimize the risk of injury or overtraining, and align with established moderate-intensity aerobic training protocols in rodent models (Ghardashi Afousi, 2023; Aghaei Bahmanbeglou et al., 2021). The final training intensity of 17–18 m/min (~70–75% Vmax) corresponds to the upper end of moderate-intensity exercise (approximately 65–75% of maximal oxygen consumption) in rats, consistent with definitions of aerobic training in rodent models. It should be noted that while we used a graded exercise test to determine Vmax and calculated training intensity relative to this measure, we did not perform post-training verification of the training effect (e.g., re-assessment of Vmax, measurement of muscle citrate synthase activity, or other markers of aerobic adaptation). This represents a limitation of the study design, as we cannot definitively confirm that the exercise stimulus was sufficient to induce a physiological training response in all animals.

Anthropometric measurements

Body weight was measured weekly using a digital scale (Apex, AKS-162W). At the end of the study, the following anthropometric indices were measured for each animal: naso-anal length (cm), abdominal circumference measured at the largest region of the abdomen, and thoracic circumference (Abubakar et al., 2022).

BMI was calculated as body weight (g) divided by the square of naso-anal length (cm²). The Lee index was calculated as the cube root of body weight (g) divided by naso-anal length (cm) (Lima et al., 2021).

Tissue and blood sample collection

Forty-eight hours after the last exercise session, rats were fasted for 12 hours and then anesthetized via intraperitoneal injection of a ketamine (90 mg/kg) and xylazine (10 mg/kg) cocktail (Rami et al., 2022). Approximately 5 mL of blood was collected directly from the left ventricle and allowed to clot. Serum was separated by centrifugation at 4000 rpm for 15 minutes and stored at -70 °C for subsequent biochemical analysis. The cardiac tissue was rapidly excised, washed with normal saline, weighed, and immediately snap-frozen in liquid nitrogen and stored at -80 °C for molecular analyses.

Biochemical analysis

Serum levels of total cholesterol (Chol), triglycerides (Tg), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were measured using commercially available kits (Pars Azmoon, Iran) according to the manufacturer's instructions.

RNA extraction and cDNA synthesis

Total RNA was extracted from approximately 100 mg of cardiac tissue using the RiboZol reagent (BioFavaran Rana, Isfahan, Iran) according to the manufacturer's protocol (Aghaei Bahmanbeglou et al., 2021). RNA concentration and purity were assessed using a spectrophotometer (Quawell Technology, San Jose, CA, USA) by measuring the absorbance ratios at 260/280 nm and 260/230 nm. RNA samples with an A260/A280 ratio greater than 1.8 and an A260/A230 ratio greater than 1.5 were considered acceptable. Subsequently, cDNA was synthesized using a cDNA synthesis kit (BioFavaran Rana, Isfahan, Iran) with oligo-dT primers and reverse transcriptase according to the manufacturer's instructions (Aghaei Bahmanbeglou et al., 2021).

Quantitative real-time PCR

The expression levels of SIRT3 and FOXO3 mRNA were measured using quantitative real-time PCR (qRT-PCR) with the SYBR Green master mix (BioFavaran Rana, Isfahan, Iran) on an ABI StepOne Plus system (Applied Biosystems, USA). The primer sequences used for amplification were as follows: FOXO3 (Forward: GCCTCATCTCAAGCTGGGT, Reverse: TGCTCTGG AGTAGGGATGCT), SIRT3 (Forward: CTGAGCTGTGCAGCT GGTATTG, Reverse: CCCACCATGACCACAACCCTA), and GAPDH as the reference gene (Forward: AGGTCGGTGTGA ACGGATTG, Reverse: TGTAGACCATGTAGTTGAGGTCA) (Schmittgen & Livak, 2008). The thermal cycling conditions were: initial denaturation at 95 °C for 5 minutes, followed by 40 cycles

of 95 °C for 30 seconds (denaturation), 58 °C for 30 seconds (annealing), and 72 °C for 30 seconds (extension). The relative gene expression was calculated using the $2^{-\Delta\Delta CT}$ method and normalized to GAPDH as the internal control. The results were expressed as fold-change relative to the HFFD group (Rao et al., 2013).

It is important to note that the present study assessed only mRNA expression levels of SIRT3 and FOXO3. While mRNA quantification provides valuable information regarding transcriptional regulation, it does not necessarily reflect corresponding changes in protein abundance, post-translational modifications, or enzymatic activity. Thus, our findings should be interpreted as reflecting transcriptional changes that may or may not translate to functional alterations in SIRT3 and FOXO3 activity.

Measurement of inflammatory cytokines

To evaluate the inflammatory status, the concentrations of tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), and interleukin-10 (IL-10) were measured in blood using commercial enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions. Briefly, after blood collection from the heart, the samples were centrifuged at 10,000 $\times$ g for 20 minutes at 4 °C, and the supernatant was collected for analysis. The levels of TNF- α , IL-1 β , and IL-10 were measured using specific rat ELISA kits (ZellBio GmbH, Germany) following the manufacturer's protocol. The absorbance was read at 450 nm using a microplate reader (BioTek, USA), and cytokine concentrations were calculated from standard curves.

Statistical analysis

All statistical analyses were performed using SPSS software version 26.0 (SPSS Inc., Chicago, IL, USA). Data are presented as mean $\pm$ standard deviation (SD). Before conducting parametric tests, the normality of data distribution was assessed using the Shapiro–Wilk test, and homogeneity of variances was evaluated using Levene's test. Differences between the two groups were analyzed using an independent samples t-test (for the obesity induction parameters). To compare the means among the three experimental groups, a one-way analysis of variance (ANOVA) was used. When the ANOVA indicated a significant difference, a post-hoc Tukey's test was performed to identify specific group differences. A P-value of less than 0.05 ($P < 0.05$) was considered statistically significant (Faul et al., 2009).

Results

Effect sizes

To complement the inferential statistics and aid interpretation of

the practical significance of our findings, effect sizes were calculated for key comparisons. For SIRT3 expression, the comparison between HFFD+Ex and HFFD groups yielded a very large effect size (Cohen's $d=6.10$), indicating a substantial difference between the groups. For FOXO3 expression, the comparison between HFFD+Ex and HFFD groups also showed a large effect size (Cohen's $d=1.54$), despite not reaching statistical significance ($P=0.795$). The effect sizes for the inflammatory markers were similarly large, with TNF- α showing $d=2.95$, IL-1 β showing $d=2.31$, and IL-10 showing $d=3.56$ for HFFD+Ex vs. HFFD comparisons. The one-way ANOVA partial η^2 values ranged from 0.738 to 0.862 for the molecular outcomes, indicating large effect sizes for the overall group differences.

Confirmation of obesity induction

To confirm the successful induction of obesity by the high-fat and high-fructose diet (HFFD), anthropometric and biochemical parameters were compared between the HFFD-fed group and the control group after the 8-week dietary period. The results are presented in Table 1.

As shown in Table 1, the HFFD group exhibited significantly higher body weight, BMI, Lee index, total cholesterol, triglycerides, and LDL levels compared to the control group ($P < 0.05$ for all parameters). Conversely, HDL levels were significantly lower in the HFFD group compared to the control group ($P < 0.001$). These findings confirm that the HFFD successfully induced obesity and associated dyslipidemia in the experimental animals.

Effects of aerobic exercise on body weight and anthropometric indices

The effects of the 6-week aerobic exercise intervention on body weight and anthropometric parameters are presented in Table 2. The HFFD group had significantly higher body weight, BMI, and Lee index compared to both the HFFD+Ex and control groups ($P < 0.05$). Importantly, the HFFD+Ex group showed a significant reduction in body weight, BMI, and Lee index compared to the HFFD group, reaching levels comparable to the control group ($P > 0.05$ for HFFD+Ex vs. control). Similarly, both abdominal and thoracic circumferences were significantly decreased in the HFFD+Ex group compared to the HFFD group ($P < 0.05$). However, the abdominal circumference to thoracic circumference (AC/TC) ratio did not differ significantly between the groups ($P > 0.05$). These results indicate that aerobic exercise effectively attenuated the obesity phenotype induced by the HFFD.

Effects of aerobic exercise on gene expression of FOXO3 and SIRT3

Table 1. Comparison of anthropometric and biochemical parameters between HFFD and control groups. Data are presented as mean±SD. HFFD: High-fat high-fructose diet; BMI: Body mass index; Chol: Cholesterol; HDL: High-density lipoprotein; LDL: Low-density lipoprotein.

Parameter	HFFD Group (n = 16)	Control Group (n = 8)	P-value
Body weight (g)	401.0 ± 7.4	297.5 ± 9.8	<0.001
BMI (g/cm²)	0.67 ± 0.065	0.44 ± 0.069	<0.001
Lee index	0.30 ± 0.043	0.26 ± 0.081	0.041
Total Chol (mg/dL)	134.0 ± 15.1	66.0 ± 11.0	0.003
Triglycerides (mg/dL)	185.3 ± 11.06	75.33 ± 13.05	<0.001
HDL (mg/dL)	2.17 ± 0.7	6.37 ± 1.42	<0.001
LDL (mg/dL)	86.73 ± 4.71	37.5 ± 4.05	<0.001

Table 2. Anthropometric parameters across experimental groups at the end of the study. Data are presented as mean±SD. *Significant difference compared to HFFD group (P<0.05). HFFD: High-fat high-fructose diet; HFFD+Ex: HFFD with aerobic exercise; control: Healthy control; AC/TC: Abdominal circumference to thoracic circumference ratio.

Parameter	HFFD (n = 8)	HFFD+Ex (n = 8)	control (n = 8)
Body weight (g)	401.0 ± 7.4	294.4 ± 10.7*	297.5 ± 9.8
BMI (g/cm²)	0.67 ± 0.65	0.44 ± 0.28*	0.44 ± 0.69
Lee index	0.30 ± 0.43	0.26 ± 0.68*	0.26 ± 0.81
Abdominal circumference (cm)	23.62 ± 0.71	21.95 ± 0.12*	20.94 ± 0.58
Thoracic circumference (cm)	17.29 ± 0.91	15.36 ± 0.67*	15.74 ± 0.46
AC/TC ratio	1.36 ± 0.31	1.43 ± 0.23	1.33 ± 0.72

The relative mRNA expression levels of FOXO3 and SIRT3 in the cardiac tissue across the experimental groups are presented in Figures 1A and 1B. One-way ANOVA revealed a significant difference in SIRT3 mRNA expression among the three groups F (2,21). Post-hoc Tukey's test indicated that the HFFD group had significantly lower SIRT3 expression compared with the Control group (mean difference: -1.76, P= 0.001). The HFFD+Ex group demonstrated significantly higher SIRT3 expression than the HFFD group (mean difference: +1.89, P=0.010). Importantly, SIRT3 expression in the HFFD+Ex group was not significantly different from the Control group (mean difference: +0.13, P=0.337), indicating that exercise effectively normalized cardiac SIRT3 expression. However, the effect size for this comparison was large (Cohen's d=1.54), suggesting that the lack of statistical significance may reflect insufficient statistical power (post-hoc power=62%) rather than a true absence of biological effect. With n=8 per group, the study is powered to detect only very large effect sizes, and a true moderate-to-large effect for FOXO3 may have been missed (Type II error). This interpretation is supported by the observation that the mean FOXO3 expression in the exercised group remained intermediate between the HFFD group and the control group, consistent with a partial restoration that failed to reach statistical significance due to limited sample size. Notably, the FOXO3 expression level in the HFFD+Ex group remained significantly lower than that of the control group (P=0.006).

A one-way ANOVA also revealed a significant difference in SIRT3 mRNA expression among the three groups F (2,21). Post-hoc Tukey's test indicated that the HFFD group had significantly lower SIRT3 expression compared to the control

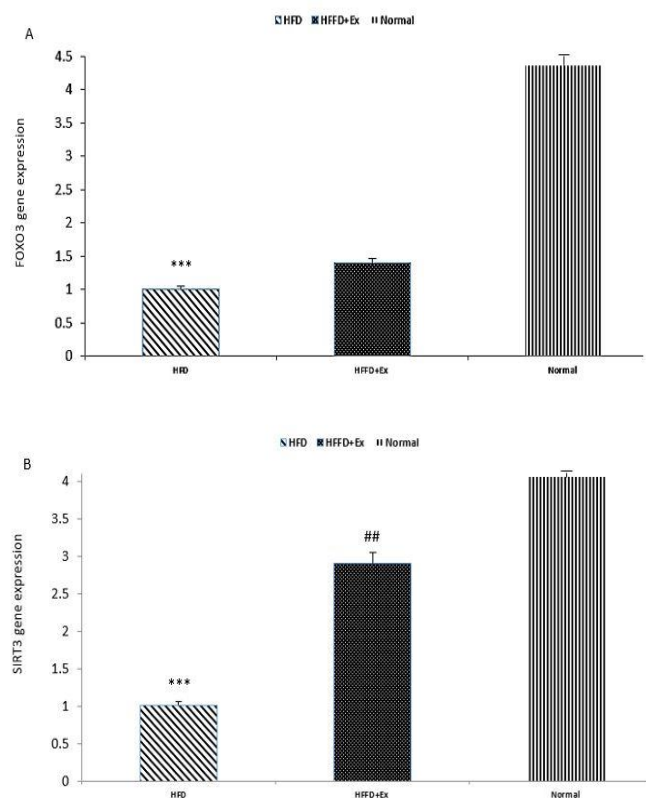


Figure 1. Relative mRNA expression of (A) FOXO3 and (B) SIRT3 in cardiac tissue across experimental groups. Data are presented as mean±SD. Statistical significance was determined by one-way ANOVA followed by Tukey's post-hoc test. For FOXO3 (A): **P<0.01 compared with control group (HFFD vs. control; HFFD+Ex vs. control); no significant difference was observed between HFFD+Ex and HFFD groups (P=0.795). For SIRT3 (B): ***P<0.001 compared with control group (HFFD vs. control); ##P<0.01 compared with HFFD group (HFFD+Ex vs. HFFD); ns indicates not significant (HFFD+Ex vs. control, P=0.337).

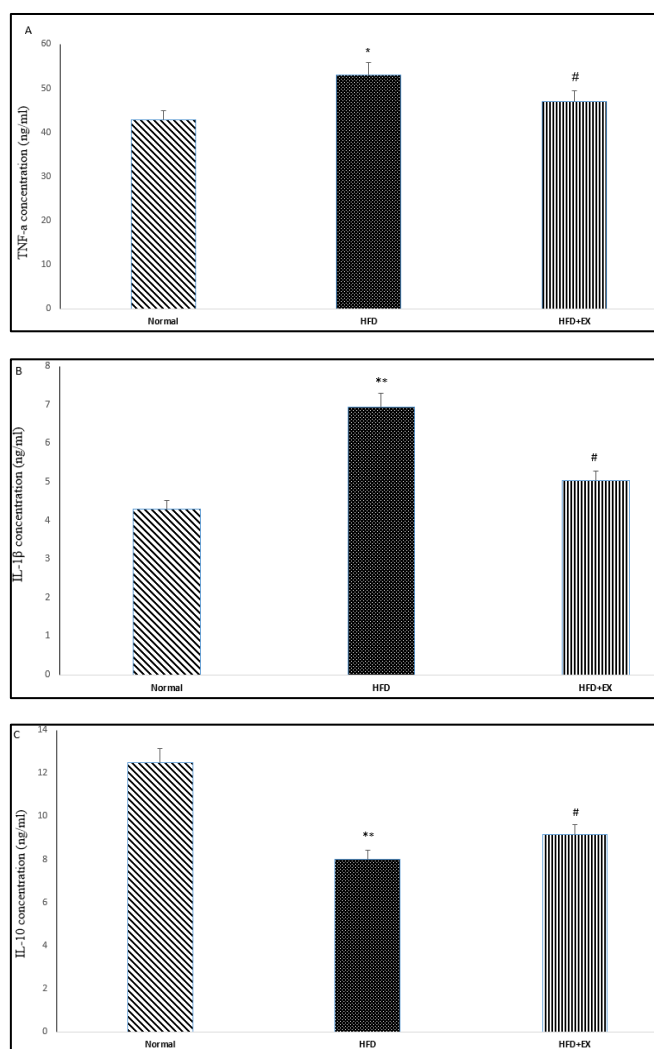


Figure 2. Concentrations of (A) TNF- α , (B) IL-1 β , and (C) IL-10 in all experimental groups. Data are presented as mean $\pm$ SD. Statistical significance was determined by one-way ANOVA followed by Tukey's post-hoc test. $P < 0.05$, $**P < 0.01$ compared with control group; # $P < 0.05$ compared with HFFD group. Note: For TNF- α (A): HFFD showed significantly higher levels than control ($P = 0.008$); HFFD+Ex showed significantly lower levels than HFFD (# $P = 0.031$). For IL-1 β (B): HFFD showed significantly higher levels than control ($P < 0.05$); HFFD+Ex showed significantly lower levels than HFFD (# $P < 0.05$). For IL-10 (C): HFFD showed significantly lower levels than control ($P = 0.009$); HFFD+Ex showed significantly higher levels than HFFD (# $P = 0.027$).

group ($P = 0.001$). Importantly, the HFFD+Ex group demonstrated a nearly 3-fold higher SIRT3 expression relative to the HFFD group (2.90 vs. 1.01), and this increase was statistically significant ($P = 0.01$). Furthermore, the SIRT3 expression in the HFFD+Ex group was restored to a level not significantly different from the control group.

Effects of aerobic exercise on inflammatory cytokines

To evaluate the inflammatory status, the concentrations of the pro-inflammatory cytokines TNF- α and IL-1 β , as well as the anti-

inflammatory cytokine IL-10, were measured in all experimental groups. The results are presented in Figures 2A, 2B, and 2C. One-way ANOVA revealed a significant difference in TNF- α concentrations among the three groups $F(2,21)$. Post-hoc Tukey's test showed that the HFFD group exhibited significantly higher TNF- α levels than the control group ($P = 0.008$), indicating enhanced inflammatory activity in obese rats. TNF- α concentrations were significantly lower in the HFFD+Ex group compared with the HFFD group ($P = 0.031$). However, TNF- α levels in the exercised group remained significantly higher than those in the control group ($P = 0.041$), suggesting partial, but not complete, normalization of this inflammatory marker.

A significant difference was also observed in IL-1 β concentrations among the three groups (one-way ANOVA, $P < 0.05$). Post-hoc analysis demonstrated that IL-1 β levels were significantly elevated in the HFFD group compared with the control group, confirming that obesity induced a pronounced pro-inflammatory response. Aerobic exercise significantly decreased IL-1 β concentrations in the HFFD+Ex group compared with the HFFD group ($P < 0.05$). Nevertheless, IL-1 β levels in the exercised animals remained higher than those of the control group, indicating that exercise attenuated, but did not completely reverse, obesity-induced IL-1 β elevation.

One-way ANOVA also revealed a significant difference in IL-10 concentrations among the three groups $F(2,21)$. Post-hoc Tukey's test indicated that the HFFD group had significantly lower IL-10 levels than the control group ($P = 0.009$), suggesting impaired anti-inflammatory capacity in obese rats. In contrast, the HFFD+Ex group showed significantly higher IL-10 concentrations than the HFFD group ($P = 0.027$). Furthermore, IL-10 levels in the exercised group were not significantly different from those of the control group ($P = 0.184$), indicating that aerobic exercise effectively restored the anti-inflammatory profile.

Discussion

The present study investigated the effects of a high-fat and high-fructose diet (HFFD) on obesity induction, cardiac gene expression of SIRT3 and FOXO3, and inflammatory status, as well as the potential modulatory role of a 6-week aerobic exercise intervention in male Wistar rats. Our findings confirmed that the HFFD successfully induced obesity, as evidenced by significant increases in body weight, BMI, Lee index, and serum lipid parameters, consistent with established animal models of diet-induced obesity (Hidayati et al., 2020). Importantly, we observed that obesity was associated with significantly reduced mRNA expression of both SIRT3 and FOXO3 in cardiac tissue, alongside increased pro-inflammatory TNF- α and IL-1 β and decreased anti-inflammatory IL-10 levels, compared to healthy

controls. Aerobic exercise effectively attenuated the obesity phenotype, significantly increased cardiac SIRT3 expression, and modulated the inflammatory response by reducing TNF- α and IL-1 β and increasing IL-10 levels. However, exercise did not significantly alter FOXO3 expression. Our findings contribute to the growing body of evidence on the molecular mechanisms linking obesity, exercise, and cardiac health. However, it is important to emphasize that our data are limited to mRNA expression levels and do not provide direct evidence for changes in SIRT3 enzymatic activity or FOXO3 protein function. The mechanistic interpretations offered below are therefore inferential and must be validated through protein-level analyses in future studies.

The significant reduction in cardiac SIRT3 mRNA expression in obese rats observed in our study aligns with the current understanding that obesity impairs SIRT3-related pathways (Sun et al., 2018; Wang et al., 2023). SIRT3, a major mitochondrial deacetylase, plays a critical role in regulating mitochondrial metabolism, energy production, and antioxidant defenses (Sun et al., 2018; Wang et al., 2023). The downregulation of SIRT3 mRNA in obesity may contribute to impaired fatty acid oxidation, increased reactive oxygen species (ROS) production, and subsequent cardiac lipotoxicity (Powell-Wiley et al., 2021; Ballal et al., 2010). However, as we measured only mRNA levels, we cannot confirm that SIRT3 protein abundance or enzymatic activity was correspondingly reduced. SIRT3 function is additionally regulated by NAD⁺ availability and post-translational modifications (Sun et al., 2018; Wang et al., 2023), and transcriptional changes may not fully reflect functional status. Thus, while our findings are consistent with SIRT3 pathway impairment in obesity, protein-level confirmation is required. Similarly, the reduced FOXO3 mRNA expression in obese rats is consistent with impaired stress resistance pathways (Sundaresan et al., 2009; Tseng et al., 2013). FOXO3 is a key transcription factor that regulates antioxidant enzyme expression and is essential for cellular stress resistance (Sundaresan et al., 2009; Tseng et al., 2013). The concurrent reduction in both SIRT3 and FOXO3 mRNA in obese hearts suggests a coordinated transcriptional suppression of protective mechanisms, potentially rendering the myocardium more vulnerable to metabolic stress. However, FOXO3 activity is primarily regulated at the post-translational level through phosphorylation (which controls nuclear export and degradation) and acetylation (which modulates DNA binding activity) (Tseng et al., 2013; Guo et al., 2020). SIRT3-mediated deacetylation of FOXO3 enhances its transcriptional activity without necessarily altering FOXO3 mRNA or protein levels (Sundaresan et al., 2009; Tseng et al., 2013). Therefore, our mRNA data alone cannot establish whether FOXO3 functional activity was impaired in obesity or enhanced by exercise. Protein-level analyses measu-

-ring FOXO3 phosphorylation, acetylation, and nuclear translocation are necessary to assess functional status. This is supported by studies showing that HFFD-induced obesity impairs the SIRT3/FOXO3 signaling axis, leading to increased oxidative damage and cardiac dysfunction (Wang et al., 2018; Bošković et al., 2021). Wang et al. (2018) demonstrated that CD38 deficiency, which activates the Sirt3/FOXO3 pathway, protects the heart from HFFD-induced oxidative stress. Furthermore, studies have shown that resveratrol, a SIRT3 activator, protects against myocardial ischemic injury in obese mice by restoring SIRT3/FOXO3a-dependent antioxidant defenses, confirming the protective role of this pathway (Sun et al., 2018; Zhu et al., 2024). The concomitant increase in TNF- α and IL-1 β and decrease in IL-10 levels in obese rats further confirms the presence of a pro-inflammatory state in the myocardium. Obesity is well-established as a state of chronic low-grade inflammation, characterized by increased production of pro-inflammatory cytokines such as TNF- α , IL-6, and MCP-1, and reduced levels of anti-inflammatory cytokines like IL-10 (Mirmiran et al., 2014; Ballal et al., 2010). TNF- α directly contributes to cardiac dysfunction by inducing oxidative stress, promoting cardiomyocyte apoptosis, and stimulating cardiac fibrosis (Meldrum, 1998). Conversely, IL-10 exerts cardioprotective effects by inhibiting the production of pro-inflammatory cytokines and suppressing inflammatory signaling pathways (Ouyang et al., 2011). The imbalance between TNF- α , IL-1 β , and IL-10 observed in our study indicates that obesity creates an inflammatory environment that may further exacerbate mitochondrial dysfunction and impair the protective SIRT3/FOXO3 axis.

A key finding of our study is that the exercised obese rats showed significantly higher cardiac SIRT3 mRNA expression compared with their sedentary obese counterparts, reaching levels comparable to healthy controls. This transcriptional response is consistent with several studies demonstrating exercise-induced upregulation of SIRT3 mRNA and protein in various cardiac pathologies (Jin et al., 2022; Donniacuo et al., 2019; Ghardashi Afousi, 2023; Zhao et al., 2018). However, it is important to note that SIRT3 is an enzyme whose function depends on its deacetylase activity, which is regulated by NAD⁺ availability and allosteric modifications, not solely by protein abundance (Sun et al., 2018; Wang et al., 2023). While increased SIRT3 mRNA may suggest enhanced SIRT3 protein expression, we cannot conclude that SIRT3 enzymatic activity was correspondingly increased without direct activity assays. The cardioprotective effects attributed to SIRT3 in our study are therefore inferred from mRNA data and require validation through protein-level and functional analyses. Jin et al. (2022) demonstrated that exercise activates the FGF21-SIRT3 axis to protect against diabetic cardiomyopathy by preserving mitochondrial integrity. Mechanistically, exercise enhances cardiac FGF21 signaling, w-

-high induces SIRT3 expression through AMPK-evoked FOXO3 phosphorylation, thereby reversing hyperacetylation and functional impairments of mitochondrial enzymes (Jin et al., 2022). Similarly, other investigations have shown that moderate and prolonged exercise increases SIRT3 expression and activates the FoxO3a pathway, leading to enhanced antioxidant defenses and reduced oxidative stress (Donniacuo et al., 2019; Zhao et al., 2018). Ghardashi Afousi (2023) also found that high-intensity interval training increased cardiac SIRT3 and FOXO3a protein expression and improved cardiac function in rats with heart failure. Zhao et al. (2018) demonstrated that short-duration swimming exercise significantly increased cardiac SIRT3 protein levels (2.7-fold) in aged mice after myocardial infarction. The significant increase in SIRT3 expression observed in our study suggests that even moderate-intensity aerobic exercise can effectively upregulate this protective mitochondrial deacetylase in the context of diet-induced obesity.

In contrast to the significant changes in SIRT3, exercise did not significantly alter FOXO3 expression in our study. While the HFFD+Ex group showed a trend toward increased FOXO3 expression (1.40 vs. 1.00), this difference was not statistically significant. This finding contrasts with studies demonstrating exercise-induced FOXO3 activation (Bošković et al., 2021; Ghardashi Afousi, 2023). However, it is important to note that FOXO3 activity is primarily regulated by post-translational modifications such as phosphorylation and acetylation, rather than transcriptional changes (Sundaresan et al., 2009; Tseng et al., 2013). Tseng et al. (2013) demonstrated that SIRT3 deacetylates FOXO3, leading to its stabilization and enhanced antioxidant gene expression. Therefore, while FOXO3 mRNA expression may not have changed significantly, the observed increase in SIRT3 expression may have enhanced FOXO3 activity through deacetylation, even without changes in gene expression. Future studies should measure FOXO3 protein levels and acetylation status to fully elucidate the effects of exercise on this pathway.

The 6-week aerobic exercise program significantly reduced TNF- α and IL-1 β levels and increased IL-10 levels in obese rats, indicating that exercise exerts anti-inflammatory effects in the heart. These findings are consistent with the well-established role of exercise in reducing systemic and tissue-specific inflammation (Petersen & Pedersen, 2005; Mathur & Pedersen, 2008). The exercise-induced reduction in TNF- α aligns with findings by Donniacuo et al. (2019), who showed that moderate and prolonged exercise reduced oxidative stress and inflammation through sirtuin pathway activation. Furthermore, the increase in IL-10 following exercise suggests that exercise promotes an anti-inflammatory phenotype, which may contribute to cardioprotection independent of SIRT3/FOXO3 modulation (Gleeson et al., 2011). The anti-inflammatory effects of exercise may be mediated through several mechanisms including reduced

adiposity, improved insulin sensitivity, and direct modulation of immune cell function (Petersen & Pedersen, 2005; Mathur & Pedersen, 2008).

The interplay between SIRT3 and inflammation is particularly relevant to our findings. TNF- α has been shown to inhibit SIRT3 expression and activity, thereby exacerbating mitochondrial dysfunction and oxidative stress (Palomer et al., 2020; Chen et al., 2021). Conversely, SIRT3 activation has been reported to suppress inflammatory signaling pathways, including NF- κ B, and to reduce the expression of pro-inflammatory cytokines (Guo et al., 2020; Palomer et al., 2020). Therefore, the exercise-induced increase in SIRT3 expression observed in our study may have contributed to the concomitant reduction in TNF- α and IL-1 β levels, creating a positive feedback loop that favors mitochondrial protection and anti-inflammatory signaling. However, given that our cytokine measurements were performed in blood rather than cardiac tissue, these interpretations reflect systemic rather than cardiac-specific inflammatory changes. Future studies should measure cardiac cytokine levels directly to confirm these mechanisms.

Conclusion

In conclusion, our findings demonstrate that HFFD-induced obesity in male Wistar rats is associated with reduced cardiac SIRT3 and FOXO3 expression, elevated TNF- α and IL-1 β levels, and decreased IL-10 levels. Notably, the exercised obese group showed significantly higher cardiac SIRT3 expression compared with the sedentary obese group, reaching levels comparable to healthy controls, and exhibited a more favorable inflammatory profile with lower TNF- α and IL-1 β and higher IL-10 concentrations. However, FOXO3 expression did not differ significantly between the exercised and sedentary obese groups. These findings suggest that aerobic exercise participation is associated with restored SIRT3 expression and reduced inflammation in the obese heart, though causal interpretation is limited by the post-test-only design. Further research with longitudinal designs is warranted to confirm these associations and explore potential mechanisms.

Limitations

Several methodological limitations should be considered when interpreting these findings. The post-test-only design limits causal inference, as baseline molecular equivalence between groups cannot be confirmed. The small sample size ($n = 8$ per group) provided limited power to detect moderate effects; post-hoc analysis revealed only 62% power for the FOXO3 comparison, suggesting the non-significant 40% increase may reflect a Type II error rather than a true absence of effect. The exclusive reliance on mRNA data without protein-level or functional measurements (SIRT3 enzymatic activity, FOXO3 phosphorylation/acetylation)

represents a major mechanistic gap, as both proteins are primarily regulated post-translationally. Additionally, inflammatory cytokines were measured in blood rather than cardiac tissue, reflecting systemic rather than cardiac-specific inflammation. The moderate-intensity exercise protocol may have been insufficient for FOXO3 transcriptional changes, and training effect verification was not performed. Finally, the study was limited to male Wistar rats, restricting generalizability. Future studies with larger samples, protein-level analyses, direct cardiac cytokine measurements, and varied exercise intensities are needed to confirm these findings.

What is already known on this subject?

The global prevalence of obesity has reached pandemic proportions, with more than 650 million adults affected and rates continuing to rise in low- and middle-income countries.

What this study adds?

These findings suggest that aerobic exercise participation is associated with restored SIRT3 expression and reduced inflammation in the obese heart, though causal interpretation is limited by the post-test-only design.

Organ Cross-Talk Tips:

- Aerobic treadmill exercise may act through skeletal muscle myokines and AMPK/FGF21 signaling; the FGF21–SIRT3 axis is known to protect cardiac mitochondria. This offers a plausible muscle-to-heart mechanism for the exercise-induced rise in cardiac SIRT3.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest regarding the publication of this manuscript. No financial or personal relationships have influenced the design, execution, or reporting of this research. All authors have reviewed

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Ethical approval The research received ethical approval under the code IR.IAU.AHVZ.REC.1402.075.

Informed consent Animal study.

Author contributions

Conceptualization: M.Kh., M.Sh.,; Methodology: M.Kh., M.Sh.,; Software: M.Kh., M.Sh.,; Validation: M.Kh., M.Sh.,; Formal analysis: M.Kh., M.Sh.,; Investigation: M.Kh., M.Sh.,; Resources: M.Kh., M.Sh.,; Data curation: M.Kh., M.Sh.,; Writing -original draft: M.Kh., M.Sh.,; Writing-review & editing: M.Kh., M.Sh.,; Visualization: M.Kh., M.Sh.,; Supervision: M.Sh.,; Project administration: M.Kh., M.Sh.,; Funding acquisition: M.Kh., M.Sh.,

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