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Original Article

# Investigating the Effect of Two Types of Aerobic Exercise on *SIRT1* and *AMPK* Gene Expression Regulation in an Animal Model of Non-Alcoholic Fatty Liver Disease

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**Abstract**

**Background and aims:** NAFLD, marked by liver fat buildup, lacks approved drug treatments. SIRT1 and AMPK pathways help reduce liver fat. This study compared the effects of HIIT and MICT on *SIRT1* and *AMPK* gene expression in mice with diet-induced NAFLD.

**Methods:** In this experimental study, 36 male C57BL/6J mice were randomly divided into NFFD, NFDHIIT, NFDMICT, HFD, HFDHIIT, and HFDMICT groups (n = 6 per group). Mice in the NFDHIIT, NFDMICT, HFDHIIT, and HFDMICT groups underwent 8 weeks of treadmill training after 12 weeks of HFD or normal diet feeding. The data were analyzed using two-way ANOVA and Tukey's post-hoc test.

**Results:** HFD significantly decreased the expression of *AMPK* (0.386 ± 0.012) and *SIRT1* (0.647 ± 0.010) genes compared to the NFFD group (*AMPK*: 0.942 ± 0.013; *SIRT1*: 1.020 ± 0.063; $P < 0.001$). In mice fed with HFD, both HIIT (*AMPK*: 0.983 ± 0.039; *SIRT1*: 1.361 ± 0.072) and MICT (*AMPK*: 0.865 ± 0.010; *SIRT1*: 1.214 ± 0.015) significantly increased gene expression compared to the HFD control group ($P < 0.001$). Although HIIT showed a higher increase in AMPK expression than MICT within the HFD groups, this difference was not statistically significant ($P = 0.219$). Similarly, under normal diet conditions, both HIIT and MICT could significantly increase *SIRT1* expression compared to the NFFD group ($P < 0.001$ and $P = 0.019$, respectively. However, no significant difference was observed between the two exercise groups in this diet condition ($P = 0.135$).

**Conclusion:** These findings suggest that aerobic exercise, particularly HIIT, may help reduce NAFLD progression through SIRT1/AMPK pathway activation.

**Keywords:** High-intensity interval training, Moderate-intensity continuous training, Metabolic pathway, Non-alcoholic fatty liver disease

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## Introduction

Non-alcoholic fatty liver disease (NAFLD) is considered one of the most common chronic liver diseases in the twenty-first century (1). NAFLD encompasses a spectrum of liver disorders, ranging from simple steatosis (the accumulation of fat in the liver without inflammation) to non-alcoholic steatohepatitis (2), which involves inflammation and hepatocellular injury (3, 4). The global prevalence of NAFLD is rapidly increasing, and according to recent statistics, approximately 25–30% of the world's population is affected by this condition (5). The rise in obesity, type 2 diabetes, insulin resistance, physical inactivity, and the consumption of processed and high-fat foods are among the most significant risk factors for NAFLD development (6). Among the regulatory pathways of energy metabolism in hepatocytes, the sirtuin 1 (SIRT1) and adenosine monophosphate-activated protein kinase (AMPK) pathways play a crucial role in improving insulin sensitivity (7), enhancing fatty acid oxidation (8), and reducing liver fat accumulation (9). Studies have shown that the activation of these genes can have beneficial effects on slowing the progression of fatty liver (10).

SIRT1, which regulates DNA expression, apoptosis, aging, and various physiological and pathological processes in organisms through the deacetylation of substrate proteins (11), is a direct target of miR-34a, which modulates the activity of AMPK (12). AMPK is a well-known regulator of energy metabolism that noticeably contributes to the development and progression of metabolic diseases (13). Upon phosphorylation, AMPK can regulate genes involved in fatty acid oxidation (14) and related transport proteins, thereby reducing hepatic steatosis (15). Therefore, the SIRT1-AMPK phosphorylation signaling pathway regulates lipid metabolic homeostasis and may serve as a therapeutic target for hepatic steatosis (16).

The fundamental and important mechanism by which exercise exerts its therapeutic effects on NAFLD is related to metabolic sensors SIRT1/AMPK (17). A recent study demonstrated that exercise can stimulate SIRT1/AMPK signaling, which can both improve pathogenesis and prevent the progression of NAFLD (18). The exercise-induced activation of SIRT1/AMPK signaling can reduce

fat accumulation, increase energy expenditure, limit de novo lipogenesis, and regulate fatty acid metabolism (19, 20). Additionally, in investigations on the effects of aerobic exercise on hepatocyte apoptosis in NAFLD, it was revealed that aerobic exercise can prevent hepatocyte apoptosis and improve mitochondrial function by activating SIRT1, thereby contributing to the reduction of disease progression (3, 21, 22). These studies indicate that aerobic exercise can play a significant role in improving liver status in NAFLD through the activation of AMPK and SIRT1 pathways, and exercise, as a low-cost, low-risk, and effective non-pharmacological intervention for NAFLD, can effectively reduce the incidence and progression of hepatic steatosis, a finding supported by some studies (23, 24).

Despite the numerous pieces of evidence regarding the beneficial effects of exercise on improving the status of NAFLD, the optimal type, intensity, and exercise pattern for activating the SIRT1/AMPK signaling pathway and improving NAFLD have not been precisely determined yet (20). In this respect, two common exercise patterns include high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT), each with its own specific advantages and challenges (25). HIIT, with its short bursts of high-intensity activity, can induce a stronger metabolic stimulus in a shorter time and activate the SIRT1/AMPK pathway through a rapid increase in oxygen consumption and mitochondrial activity (26). On the other hand, MICT, with moderate intensity and longer duration, offers greater stability and is a more tolerable and safer approach for individuals with NAFLD and metabolic disorders (27). These differences have made the practical choice between HIIT and MICT for rehabilitation programs in patients with NAFLD challenging.

Moreover, limited studies have comparatively examined the molecular pathways activated by HIIT and MICT in the liver tissue, particularly the SIRT1/AMPK pathway, which plays a key role in regulating fatty acid oxidation and reducing liver fat. Accordingly, the present study aims to compare the effects of eight weeks of HIIT or MICT combined with diet on the expression of *SIRT1* and *AMPK* genes in male rats with HFD-induced NAFLD.

## Materials and Methods

The statistical population of this experimental study consisted of male C57BL/6J mice aged 6–8 weeks and weighing 21–23 g, which were purchased from the Pasteur Institute of Tehran and housed at Histogeneotech, a knowledge-based company (Fanavaran Baft va Jen Pasargad). The sample size was determined based on a priori power analysis using G*Power software (version 3.1.9.7), with a significance level (α) of 0.05, a power (1−β) of 0.80, and an estimated large effect size (f = 0.4), resulting in a total of 36 mice (n = 6 per group) to ensure sufficient statistical power (28). All animal procedures were conducted in accordance with the ethical principles outlined in the Helsinki Declaration and the national guidelines for laboratory animal care. The entire protocol was reviewed and approved by the Biomedical Research Ethics Committee of Islamic Azad University, North Tehran Branch (IR.IAU.TNB.REC.1403.013).

After transferring the animals to the laboratory, they were housed in transparent polycarbonate cages with dimensions of 42 cm × 26.5 cm × 15 cm, at a temperature of 22 ± 2°C, relative humidity of 55 ± 5%, and a 12:12-hour light-dark cycle with adequate ventilation. Throughout the study, the animals were daily fed pelletized food produced by Behparvar Karaj Company, according to Tables 1 and 2 (adjusted based on weekly weighing), and had ad libitum access to drinking water provided via bottles. For bedding, coarse sawdust made from light-colored, dust-free softwood was used, which was spread to a depth of 3–5 cm from the bottom of the cages and was replaced twice weekly throughout the study period.

After a two-week acclimatization period to the new environment and familiarization with the intervention protocol, the mice were randomly assigned to six groups with six mice in each group. They included normal-fat diet (NFD) control, NFD + HIIT (NFDHIIT), NFD + MICD (NFDMICT), HFD control, HFD + HIIT (HFDHIIT), and HFD + MICT (HFDMICT).

To minimize selection bias and ensure uniform distribution of animals across experimental groups, the random allocation process was performed in a stepwise and rigorous manner. First, after a two-week acclimatization period, all 36 male C57BL/6J mice were assessed for weight, age, and general health to confirm no significant initial differences. Next, the mice were randomly divided into two primary dietary groups using a random number table and stratified randomization based on weight: NFD (n = 18) and HFD (n = 18). Subsequently, each dietary group was randomly allocated to three subgroups using the same randomization method: control (no exercise), HIIT, and MICT, resulting in six final groups with six mice in each: NFD, NFDHIIT, NFDMICT, HFD, HFDHIIT, and HFDMICT. Random assignment was conducted by an independent researcher who was not involved in the intervention or data analysis phases in order to ensure objectivity and prevent allocation bias.

The HFD consisted of 60% fat, 20% carbohydrate, and 20% protein, providing 4057 kcal/kg. The NFD contained 70% carbohydrate, 10% fat, and 20% protein. The compositions of the diets are presented in Tables 1 and 2.

Following 12 weeks of feeding with either an HFD or NFD, the mice in the NFDHIIT, NFDMICT, HFDHIIT, and HFDMICT groups underwent 8 weeks of treadmill training (Navid 10-Line Model, Pishro Andisheh Sanat Engineering Company). In this study, the MICT protocol was implemented over 8 weeks, 5 sessions per week. Each session began with a 10-minute warm-up at an intensity of 45–50% of maximum rate of oxygen consumption ($VO_2max$), equivalent to a speed of 5 m/min. Subsequently, the mice exercised for 35 minutes

at an intensity of 70–75% $VO_2$max, corresponding to a speed range of 14–17.5 m/min, which was progressively increased over the consecutive weeks. In the first week, the training commenced at a speed of 14 m/min, and the speed was increased by approximately 0.5–1 m/min each week, reaching 17.5 m/min by the eighth week. The total duration of each session was 45 minutes, and the total distance covered per session increased from 540 m in the first week to 662.5 m in the eighth week. This gradual increase in training intensity and volume was designed to apply the principle of progressive overload and to achieve optimal adaptation in the animal model while maintaining safety and preventing injury. This protocol (Table 3) was selected to establish a training volume and intensity consistent with MICT requirements in obesity and insulin resistance models and was aligned with similar research in the field of aerobic exercise in animal models (15).

In this study, the HIIT protocol was implemented over 8 weeks, with 5 sessions per week. Each session began with a 10-minute warm-up at an intensity of 45–50% $VO_2$max at a speed of 5 m/min. Following the warm-up, the main phase of the session consisted of 10 intervals of 2 minutes at moderate intensity (70–75% $VO_2$max) with speeds ranging from 14 m/min to 17.5 m/min, alternated with 9 intervals of 1 minute at high intensity (95–100% $VO_2$max) with speeds ranging from 24 m/min to 27.5 m/min. The intensity and speed of the training were progressively increased each week, starting with a moderate intensity speed of 14 m/min and a high-intensity speed of 24 m/min in the first week, reaching 17.5 m/min and 27.5 m/min, respectively, by the eighth week. The total duration of each training session was approximately 38.6 minutes in the first week, gradually increasing to 39.5 minutes by the eighth week. Additionally, the distance covered in each session increased from 540 m in the first week to 662.5 m in the eighth week. This design adhered to the principle of progressive overload and optimal adaptation in the animal model to enhance aerobic capacity and to provide an appropriate training stimulus (Table 4). The HIIT protocol used in this study was in line with that of previous validated research on HIIT in animal models and aimed at establishing an effective comparison with the MICT protocol in evaluating physiological effects (20).

The fresh method was utilized for euthanasia and tissue sampling for cellular and molecular studies. In compliance with ethical considerations, the rats were anesthetized after a minimum 8-hour fast and 48 hours following the last intervention using chloroform solution. Following thoracotomy, the blood sample was collected from the left

**Table 1.** Composition and Caloric Content of the NFD and HFD

| Ingredients | NFD (g) | NFD (kcal) | HFD(g) | HFD(kcal) |
|---|---|---|---|---|
| Casein (80 mesh) | 200 | 800 | 200 | 800 |
| L-cystine | 3 | 12 | 3 | 12 |
| Corn starch | 315 | 1260 | 0 | 0 |
| Maltodextrin 10 | 35 | 1400 | 125 | 500 |
| Sucrose | 350 | 1400 | 68.8 | 272 |
| Cellulose (BW200) | 50 | 0 | 50 | 0 |
| Soybean oil | 25 | 225 | 25 | 225 |
| Lard (animal fat) | 20 | 180 | 245 | 2205 |
| Mineral mix (S10026) | 10 | 0 | 10 | 0 |
| Dicalcium phosphate | 13 | 0 | 13 | 0 |
| Calcium carbonate | 5.5 | 0 | 5.5 | 0 |
| Potassium citrate | 16.5 | 0 | 16.5 | 0 |
| Vitamin mix (V10001) | 10 | 40 | 10 | 40 |
| Choline bitartrate | 2 | 0 | 2 | 0 |
| FD&C Yellow No. 5 | 0.05 | 0 | 0 | 0 |
| FD&C Blue No. 1 | 0 | 0 | 0.05 | 0 |
| **Total** | 1055.05 | 4057 | 773.85 | 4057 |

*Note*. NFD: Normal-fat diet; HFD: High-fat diet.

**Table 2.** Macronutrient Composition and Energy Content of NFD and HFD

| Nutrient | NFD (%g) | NFD (%kcal) | HFD (%g) | HFD (%kcal) |
|---|---|---|---|---|
| Protein | 19 | 20 | 26 | 20 |
| Carbohydrate | 67 | 70 | 26 | 20 |
| Fat | 4 | 10 | 35 | 60 |
| Total | 100 | 100 | 100 | 100 |
| Energy density | 3.8 kcal/g | | 5.2 kcal/g | |

*Note*. NFD: Normal-fat diet; HFD: High-fat diet.

**Table 3.** Eight-Week MICT Protocol

| Week | Warm-up (45-50% of Maximum Oxygen Consumption) | | Moderate Intensity (70-75% of Maximum Oxygen Consumption) | | Total Distance (m) | Total Time (Minute) |
|---|---|---|---|---|---|---|
| | Speed (m/min) | Time (Minute) | Speed (m/min) | Time (Minute) | | |
| 1 | 5 | 10 | 14 | 35 | 540 | 45 |
| 2 | | | 14.5 | | 557.5 | |
| 3 | | | 15 | | 575 | |
| 4 | | | 15.5 | | 592.5 | |
| 5 | | | 16 | | 610 | |
| 6 | | | 16.5 | | 627.5 | |
| 7 | | | 17 | | 645 | |
| 8 | | | 17.5 | | 662.5 | |

*Note*. MICT: Moderate-intensity continuous training.

ventricle of the heart using a 3 cc syringe. The collected sample was placed into plain 12 mm×100 mm tubes and ethylenediaminetetraacetic acid tubes for serum and plasma separation and then centrifuged in a refrigerated centrifuge at 4˚C at 3,000 rpm for 15 minutes. The clear supernatant was carefully collected using a 100 µL sampler, transferred into 2 mL microtubes for biochemical analyses, and stored at -80˚C until measurement. Immediately after cardiac blood collection, the tissues were rapidly excised and washed with phosphate-buffered saline. Liver tissue was longitudinally sectioned from the anterior lobe using a scalpel blade, placed in microtubes, snap-frozen in liquid nitrogen, and stored at -80˚C until analysis.

The real-time polymerase chain reaction (RT-PCR) technique was used to assess the gene expression of *SIRT1* and *AMPK*. Initially, primer design was conducted, followed by total RNA extraction from the tissues, which was then converted into complementary DNA (cDNA). The quality and purity of the extracted RNA were evaluated by measuring the absorbance ratios at 260/280 and 260/230 using a NanoDrop spectrophotometer, and only samples with appropriate quality (ratios between 1.8 and 2.0) were selected for cDNA synthesis. Additionally, RNA integrity was checked using 1% agarose gel electrophoresis to ensure the absence of RNA degradation.

Primers were designed using AlleleID software and synthesized by CinnaGen Company. In addition, each primer was verified using NCBI BLAST to ensure sequence specificity. Further, primer validation was performed by generating a standard curve, and primer efficiencies within the range of 90–110% were confirmed. Furthermore, melting curve analysis was conducted to verify amplification specificity.

RT-PCRs were performed using an ABI system (Applied Biosystems, USA). For each well of a 96-well plate, a 25 µL reaction mixture was prepared, containing 12.5 µL of SYBR Green Master Mix, 2 µL of each gene-specific primer, 4 µL of cDNA, and 6.5 µL of distilled water. The thermal cycling program included an initial denaturation at 95˚C for 10 minutes, followed by 40 cycles of denaturation at 95˚C for 15 seconds and annealing/extension at 60˚C for 1 minute. Subsequently, a melting curve stage was conducted as 95˚C for 15 seconds, 60˚C for 30 seconds, and 95˚C for 15 seconds. The temperature was decreased from 95˚C to 60˚C at a ramp rate of 0.03˚C per second, taking approximately 20 minutes. After completion, amplification and melting curves were analyzed using ABI SDS software. For gene expression analysis, the difference between the Ct values of the target gene and the reference gene was calculated as ΔCt, followed by computing the relative expression using the 2^-ΔΔCt method. In this study, GAPDH was utilized as the internal control gene. Each PCR run consisted of 40 cycles. The applied primers are provided in Table 5.

After the laboratory analysis of the samples, the data were described using statistical indices, including means and standard deviations (SD). The Shapiro-Wilk test was employed to assess the normality of data distribution. Subsequently, a two-way analysis of variance (ANOVA) for independent groups was performed, and when a significant group effect was detected, pairwise comparisons were conducted using Tukey's post hoc test. A significance level of $P \leq 0.05$ was considered, and the obtained data were analyzed using SPSS 22 and Excel software.

## Results

The values (mean ± SD) of the variables studied in the experimental groups are provided in Table 6.

To assess the normality of data distribution, the Shapiro-Wilk test was performed separately for each variable (AMPK and SIRT1) and in each independent group. The

**Table 4.** Eight-Week HIIT Protocol

| Week | Warm-up (45-50% of Maximum Oxygen Consumption) | | Moderate (70-75% of Maximum Oxygen Consumption) | | High (95-100% of Maximum Oxygen Consumption) | | Total Distance (m) | Total Time (Minute) |
|---|---|---|---|---|---|---|---|---|
| | Speed (m/min) | Time (Minute) | Speed (m/min) | Time (Minute) | Speed (m/min) | Time (Minute) | | |
| 1 | 5 | 10 | 14 | 2×10 | 24 | 1×9 | 540 | 38.6 |
| 2 | | | 14.5 | | 24.5 | | 557.5 | 38.8 |
| 3 | | | 15 | | 25 | | 575 | 39 |
| 4 | | | 15.5 | | 25.5 | | 592.5 | 39.1 |
| 5 | | | 16 | | 26 | | 610 | 39.2 |
| 6 | | | 16.5 | | 26.5 | | 627.5 | 39.3 |
| 7 | | | 17 | | 27 | | 645 | 39.4 |
| 8 | | | 17.5 | | 27.5 | | 662.5 | 39.5 |

*Note*. HIIT: High-intensity interval training.

**Table 5.** Primer Sequences for *Sirt1* and *AMPK* Gene Expression

| Gene | Forward Primer Sequence | Reverse Primer Sequence |
|---|---|---|
| *Sirt1* | GCTGGAACAGGTTGCGGGA | GACAGCTTCACAGTCAACTTTGT |
| *AMPK* | TGGCACAGGAGAACTACAAGAT | CGTGTGGTGATCTTGGTTCT |
| *GAPDH* | AACCCATCACCATCTTCCAG | CCAGTAGACTCCACGACATAC |

results demonstrated that all *P*-values were above the significance level of 0.05, indicating a normal distribution of data in all groups. Therefore, the normality of data distribution in each group was confirmed separately, and parametric tests, including two-way ANOVA and Tukey's post-hoc test, were used, respectively.

The results (Table 7) indicated that an HFD significantly reduced the gene expression levels of *AMPK* and *SIRT1* ($P<0.001$), with approximately 68% and 41.6% of the variations in these genes attributed to the dietary intervention, respectively. Performing exercise, whether HIIT or MICT, could significantly increase the gene expression levels of AMPK and *SIRT1* ($P<0.001$), explaining 76% and 83.4% of the variations, respectively. Moreover, the interaction between diet type and exercise modality had a significant effect on gene expression ($P<0.001$), such that 67.1% and 27.2% of the changes in *AMPK* and *SIRT1* expression, respectively, were due to this interaction, demonstrating the strong synergistic effect of these two factors.

Tukey's post hoc test was used to perform pairwise comparisons among the studied groups regarding changes in *AMPK* gene expression. Tukey's post hoc analysis (Figure 1) revealed that an HFD significantly reduced *AMPK* gene expression compared to a normal diet ($P<0.001$). Additionally, both HIIT and MICT under the HFD condition led to a significant increase in *AMPK* expression compared to the HFD control group ($P<0.001$), with this increase being greater in the HIIT group; however, the difference between HIIT and MICT was not statistically significant ($P=0.219$).

The results of Tukey's post hoc test (Figure 2) also confirmed that under normal diet conditions, both HIIT and MICT led to a significant increase in *SIRT1* gene expression compared to the control group ($P<0.001$ and $P=0.019$, respectively). Additionally, an HFD significantly reduced *SIRT1* expression compared to the normal diet ($P<0.001$). Under HFD conditions, both HIIT and MICT could noticeably increase *SIRT1* expression compared to the HFD control group ($P<0.001$), with this increase being greater in the HIIT group.

## Discussion

Our results revealed that eight weeks of HIIT and MICT had different effects on hepatic *AMPK* gene expression in mice with HFD-induced NAFLD. There was no significant

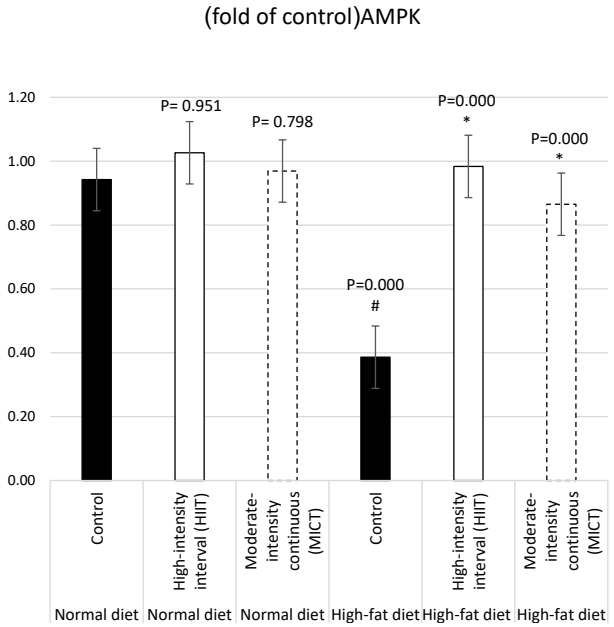


**Figure 1.** *AMPK* Gene Expression Levels
*Note*. HFD: High-fat diet; NFD: Normal-fat diet; Symbol *: compared to HFD control; Symbol #: compared to NFD control. One symbol: $P<0.01$

**Table 6.** Mean ± SD values of the Studied Indices

| Variable | NFFD | NFDHIIT | NFDMICT | HFD | HFDHIIT | HFDMICT |
|---|---|---|---|---|---|---|
| Body weight (g) | 29.690 ± 0.066 | 29.820 ± 0.095 | 31.300 ± 0.060 | 36.160 ± 0.040 | 31.300 ± 0.080 | 31.520 ± 0.038 |
| Liver weight (g) | 0.982 ± 0.049 | 0.954 ± 0.049 | 0.950 ± 0.060 | 1.095 ± 0.016 | 1.011 ± 0.010 | 1.017 ± 0.028 |
| *AMPK* expression | 0.942 ± 0.013 | 1.026 ± 0.023 | 0.969 ± 0.064 | 0.386 ± 0.012 | 0.983 ± 0.039 | 0.865 ± 0.010 |
| *SIRT1* expression | 1.020 ± 0.063 | 1.474 ± 0.017 | 1.284 ± 0.013 | 0.647 ± 0.010 | 1.361 ± 0.072 | 1.214 ± 0.015 |

*Note*. SD: Standard deviation; NFFD: Normal-fat diet control group; NFDHIIT: Normal-fat diet with high-intensity interval training; NFDMICT: Normal-fat diet with moderate-intensity continuous training; HFD: High-fat diet control group; HFDHIIT: High-fat diet with high-intensity interval training; HFDMICT: High-fat diet with moderate-intensity continuous training.

**Table 7.** Two-Way ANOVA Results for *AMPK* and *SIRT1* Expression

| Variable | Source | Sum of Squares | Mean Square | F | *P* | $\eta^2$ | Power |
|---|---|---|---|---|---|---|---|
| AMPK | Model (corrected) | 1.430 | 0.286 | 35.563 | <0.001* | 0.881 | 1.000 |
| | Diet | 0.412 | 0.412 | 51.188 | <0.001* | 0.681 | 1.000 |
| | Exercise | 0.625 | 0.313 | 38.864 | <0.001* | 0.764 | 1.000 |
| | Diet × Exercise | 0.393 | 0.197 | 24.450 | <0.001* | 0.671 | 1.000 |
| SIRT1 | Model (corrected) | 2.200 | 0.440 | 29.248 | <0.001* | 0.859 | 1.000 |
| | Diet | 0.258 | 0.258 | 17.126 | <0.001* | 0.416 | 0.978 |
| | Exercise | 1.808 | 0.904 | 60.074 | <0.001* | 0.834 | 1.000 |
| | Diet × Exercise | 0.135 | 0.067 | 4.482 | 0.022* | 0.272 | 0.711 |

*Note*. *Significant at $P<0.01$. AMPK: Adenosine monophosphate-activated protein kinase; SIRT1: Sirtuin 1.

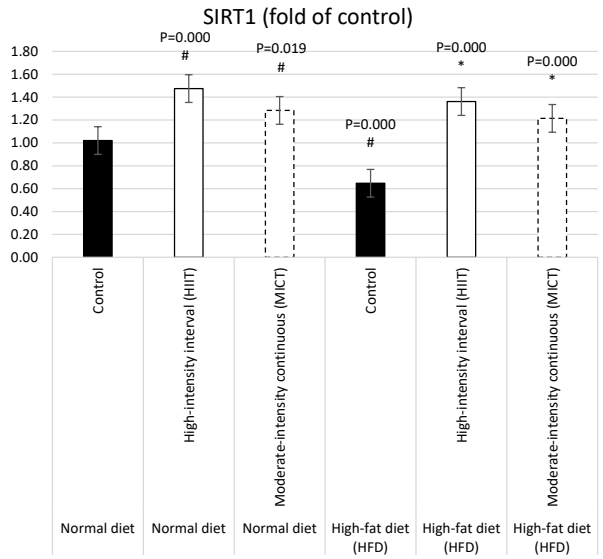


**Figure 2.** *SIRT1* Gene Expression Levels
*Note*. HFD: High-fat diet; NFD: Normal-fat diet; Symbol *: compared to HFD control; Symbol #: compared to NFD control. One symbol: $P<0.01$

change among the intervention groups under normal diet conditions; however, the HFD control group showed a significant decrease compared to the normal diet control. Additionally, both HIIT and MICT significantly increased *AMPK* expression in the HFD groups in comparison to the HFD control, with a more pronounced increase observed in the HIIT group. These findings indicate that exercise training can activate energy metabolism-related pathways in the liver tissue. Consistent with the results of previous studies, *AMPK* plays a key role in enhancing fatty acid oxidation and inhibiting lipogenesis, and its activation can be effective in managing NAFLD (15, 23, 29). The findings of Ashiqueali et al and Seddighi khovidak et al also indicated *AMPK* and *SIRT1* activation in response to HIIT (20, 21), aligning with the results of the present study. Furthermore, a study by En Li Cho et al highlighted the PPARα/SIRT1/AMPK axis, emphasizing that aerobic exercise can activate the AMPK pathway through miR-34a inhibition and the upregulation of SIRT1 and PPARα (23). Although our findings indicated increased *AMPK* gene expression in response to HIIT and MICT in the liver of mice with NAFLD, some studies reported differing or contradictory results. From the perspective of cellular function, AMPK activation not only promotes fatty acid oxidation and inhibits the lipogenesis pathway but also plays a role in improving cellular energy performance and reducing oxidative stress through the activation of mitochondrial biogenesis. It appears that the increased expression of the *AMPK* gene in the present study contributed beyond controlling steatosis, contributing to the overall improvement of liver health (19). Exercise induces significant changes in cellular metabolism, which are especially more meaningful and stronger under HFD conditions. Among them, HIIT, due to its variable intensity, sudden fluctuations in oxygen consumption, and abrupt energy demands, causes a severe decrease in adenosine monophosphate (ATP) and an increase in the AMP-to-ATP ratio (29). These conditions directly activate AMPK as the primary energy sensor. This activation not only leads to increased *AMPK* gene expression but also subsequently stimulates other downstream pathways, including enhanced fatty acid oxidation, reduced lipid synthesis, and increased glucose uptake (30). Similar pathways are activated in MICT; however, due to the relative stability in intensity and physiological stress, the extent and rate of AMPK stimulation are more limited.

Another finding of this study was the effect of eight weeks of HIIT and MICT on the expression of the *SIRT1* gene in the liver tissue of mice with NAFLD induced by an HFD. The results showed that both types of exercise, under normal diet and HFD conditions, significantly increased *SIRT1* expression, but HIIT had a more considerable effect than MICT. The significant reduction of *SIRT1* in the HFD control group, followed by its increase in the exercise groups, reflects the protective and therapeutic role of exercise in restoring the function of this energy-sensing gene. These findings demonstrated that exercise, not only in normal dietary conditions but especially in metabolically challenging conditions caused by an HFD, can restore the function of genes such as *SIRT1*, which plays a key role in regulating energy metabolism, insulin resistance, fat oxidation, and inflammatory responses. This finding conforms to the results of previous studies. Seddighi Khovidak et al reported that HIIT combined with calorie restriction increases SIRT1 in the liver (20). Likewise, Zou et al and Wu et al concluded that exercise inhibits miR-34a, leading to the release of SIRT1 inhibition and activation of the AMPK and PPARα pathways, thereby reducing oxidative stress and liver inflammation (17, 18). These pathways can improve metabolism and insulin resistance in the liver by increasing intracellular nicotinamide adenine dinucleotide ($NAD^+$), deacetylating components of the insulin signaling pathway, reducing nuclear factor kappa-light-chain-enhancer of activated B cell-dependent inflammation, and enhancing lipophagy (5). Liu et al found that aerobic exercise decreased *SIRT1* expression, possibly due to chronic oxidative stress and $NAD^+$ metabolism dysfunction under HFD conditions (19). These discrepancies are likely due to differences in exercise intensity, duration, the metabolic status of the animals, and gene expression measurement methods. HIIT, due to causing a severe energy drop and greater stimulation of ATP-sensitive pathways, can increase *SIRT1* expression more than MICT (10). The high intensity of HIIT leads to stronger activation of the hypoxia axis, mechanical stress, and increased secretion of catecholamines, all playing a role in the upstream pathways activating SIRT1 (6). Under HFD conditions, lipid metabolites, such as ceramides and diacylglycerol, inhibit insulin signaling and increase hepatic insulin resistance. Exercise activates SIRT1, which leads to the deacetylation of insulin receptor substrate 2 and other components of the insulin signaling pathway, thereby restoring insulin signaling at the cellular level. This direct regulation of insulin signaling via SIRT1 becomes

particularly important when the liver is damaged by an HFD and is not as critical under normal diet conditions (1, 11). The disruption of the circadian rhythms of hepatic genes, including *SIRT1*, has been observed in HFD–induced fatty liver models. Regular exercise (especially high-intensity training that strongly stimulates the cellular clock) can help reset these genetic oscillations and restore the physiological timing of gene expression, such as *SIRT1*. This rhythm regulation is less relevant in healthy livers but is essential in livers damaged by an HFD (27).

## Conclusion

Based on the findings of this study, both types of aerobic exercise (i.e., HIIT and MICT) positively influenced the regulation of *AMPK* and *SIRT1* gene expression in the liver tissue of mice with HFD-induced NAFLD. The increased expression of *AMPK* and *SIRT1* genes in the exercise intervention groups compared to the high-fat control group underscores the active role of these molecular pathways in response to aerobic training and in the prevention of NAFLD progression. The significant reduction in the expression of these genes in the high-fat control group compared to the normal diet control group also highlights the importance of energy-demanding cellular pathway disruptions under unhealthy nutritional conditions.

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### Competing Interests

The authors declare that there is no conflict of interests.

### Ethical Approval

The entire study protocol was reviewed and approved by the Biomedical Research Ethics Committee of Islamic Azad University, North Tehran Branch (IR.IAU.TNB.REC.1403.013).

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