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Synergistic Effects of Aerobic Training and Capsaicin on Visceral Adipose Tissue SIRT1 Gene Expression and Insulin Resistance in Rat Fed a High-Fat Diet.

Elham Heydarzadeh[1], *Ahmad Abdi*[2*], *Masoomeh Alsadat Mirshafaei*[3], *Rostam Abdi*[4]

[1]Department of Sport Physiology, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran.

[2]Department of Sport Physiology, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran.

[3]Department of Physical Education and Sport Science, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran.

[4]Department of Food Science and Technology, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran.

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Abstract

Background: Metabolic dysregulation may cause tissue damage in obesity-related diseases. The effects of SIRT1 on metabolism may provide a therapeutic target for treating obesity-related diseases. This study aimed to investigate the effects of aerobic training and Capsaicin (Cap) on visceral adipose tissue SIRT1 gene expression and insulin resistance in rats fed a high-fat diet (HFD).

Methods: 40 male Wistar rats were fed a normal diet (ND, n = 8) or HFD (n = 32) for 8 weeks. After 8 weeks, all rats were divided into five groups: ND, HFD, high-fat diet-training (HFDT), high-fat diet-capsaicin (HFDCap), and high-fat diet-training-capsaicin (HFDTCap). Training groups performed a progressive aerobic running program (at 15-25 m/min, 30-60 min/day, and 5 days/week) on a motor-driven treadmill for eight weeks. Capsaicin (4 mg/kg/day) was administered orally, by gavage, once a day.

Results: This study showed that SIRT1 expression decreased and HOMA-IR increased in the HFD group compared to the ND group. Also, the expression of SIRT1 in HFDT, HFDCap, and HFDTCap groups significantly increased compared to HFD. The expression of SIRT1 in HFDTCap also significantly increased compared to HFDT and HFDCap groups. There was a significant decrease in HOMA-IR levels in all experimental groups.

Conclusion: Possibly, eight weeks of progressive training combined with capsaicin administration has an effect on the glucose homeostasis of HFD rats by increasing the expression of SIRT1 and decreasing HOMA-IR.

Keywords: Exercise, Capsaicin, SIRT1, Insulin Resistance, Obesity

* **Correspond Author**: a.abdi58@gmail.com

Introduction

Adipose tissue plays a crucial role in metabolic homeostasis, which not only acts as energy storage but also functions as an essential endocrine organ to secrete a variety of adipokines and hormones (1). It has been shown that there is a close relationship between obesity and insulin resistance, and obesity-induced insulin resistance is one of the most critical risk factors for developing type 2 diabetes (2). In addition, obesity is a significant risk factor responsible for an ever-growing list of disorders, including non-alcoholic fatty liver disease, cardiovascular disease, chronic kidney disease, and cancers (3). These disorders may all arise from a pathological accumulation of fat (4). Evidence has shown that adipose Sirt1 has essential effects on the development of obesity and insulin resistance (5, 6). SIRT1 is a highly conserved NAD+-dependent protein deacetylase, which regulates cell senescence, inflammation, autophagy, insulin sensitivity, glucose and lipid metabolism (5). Selective overexpression of Sirt1 in adipose tissue enhances energy homeostasis and prevents metabolic disorders, such as blunted insulin sensitivity, suggesting that adipose Sirt1 is a key player in maintaining systemic energy homeostasis and insulin sensitivity (6). Sirt1 gene expression in adipose tissue and peripheral blood mononuclear cells was significantly suppressed in obese subjects compared to control subjects, and the down-regulation of Sirt1 may contribute to obesity-associated metabolic abnormalities (7). It was reported that adipocyte-specific Sirt1 deficiency led to increased whole-body adiposity and insulin resistance, and obesity in mice (8). A recent study showed that inhibition of SIRT1 in HFD mice increased insulin resistance (9). However, contradictory results were also observed (10).

Some anti-obesity interventions, both pharmaceutical and surgical, have been approved by international organizations (5). Bariatric surgeries and drugs can reduce fat accumulation and increase energy expenditure. However, these treatments are expensive and carry other health risks, such as mental disorders. Therefore, lifestyle interventions have been proposed as a safe and effective anti-obesity strategy. One of the interesting interventions is sports training. Previous studies showed that exercise training increases AMPK/SIRT1/PGC-1α/FOXO3 signaling mechanism in gastrocnemius muscles, soleus muscles, and cardiac muscle (11). The activation of SIRT1/AMPK signaling induced by exercise could decrease lipid accumulation, increase energy expenditure, limit lipogenesis, and upregulate fatty acid metabolism (12). In addition to exercise training, studies have shown that the activity of

adipose tissue is affected by various food compounds, such as capsaicin, found in red pepper. Upregulation of SIRT1 with capsaicin consumption was associated with improved expression of thermogenic proteins and improved metabolic syndrome in white adipose tissue (13). It was also shown that the combination of capsaicin and Capsiate could increase the expression of UCP-1, PGC-1α, AMPK, SIRT1, and TRPV1 and is a potential treatment method for obesity (14). However, the physiological effects of capsaicin and adaptations caused by exercise activity, the simultaneous effect of exercise activity, and capsaicin on this SIRT1 in obese rat model have been less investigated. Therefore, this study aims to investigate the effect of aerobic exercise with capsaicin on SIRT1 gene expression in visceral adipose tissue and insulin resistance in HFD rats.

Material and methods

Animals

All experiments were conducted based on the Iranian convention for protecting vertebrate animal policy; the Ethics Committee of the Sciences, Institute of Physical Education and Sports Sciences (IR.SSRC.REC.1398.125) approved the protocol. 40 male Wistar rats (5 weeks old, 147.68±9.14–135 gr weight) were acquired from the Pasteur Institute and transported to the laboratory. Four rats were housed per cage (46-L) with a 12:12-h light/dark cycle. Temperature and humidity were maintained at 22 ℃ ± 1.4 ℃ and 50% ± 5%, respectively. Diets (pellet form) and water were provided ad libitum.

Generating obese animal

After the rats adapted to the new environmental conditions (after a week), the rats were divided into two groups: regular diet (n=8, ND) and high-fat diet (n=32, HFD). ND group rats were fed a standard diet for eight weeks (23% protein, 65% carbohydrate, and 12% fat). Meanwhile, the rats in the HFD group used a high-fat diet. The high-fat meal contained 17% protein, 43% carbohydrate, and 40% fat (15). After eight weeks, all rats were divided into 5 groups: normal diet (ND), high-fat diet (HFD), high-fat-training (HFDT), high-fat-capsaicin (HFDCap) and high-fat-training -capsaicin (HFDTCap).

Training protocol

Before starting the main training and for familiarization, the rats ran on the treadmill for

five minutes at a speed of 8-10 m/min with a zero slope during five sessions in one week. The aerobic training program consisted of running on a treadmill with a 0% incline for eight weeks and five days a week. In the first week, the rats performed an aerobic exercise program on a treadmill with an intensity of 15 meters per minute for 30 minutes. After that, the activity intensity increased from 15 m/min to 25 m/min in the seventh week, and the activity time increased to 60 minutes (Tab 1) (16).

Table 1. Training protocol

Week	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***
Intensity (m)	*15*	*16*	*18*	*20*	*21*	*23*	*25*	*25*
Time (min)	*30*	*35*	*40*	*45*	*50*	*55*	*60*	*60*

Prepare and use capsaicin

Capsaicin (95% pure) was purchased from Sigma-Aldrich. Capsaicin solution (4 mg/ml) was prepared in 0.9% saline. This compound was used as gavage once daily with a dose of 4 mg/kg/day for eight weeks (17).

Biopsy

Forty-eight hours after the last training session, rats were anesthetized with intraperitoneal administration of a mixture of ketamine (30–50 mg/kg body weight) and xylazine (3–5 mg/kg body weight). Epidermal adipose tissue (EAT) was removed and washed with saline, then underwent freeze clamping. The supernatant was separated and placed into special tubes cooled with liquid nitrogen and preserved in a freezer at a temperature of -80 °C until the time of measuring.

Insulin and glucose

The ELISA method measured plasma insulin and glucose using a special kit (ZellBio GmbH- Germany). Homeostatic model assessment for insulin resistance (HOMA-IR) was calculated as fasting blood glucose fasting insulin/22.5 [17].

SITR1 RNA isolation, cDNA synthesis, and real-time PCR

The total RNA was extracted from 20mg of VAT using RNA purification kits (QIAGEN Rneasy protects mini kit). Complementary DNA (cDNA), utilizing an extended cDNA

synthesis kit (One Step SYBR TAKARA), was measured based on the standard manufacturer's protocol. Expected fragment size and gene bank accession numbers are listed in Table 1. PCR was carried out on the Rotor-Gene 6000 Real-time PCR system (Corbett Life Science) with cycler conditions listed in Table 3.

The melting curve was analyzed at the end of the PCR cycle to determine the validity of the expected PCR product. The cyclic thermal protocol used by the Rotor-Gene device in Real-Time PCR was 95° for 10 min, 95° for 2 min, and 40 cycles at 94° for 10 s and 60° for 40 s. After the PCR steps, temperatures 50° to 99 °C were used to prepare the melting curve and analyze the primer properties. RNA polymerase was used as a control gene to determine the expression of RBP4. Reaction cycle thresholds were extracted and recorded using Rotor-Gene 6000 Real-Time PCR software. A comparative DDCT method was used to quantify the expression of TCFmRNA. The relative levels of mRNA were analyzed by the 2–DDCt method.

Table 2. Oligonucleotide primer sequences and real-time PCR amplification parameters.

Genes	Sequence (5' → 3')
B2m forward	5'- CGTGCTTGCCATTCAGAAA -3'
B2m reverse	5'-ATATACATCGGTCTCGGTGG -3'
SIRT1 forward	5'- TCCTGTGGGATACCTGACTT-3'
SIRT1 reverse	5'- AAAGGAACCATGACACTGAATGA-3'

Table 3. Real-time cycler conditions.

Steps		Time	Temperature
PCR initial activation step		10 min	95 °C
Denaturation		2 min	95 °C
40 Cycle	Combined annealing/extension	10s	94 °C
Melting Curve		5 min	50 to 99°C
Cooling		End	40 °C

Statistical Analysis

The Shapiro-Wilk test was used to ensure normal data distribution, and the Levin test was used to ensure homogeneity of variances. Descriptive statistics were used to describe the data and draw graphs, and ANOVA was used to compare the groups in the studied

variables. A significant level was considered P≤0.05. All statistical analysis was performed using 26 SPSS software.

Results

Table 4 indicated the mean changes in weight, glucose, and insulin in different groups (Tab 4).

Table 4. Results of descriptive and inferential statistics related to research variables

	Weight (g)			**Glucose**	**Insulin**
	Pre-test	After induction of obesity	Post test		
ND	145.33 ± 11.89	297.56 ± 30.00	323.56±32.94	100.75±5.62	6.88±0.32
HFD	143.22 ± 7.25	456.11±81.8[a]	479.44±29.3 [a]	175.61±9.66	8.36±0.57
HFDT	149.11 ± 6.13	469.56±37.5 [a]	429.7±32.7 [ab]	147.82±10.09	7.26±0.33
HFDCap	148.67 ± 11.19	482.89±51.6 [a]	433.67±40.1 [ab]	146.75±11.01	7.24±0.21
HFDTCap	151.22 ± 8.98	482.22±50.3 [a]	396.67±32.1 [ab]	132±12.47	6.14±0.40
Between Group p	0.394	0.0001*	0.0001*	0.0001*	0.0001*

* Difference Between Group, a Difference with ND, b Difference with HFD group, c Difference with HFDTCap group.

The research results showed that induction of HFD decreased the expression of SIRT1 (p = 0.016) and increased HOMA-IR (p = 0.028). Based on the data analysis, a significant difference was observed in the rate of changes in SIRT1 expression in EAT among different groups (p = 0.0001, F = 4.124). Furthermore, the results of the Tukey post hoc test indicated a significant increase in the rate of changes in SIRT1 expression of EAT in HFDT (p = 0.023), HFDCap (p = 0.028) and HFDTCap (p = 0.0001) groups compared to HFD group; and HFDTCap group was observed compared to HFDT and HFDCap (p = 0.022) groups (P=0.019) (Figure 1).

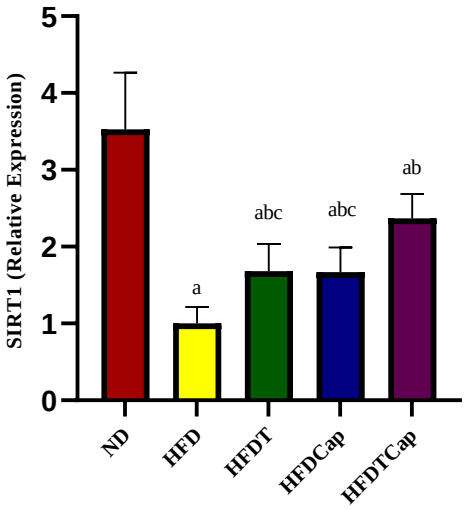

Figure 1. Changes in SITR1 levels in different groups using ANOVA (level of p <0.05). a Difference with ND, b with the HFD group, and c with the HFDTCap group. Normal diet (ND), high-fat diet (HFD), high-fat diet training (HFDT), high-fat diet-capsaicin (HFDCap), and high-fat diet-training-capsaicin (HFDTCap).

Another result of this study using the ANOVA test was a significant difference in the amount of HOMA-IR changes between different groups (P=0.0001, F=17.298). In addition, the results of the post hoc test showed that there was a significant decreased in HFDT (p = 0.0021), HFDCap (p=0.0045), and HFDTCap (p=0.0016) groups compared to the HFD group; and HFDTCap group was observed compared to HFDT and HFDCap (p=0.018) groups (P=0.009) (Figure 2).

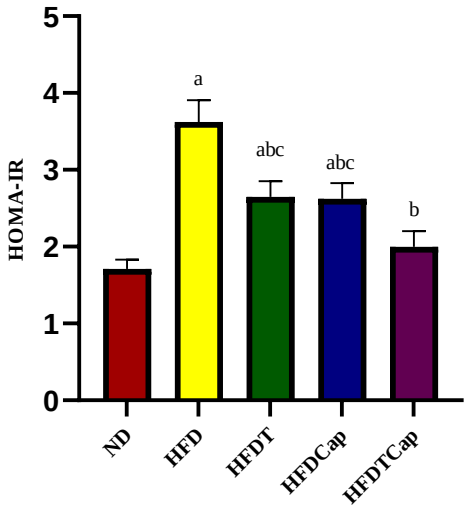

Figure 1. Changes in HOMA-IR levels in different groups using ANOVA (level of $p < 0.05$). a Difference with ND, b with the HFD group, c with the HFDTCap group. Normal diet (ND), high-fat diet (HFD), high-fat diet training (HFDT), high-fat diet-capsaicin (HFDCap), and high-fat diet-training-capsaicin (HFDTCap).

Discussion

The present study showed that aerobic exercise increased the expression of SIRT1 in adipose tissue and decreased the HOMA-IR index in HFD rats. In line with the present study, Habibi et al. (2020) showed that swimming training by increasing the expression of SIRT1 caused a significant decrease in inflammatory cytokines and tissue damage and improved glucose homeostasis (18). Also, Omidfar et al. (2021) showed that high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT) or continuous endurance training (CET) programs increased the expression of SIRT1 and improved insulin resistance in HFD rats (19). SIRT1/AMPK signaling is an energy-sensitive pathway that regulates various physiological responses, including catabolic metabolism, angiogenesis, cell survival, and insulin resistance (20). SIRT1 activation can regulate lipid metabolism (21), reduce inflammation (22), and reduce endoplasmic reticulum stress and insulin resistance (23). In addition, this pathway was identified as a regulator of exercise-induced adaptation (12). A study showed that swimming reduced the suppression of the SIRT1/AMPK pathway caused by high-fat diets (24). Endurance exercises have been shown to increase mitochondrial biogenesis and SIRT1 content and activity (25), and it has also been shown that SIRT1 deacetylates PGC-1α (26). Therefore, SIRT1 can cause mitochondrial biogenesis by

deacetylation of PGC-1α. An increase in mitochondrial efficiency can also play a role in improving insulin resistance conditions in HFD rats.

Among other results of the present study, insulin resistance improved, and SIRT1 expression increased following capsaicin consumption in HFD rats. The increased SIRT expression following capsaicin administration may be due to AMPKα phosphorylation. Previous studies mentioned that phosphorylation of AMPKα could activate SIRT1 directly and that SIRT1 also played a significant role in regulating lipid metabolism (13). Fan et al. (2019) showed that the combination of capsaicin and capsiate greatly elevated the phosphorylation of AMPKα and AMPKβ1 and SIRT1 as well, which contributed to decreased lipid accumulation in 3T3-L1 adipocytes (14). Studies have shown that following the activation of SIRT1 improves insulin sensitivity in the liver, skeletal muscle, and adipose tissue and confers protection to pancreatic beta-cell function and quality. There seems to be a close relationship between SIRT1 changes and insulin resistance. Many biological processes, such as mitochondrial biology and fatty acid oxidation, are regulated by the AMPK/SIRT1/PGC-1α signaling pathway (28). In addition, Gong et al. (2022) stated that capsaicin is able to activate the TRPV1 channel, and this pathway regulates lipid metabolism, fasting plasma glucose and insulin resistance (29). Also, Liang et al. (2021) demonstrated the modulatory effects of capsaicin on glucose homeostasis in cell models, animal models, and human trials through TRPV1-dependent and TRPV1-independent pathways (30).

Among other results of the present study, there was a significant increase in the expression of SIRT1 in adipose tissue and a decrease in the HOMA-IR index in the combined group (ObeTCap) compared to the aerobic exercise and capsaicin groups. Exercise interventions and phytochemical compounds seem to improve metabolic disorders in obese rats through changes in the amount and type of adipose tissue fat (31). In this regard, Mostafavian et al. (2020) showed that aerobic exercise combined with capsaicin increased FNDC5 and irisin in visceral fat tissue, had a double effect on the factors affecting the browning of fat tissue, and thereby increased the rate of metabolism. It can reduce the complications caused by obesity (33). Also, another study showed that training with capsaicin increases the expression of PGC-1α in HFD mice and can partially control obesity-related diseases (33). These results indicate the synergistic effects of aerobic exercise and capsaicin on SIRT1 and insulin resistance in HFD rats.

Conclusion

Exercise training and capsaicin affected the expression of SIRT1 in visceral adipose tissue in rats, thereby reducing insulin resistance in HFD rats. Therefore, the use of capsaicin and other biologically active compounds along with aerobic physical activity, is an interesting and effective strategy to improve glucose homeostasis caused by high-fat diets. Human studies are necessary to investigate the effect of capsaicin and sports activity on this pathway.

Declarations

Compliance with ethical guidelines

This research was carried out with the approval of the Ethics Committee of the Research Institute of Physical Education and Sports Sciences with code IR.SSRC.REC.1398.125.

Conflicts of interest

The authors declare that they have no competing interests.

Acknowledgments

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