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# The effect of high-intensity interval, aerobic, resistance and spirulina supplement consumption on levels of UCP-1, TRPV1 and HOMA-IR in the white adipose tissue of diabetic rats

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**FATHI, M., A. RASHIDLAMIR, S. KHAIRABADI, K. HEJAZI: The effect of high-intensity interval, aerobic, resistance and spirulina supplement consumption on levels of UCP-1, TRPV1 and HOMA-IR in the white adipose tissue of diabetic rats.Vet.arhiv 93, 97-116, 2023.**

**ABSTRACT**

The purpose of this study was to investigate the effect of high-intensity interval, aerobic, and resistance training, and spirulina supplement consumption on the levels of Uncoupling Protein-1 (UCP1), Transient Receptor Potential Vanilloid (TRPV1) and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) in the white adipose tissue of diabetic rats. A total of 42 male rats with diabetes type II were randomly assigned into seven groups as follows: aerobic training (n=6), resistance training (n=6), interval training (n=6), aerobic training combined with supplementation (n=6), resistance training combined with supplementation (n=6), interval training combined with supplementation (n=6) and control (n=6) groups. The training groups carried out the training (8 weeks/5 days) on a rodent treadmill and ladder. The paired sample t-test and one-way ANOVA were employed for data analysis. The three kinds of training, with and without supplementation, significantly reduced mass, glucose, insulin, and insulin resistance. In the three supplementation-combined training groups the difference on the HOMA-IR index was significant; however, the decline was larger in the resistance training with supplementation group. The concentration of UCP-1 and TRPV1 proteins significantly increased in all training groups, with and without supplementation. Nonetheless, the significant increase in the UCP-1 levels in the interval training with supplementation group was more than in the other groups. Furthermore, the TRPV1 protein levels were higher in the resistance training with supplementation group. Eight weeks of training, with and without Spirulina supplementation, reduced insulin resistance and gave rise to significant changes in UCP-1 and TPRV1 concentrations.

**Key words:** diabetes; aerobic training; resistance training; interval training; spirulina supplementation

## Introduction

Diabetes mellitus is one of the most prevalent and complicated problems of modern societies, and causes many socioeconomic difficulties (SRINIVASAN and RAMARAO, 2007). Diabetes and obesity are two kinds of metabolic impairment (DESPRÉS et al., 2001). Obesity and insulin resistance play crucial roles in the pathogenicity of diabetes type II and cardiovascular diseases (HØJLUND and BOSTRÖM, 2013; HOTTA et al., 2012). The brown adipose tissue is recognized as a

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therapeutic goal for obesity. The activation of the brown adipose tissue can be accompanied by useful metabolic effects for humans, and may improve the obesity complications such as diabetes type II or changes in pathological glucolipotoxicity, which is the main reason for peripheral insulin resistance and impaired insulin secretion due to the destruction of the beta cells of the pancreas (PEIRCE and VIDAL-PUIG, 2013). Non-shivering thermogenesis is the most important function of brown adipose. This function is regulated by the mediation of UCP-1, which is situated in the inner membrane of the mitochondria and gives rise to the separation of the electron transport chain from energy generation and, as a result, the release of potential energy in the form of heat (ALBERDI et al., 2013; BARBERÁ et al., 2001). UCP-1 is located in the inner membrane of the mitochondria of brown adipose tissue cells, as well as subcutaneous cells. This protein shapes a channel that enables proton leak to extracellular space, and hence the energy to be used for ATP synthesis is excreted thermally (SLUSE et al., 2006). Through producing non-shivering heat in brown and brown-looking white fats, this protein increases energy use, metabolic malfunctioning, and energy excretion (NEDERGAARD et al., 2001).

UCP-1 synthesis in the adipose tissue plays a leading role in resistance against and precluding lipid accumulation, mass gain, obesity, and diabetes. A study on genetically-modified rats with higher UCP-1 levels in their adipose tissue showed that these rats were resistant to obesity derived from fat food, and diabetes (KOPECKY et al., 1995). FELDMANN et al. (2009) reported that the elimination of UCP-1 in animal samples led to obesity, besides reducing energy efficiency and thermogenesis, while the induction of UCP-1 expression in white adipose tissue could be employed as a therapeutic method for resisting obesity and diabetes type II. Another factor with a part in metabolism and energy hemostasis is the Transient Receptor Potential Vanilloid 1 (TRPV1), usually called the capsaicin receptor. These receptors can increase thermogenesis in the fat tissue. Capsaicin receptors are, in fact, channels for the passage of positive ions, and their stimulation leads to heat generation and pain reduction (BHAVE et al., 2003). Moreover, TRP channels have a crucial role in metabolism regulation and energy hemostasis. Several studies have examined the relationships of obesity and TRPV1 with controlling appetite and mass, regulating pancreas function, balancing adiponectin, and leptin signaling (SURI and SZALLASI, 2008). The activation of the TRP channel reduces visceral fat in high-fat diet conditions, and generates heat in brown adipose tissue (SURI and SZALLASI, 2008). LEE et al. (2015) observed that TRPV1 inhibition in rats with high-fat diets increased mass, the Fat Mass Index, and insulin resistance, in addition to decreasing the uptake of insulin-stimulated glucose in the white and brown adipose tissues. Clinical studies reveal that the UCP-1 and TRPV1 levels are lower in obese and diabetic individuals (SURI and SZALLASI, 2008).

The Spirulina supplement is antioxidant-enriched, and possesses some nutrients such as phycocyanin, chlorophyll, polysaccharides, and sulpholipids. Studies show that Spirulina is effective in preventing obesity and diabetes type II since, in addition to its low calories, it contains large amounts of vitamins (LITTLE et al., 2010). Spirulina supplement consumption reduces white adipose tissue while increasing the brown adipose tissue (REISI et al., 2013). Investigations disclose that both body activation and Spirulina consumption increase UCP-1 and mitochondrial biogenesis in brown adipose tissue (BOUAZIZ et al., 2015). However, there are few studies addressing the simultaneous effect of Spirulina consumption and physical activity in relation to cellular and molecular mechanisms, mass loss, and insulin resistance enhancement (HERNANDEZ LEPE, 2018; HOZAYEN et al., 2016; MISRA et al., 2008). Insulin resistance is the paramount diabetes-causing factor. The main mechanisms that trigger insulin resistance are the negative regulation of Insulin Receptor Substrate 1 (IRS1), and a reduction in insulin signaling due to the overgrowth of free fatty acids in the blood circulation. A reduction in insulin signaling in the skeletal muscles impairs glucose removal by the muscular tissue. This leads to the development of peripheral insulin resistance and compensatory hyperinsulinemia

(NIKROO et al., 2020; HENRIKSEN 2002). Recently, researchers have made efforts to activate and increase brown adipose tissue as an approach to precluding, as well as treating excess weight, obesity, and their associated diseases (BONET et al., 2013; BOSTRÖM et al., 2012). Various studies have recognized the use of edible supplements and diverse sports training as helpful in activating this tissue (ALBERDI et al., 2013).

One of the newest studies demonstrates the important point that body activation brings changes in the adipose tissue phenotype, activates the signaling pathway of the thermogenesis process in fat tissue using UCP-1 and TRPV1, and, thus, alters the adipose tissue type and reduces the Body Fat Index (BFI) (CHRISTIE et al., 2018; MOSTAFAVIAN et al., 2020). Many previous studies on human and animal samples revealed that physical activities with and without diet modification improve HOMA-IR, as well as other effective pathogenic factors contributing to some diseases, such as diabetes, hyperglycemia, and obesity (HOUGHTON et al., 2018). In this respect, only a few studies have compared different kinds of physical activities and simultaneous supplementation in the metabolic pathways involved in these processes. Although several human and animal studies have displayed the positive effects of Spirulina on mass loss, its mechanism of action and the active ingredient which helps with mass-loss and improves insulin sensitivity are not properly recognized. Thus, UCP-1, TRPV1, and Spirulina supplement consumption are among the factors whose high levels may increase brown adipose tissue and reduce the prevalence of obesity and diabetes type II. Generally, since investigating and treating the pathogenesis-involved molecular pathways is difficult and sometimes impossible in human samples, the necessity for animal and laboratory studies is highlighted. Although studies on rats have confirmed the positive effect of physical activities on diabetes, the independent effect of different kinds of activity and supplementation with varying energy systems has not yet been specified. Likewise, the micromolecule factors that engage with the metabolic pathways associated with the phenotype variations from white to brown tissues, such as UCP-1, TRPV1, and HOMA-IR, require further investigation. The purpose of this study was to investigate the effect of high-intensity interval, aerobic, resistance and spirulina supplement consumption on the levels of UCP1, TRPV1 and HOMA-IR in the white adipose tissue of diabetic rats.

## Materials and methods

*Study design and animal*. In this experimental study, 42 male, adult, 8-week-old Wistar rats with an average mass of 125-150 gr were purchased from the Animal Laboratory of the Mashhad University of Medical Sciences and considered as the research sample. The rats could freely access food manufactured by Behparvar Company (Table 1) and water, and were kept in controlled conditions of light (12 hours of light and 12 hours of dark) temperature (22±3ºC), and humidity (~45%). The forty-two rats were randomly assigned into seven groups: 1- control (n=6), 2-aerobic training (n=6), 3-resistance training (n=6), 4-interval training (n=6), 5-aerobic training with supplementation (n=6), 6-resistance training with supplementation (n=6), and 7-interval training with supplementation (n=6) (Figure 1). All housing, induction, training, and euthanasia stages were conducted according to the regulations of the ethics committee for biomedicine studies of Ferdowsi University of Mashhad. The present study was approved by the ethics committee of Ferdowsi University of Mashhad, under the Code no. IR.UM.REC.2020.53293.

*Induction of diabetes by Streptozotocin in rats*. For diabetes induction in rats, after 8 hours of food deprivation, the Streptozocin medicine, dissolved in buffer sodium citrate with a PH of 4, 50mg per Kg, was injected by the intraperitoneal method. The rats were afflicted 48 hours after the injection. For diabetes confirmation, four days after the Streptozocin injection, small wounds were made in the animals' tails, and a blood drop was put on glucometer strips and read by a glucometer device. Glucose levels higher than 300mg/deciliters were considered as the diabetes index (CALCUTT, 2004). Streptozotocin was supplied by the Pharmacia Company. Streptozotocin is available for intravenous use as a dry-frozen, pale yellow, sterilized product.

Table 1. Standard food content manufactured by Behparvar Company

| Contents | Protein | Fat | Carbohydrate | Fiber | Ash | Calcium | Phosphorus | Salt | Humidity | Lysine | Methionine | Methionine + cysteine | Reunin + Tryptophan | Other materials | Kcal energy in grams |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Standard (%) | 20 | 3.5 | 25 | 14.5 | 10 | 1 | 0.7 | 0.5 | 10 | 1.15 | 0.33 | 0.63 | 0.95 | 11 | 3.9 |

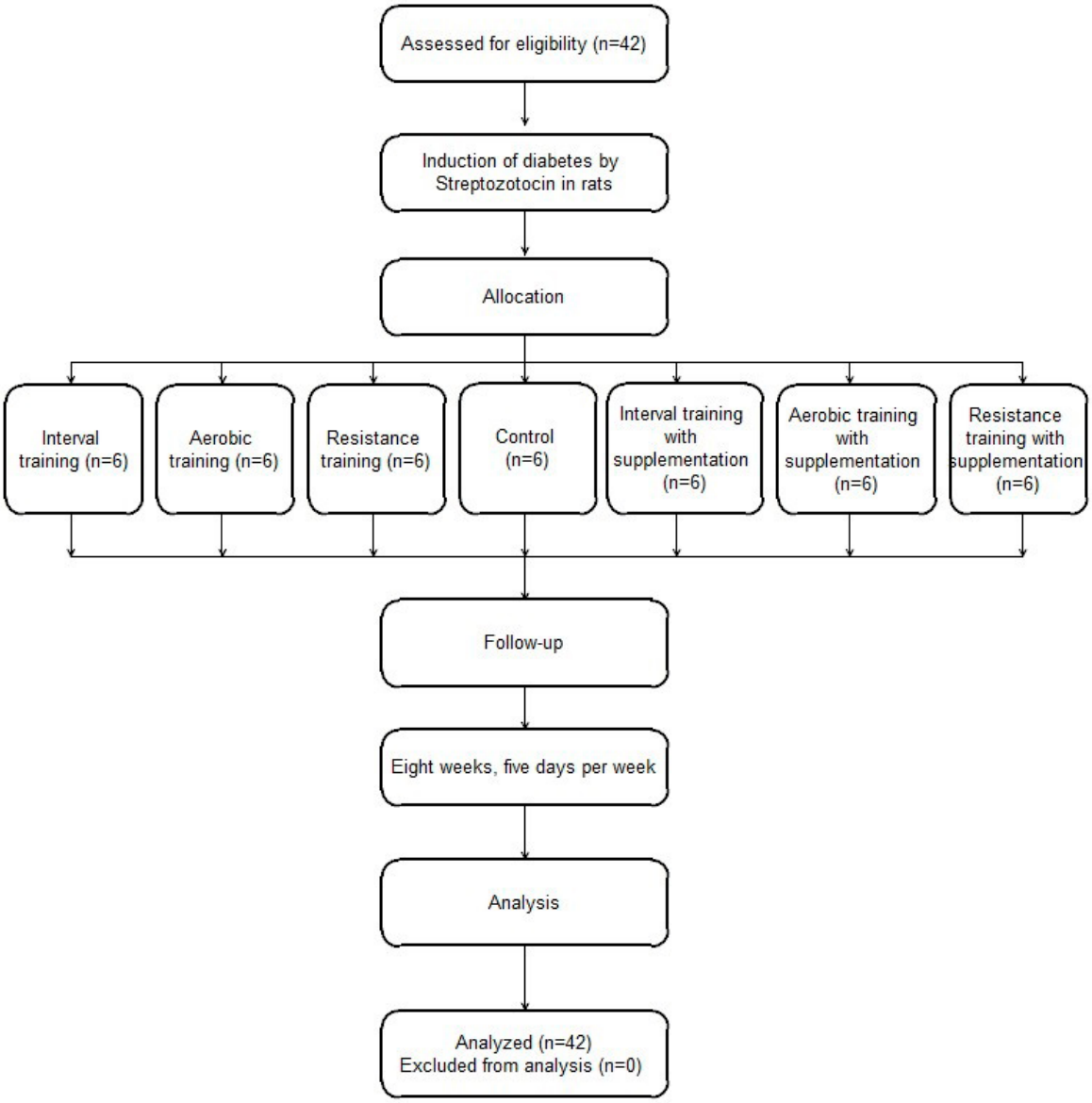


Fig.1. Flow diagram of study design

*Classification of exercise training types. High intensity interval training.* The high intensity interval training of the rodents was conducted for 8 weeks, five days per week, with one 25-minute session a day. It included a 5-minute warm up, four 4-minute intervals comprising 3 minutes of high-intensity activation and 1 minute of low-intensity activation, and a 5-minute cooling down period on the treadmill (Table 2).

Table 2. Prescription details of the HIIT exercise training

| | | The main body of the exercise training (4 alternation) | | |
|---|---|---|---|---|
| Exercise component | Warm up | High-intensity interval training | Low-intensity interval training | Cool down |
| Duration (min) | 5 min | 3 min | 1 min | 5 min |
| Intensity ($VO_{2max}$) | 30-40 % | 85-90 % | 30-35 % | 30-40 % |
| Intensity (m/s) | 0.19-0.25 | 0.53-0.56 | 0.19-0.22 | 0.19-0.25 |

HIIT: High-intensity interval training

*Resistance training.* The resistance training encompassed climbing a 1-meter ladder, with 26 steps, an 85º slope, and a 2-cm inter-step distance, carrying a load. The resistance training was performed for 8 weeks, 5 days per week, and 15 climbs per session, with a 1-minute rest between two climbs, and an average intensity of 40-60% of the maximum load test. The extra load was determined and carried according to the body mass of the rats. Before the maximum load test and the 8-week resistance training, the rats performed ladder-climbing for 5 successive days to learn the activity. The maximum load test began with a load of 75% of body mass, and the rats were given a 2-minute rest time in a dark environment before the next climb. For the next climb, 15% of body mass was added as a load to the former mass. This increase in the load continued until the rats could not climb the ladder, and 6 climbs were completed maximally. The maximum load test was performed at the beginning of the first and fourth weeks, and at the end of the eighth week. The load applied per climb was 15, 25, and 40% of the body mass, respectively. The masses were stuck on using a strip cloth 1cm below the hair-growing part of the tail, and the rats climbed the ladder with no stimulation (LEE and FARRAR, 2003; NIKROO et al., 2020) (Table 3).

Table 3. Prescription details of the resistance exercise training.

| First Week | | Second Week | | Third Week | | Fourth Week | |
|---|---|---|---|---|---|---|---|
| Climbs* (Repetition) | % of ML | Climbs (Repetition) | % of ML | Climbs (Repetition) | % of ML | Climbs (Repetition) | % of ML |
| 2 | 0 | 1 | 0 | 2 | 0 | 1 | 0 |
| 3 | 20% | 1 | 20% | 1 | 20% | 1 | 20% |
| 2 | 30% | 4 | 30% | 2 | 30% | 1 | 30% |
| 2 | 40% | 3 | 40% | 2 | 40% | 2 | 40% |
| 2 | 30% | 4 | 30% | 1 | 50% | 2 | 50% |

Table 3. Prescription details of the resistance exercise training. (continued)

| First Week | | Second Week | | Third Week | | Fourth Week | |
|---|---|---|---|---|---|---|---|
| Climbs* (Repetition) | % of ML | Climbs (Repetition) | % of ML | Climbs (Repetition) | % of ML | Climbs (Repetition) | % of ML |
| 2 | 20% | 1 | 20% | 2 | 40% | 2 | 40% |
| 2 | 0 | 1 | 0 | 2 | 30% | 2 | 30% |
| - | - | - | | 1 | 20% | 1 | 20% |
| - | - | - | | 2 | 0 | 1 | 0 |
| Fifth Week | | Sixth Week | | Seventh Week | | Eighth Week | |
| Climbs (Repetition) | % of ML | Climbs (Repetition) | % of ML | Climbs (Repetition) | % of ML | Climbs (Repetition) | % of ML |
| 1 | 0 | 1 | 0 | 2 | 0 | 1 | 0 |
| 1 | 20% | 1 | 30% | 2 | 40% | 2 | 40% |
| 2 | 30% | 2 | 40% | 3 | 50% | 4 | 50% |
| 2 | 40% | 3 | 50% | 3 | 60% | 5 | 60% |
| 3 | 50% | 2 | 60% | 3 | 50% | 2 | 50% |
| 2 | 40% | 3 | 50% | 1 | 40% | 1 | 40% |
| 2 | 30% | 1 | 40% | 1 | 0 | 1 | 0 |
| 1 | 20% | 1 | 30% | - | - | - | - |
| 1 | 0 | 1 | 0 | - | - | - | - |

*1 min time interval between climbs; ML: maximal load

*Aerobic training.* The aerobic training was rodent-specific treadmill running (manufactured by Borj Sanat Co.; Model T.S 8000, made in Iran) with velocity and slope controlling capacity. The aerobic training was executed for 8 weeks, 5 days per week, 60 minutes a day, with an intensity of 40-60% of the maximum running velocity test, and with a zero slope. The exercise time increased incrementally from 18 minutes at the onset of the first week to 60 minutes at the end of the fourth week, and lasted until the eighth week. Before the maximum running velocity test and the 8-week aerobic training, the rats performed treadmill running for a week, 10 minutes a day at 0.3 k/hr (5m/min) velocity, to learn the activity. The maximum velocity test started after a 30-minute rest on the treadmill. The initial speed was 5m/min, and 3m/min velocity was added to the treadmill speed every three minutes. The acceleration of the treadmill continued until the rats were exhausted, and the recorded velocity was considered as the maximum running speed. This test was conducted at the beginning of the first and fourth weeks, and at the end of the eighth week (NIKROO et al., 2020). After the completion of the training program and for cooling down, the device was slowed down until it reached zero velocity. It is worth mentioning that, apart from the main training protocols on the experimentation days, all the biological conditions of the control group were similar to the training groups (Table 4).

Table 4. Prescription details of the aerobic exercise training.

| Weeks | Session | Time (min) | % of MRS | Slope | Weeks | Session | Time (min) | % of MRS | Slope |
|---|---|---|---|---|---|---|---|---|---|
| 1th | 1 | 18 | 40 % | 0 | 5th | 21 | 60 | 60 % | 0 |
| | 2 | 21 | 40 % | 0 | | 22 | 60 | 60 % | 0 |
| | 3 | 24 | 43 % | 0 | | 23 | 60 | 60 % | 0 |
| | 4 | 27 | 43 % | 0 | | 24 | 60 | 60 % | 0 |
| | 5 | 30 | 46 % | 0 | | 25 | 60 | 60 % | 0 |
| 2nd | 6 | 32 | 46 % | 0 | 6th | 26 | 60 | 60 % | 0 |
| | 7 | 34 | 49 % | 0 | | 27 | 60 | 60 % | 0 |
| | 8 | 36 | 49 % | 0 | | 28 | 60 | 60 % | 0 |
| | 9 | 38 | 52 % | 0 | | 29 | 60 | 60 % | 0 |
| | 10 | 40 | 52 % | 0 | | 30 | 60 | 60 % | 0 |
| 3th | 11 | 42 | 55 % | 0 | 7th | 31 | 60 | 60 % | 0 |
| | 12 | 44 | 55 % | 0 | | 32 | 60 | 60 % | 0 |
| | 13 | 46 | 58 % | 0 | | 33 | 60 | 60 % | 0 |
| | 14 | 48 | 58 % | 0 | | 34 | 60 | 60 % | 0 |
| | 15 | 50 | 60 % | 0 | | 35 | 60 | 60 % | 0 |
| 4th | 16 | 52 | 50 % | 0 | 8th | 36 | 60 | 60 % | 0 |
| | 17 | 54 | 50 % | 0 | | 37 | 60 | 60 % | 0 |
| | 18 | 56 | 55 % | 0 | | 38 | 60 | 60 % | 0 |
| | 19 | 58 | 55 % | 0 | | 39 | 60 | 60 % | 0 |
| | 20 | 60 | 60 % | 0 | | 40 | 60 | 60 % | 0 |

MRS: Maximum Running Speed

*Spirulina-consuming*. The Spirulina-consuming groups received the supplement for eight weeks, 15 mg per day for every kilogram of body mass, through nasogastric tubes (PANDEY et al., 2011). Since Spirulina contains protein in large amounts, in order to provide an equal protein and carbohydrate content in each diet, the amounts of casein and cornstarch were adjusted properly. The spirulina was supplied by ES Biotech Co. (Cheonan, Korea) for use in this study.

*Sampling methods*. After 48 hours of non-training and nightly fasting, the rats were weighed and then anesthetized by the Xylazine (8mg/kg) and Ketamine (75mg/kg) compound through an intraperitoneal injection. After their anesthesia was confirmed by failure to pull back the foot, a 5-6cm incision was made in the abdominal area of the rats. Next, 5ml of blood was drawn from the right ventricle of every rat by a syringe and poured immediately into lab tubes containing no anticoagulation substance. Some of the white adipose tissue was washed thoroughly in natural saline solution so that extra blood on the tissue was cleared. The blood samples were centrifuged for serum separation. Finally, the white adipose tissue and serum samples were transferred to a freezer at -70ºC for later measurements. For RNA extraction, 40-50 mg of the white adipose tissue was homogenized with 750 µL of RIPA, and all phases were carried out in ice containers. In the last phase, the sample was drawn from the tube by a sampler and poured into a 1.5mm microtube. The microtube was centrifuged at a velocity of 20000 RPM and a temperature of 4ºC. After completion of the centrifuge, the microtubes were removed from the device, and the supernatants were drawn

off by a sampler and poured into a new microtube. Then the samples were frozen at -70ºC. The serum concentration of glucose was measured by the glucose oxidase method using kits manufactured by Azmoon Pars Co., and the serum insulin level was measured by the ELISA method and kits produced by Mercodia Sweden Co., with a sensitivity of 0.021ng/l. According to the formula below, HOMA-IR was used for insulin resistance evaluation. It was obtained on the basis of the product of fasting blood glucose concentration (mMol/l) and fasting insulin concentration (micro-unit/mm) divided by 22.5. The UCP1 level was measured by a rat-specific ELISA kit (EASTBIOPHARM, under America license) with the Cat.No: CK-E91412 and Intra-Assay: CV<10% serial No. The TRPV1 level was also measured by rat-specific ELISA kits (EASTBIOPHARM, under the license of America) with Cat.No: CK-E91266 and Intra-Assay: CV<10% serial No.

*Statistical analysis*. The collected data were analyzed by the SPSS 16 software at the significance level of 0.05 (P≤0.05). After ensuring the normal distribution of data by the Shapiro-Wilk statistical test, and variance homogeneity by the Levine's test, the researcher employed the statistical one-way ANOVA and paired sample t-test to investigate the differences between the intergroup and intragroup means. Likewise, the post hoc Tukey test was used for pairwise comparison of means.

## Results

*Body mass changes*. The intragroup comparisons indicated significant declines in the mean body mass after 8 weeks of aerobic training, with/without supplementation, resistance training with/without supplementation, and interval training with/without supplementation. According to Table 5, these 6 groups were significantly different from the control group in their average mass. As seen in Table 5, the results of the post hoc Tukey test showed that there were significant differences between the body mass means of the following groups (Figure 2):

Table 5. The comparison of between groups in weight

| | | Stages | | | | | |
|---|---|---|---|---|---|---|---|
| Variables | Groups | Pre-test (Mean ± SD) | Post-test (Mean ± SD) | P Value | Shapiro-Wilk Test | ANOVA F | P Value |
| Weight (g) | AT | 185.4±5.5 | 181.4±4.1$^{a*g*}$ | 0.001$^{**}$ | 0.184 | 3.14 | 0.001* |
| | RT | 186.3±4.1 | 182.4±3.8$^{a*b*}$ | 0.001$^{**}$ | 0.325 | | |
| | HIIT | 185.1±6.3 | 179.2±6.4$^{a*}$ | 0.001$^{**}$ | 0.543 | | |
| | AT+ SSC | 186.7±5.5 | 181.6±4.00$^{a*i*}$ | 0.001$^{**}$ | 0.263 | | |
| | RT+ SSC | 187.1±5.2 | 181.3±4.3$^{a*j*}$ | 0.001$^{**}$ | 0.939 | | |
| | HIIT+ SSC | 184.2±3.6 | 177.1±4.3$^{a*}$ | 0.001$^{**}$ | 0.490 | | |
| | C | 186.4±6.7 | 191.2±3.6 | 0.241 | 0.247 | | |

Values are expressed as Mean±SD (n = 6 per group).
a, vs. Control; b, vs. RT- HIIT+ SSC; c, vs. HIIT- RT+ SSC; d, vs. RT- RT+ SSC; e, vs. HIIT- HIIT+ SSC; f, vs. HIIT- RT+ SSC; g, vs. AT- HIIT+ SSC; I, vs. AT+ SSC- HIIT+ SSC; j, vs. RT+ SSC- HIIT+ SSC
$^{*}$ P<0.05 was considered the significance level.$^{**}$ significance levels for paired sample t-test
Abbreviations: AT =Aerobic Training; RT =Resistance Training; HIIT= High-Intensity Interval Training; AT+ SSC= Aerobic Training + Spirulina supplement consumption; RT+ SSC= Resistance Training + Spirulina supplement consumption; HIIT+ SSC= High-Intensity Interval Training + Spirulina supplement consumption; C= Control SD= Standard Deviation;

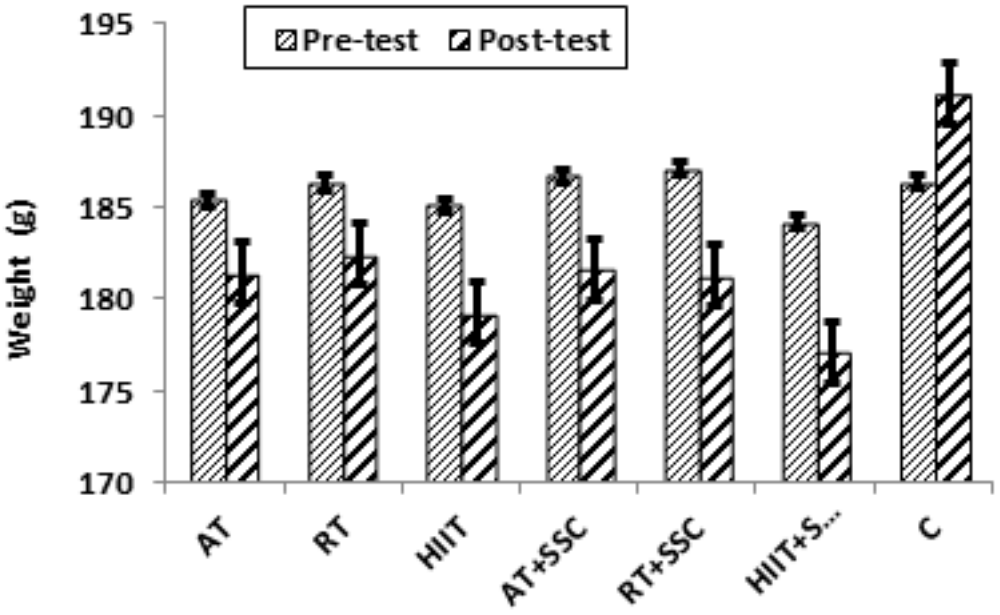


Fig. 2. Changes of weight in diabetic rats (AT =Aerobic Training; RT =Resistance Training; HIIT= High-Intensity Interval Training; AT+ SSC= Aerobic Training + Spirulina supplement consumption; RT+ SSC= Resistance Training + Spirulina supplement consumption; HIIT+ SSC= High-Intensity Interval Training + Spirulina supplement consumption; C= Control)

aerobic training (181.4±4.1g) and interval training with supplementation (177.1±4.3 g)

resistance training (182.4±3.8 g) and interval training with supplementation (177.1±4.3 g)

aerobic training with supplementation (181.6±4.00 g) and interval training with supplementation (177.1±4.3 g)

resistance training with supplementation (181.3±4.3 g) and interval training with supplementation (177.1±4.3 g)

*Changes in UCP-1 and TRPV1 protein concentrations.* As seen in Table 6, the intergroup comparisons indicated a significant increases in the mean UCP1 concentration after 8 weeks of aerobic training with/without supplementation, resistance training with/without supplementation, and interval training with/without supplementation, compared to the control group.

As seen in Table 6, the results of the post hoc Tukey test showed that there were significant differences between the mean UCP1 concentrations of the following groups (Figure 3).

Table 6. The comparison of between groups in UCP-1, TRPV1 and HOMA-IR

| Variables | Groups | | | ANOVA F | P Value |
|---|---|---|---|---|---|
| | Groups | Mean ± S.D | Shapiro-Wilk Test | | |
| UCP-1 (ng/ml) | AT | 5.63±0.18$^{a*g*}$ | 0.520 | 3.12 | 0.001* |
| | RT | 5.68±0.42$^{a*b*}$ | 0.621 | | |
| | HIIT | 5.72±0.15$^{a*c*}$ | 0.838 | | |
| | AT+ SSC | 5.75±0.32$^{a*i*}$ | 0.486 | | |

Table 6. The comparison of between groups in UCP-1, TRPV1 and HOMA-IR (continued)

| Variables | Groups | | | ANOVA F | P Value |
|---|---|---|---|---|---|
| | Groups | Mean ± S.D | Shapiro-Wilk Test | | |
| UCP-1 (ng/ml) | RT+ SSC | 5.78±0.22$^{a*}$ | 0.843 | 3.12 | 0.001* |
| | HIIT+ SSC | 5.83±0.65$^{a*}$ | 0.919 | | |
| | C | 4.10±0.21 | 0.952 | | |
| TRPV1 (ng/l) | AT | 2.45±0.32$^{a*}$ | 0.253 | 8.24 | 0.001* |
| | RT | 2.41±0.74$^{a*d*}$ | 0.698 | | |
| | HIIT | 2.43±0.9$^{a*}$ | 0.362 | | |
| | AT+ SSC | 2.44±0.12$^{a*}$ | 0.328 | | |
| | RT+ SSC | 2.47±0.17$^{a*}$ | 0.544 | | |
| | HIIT+ SSC | 2.45±0.24$^{a*}$ | 0.966 | | |
| | C | 2.22±0.42 | 0.865 | | |
| FBS (mg/dl) | AT | 318.5±16.43 $^{a*h*g*}$ | 0.451 | 4.37 | 0.001* |
| | RT | 318.1±18.3$^{a*d*b*}$ | 0.471 | | |
| | HIIT | 314.5±15.8$^{a*e*c*}$ | 0.567 | | |
| | AT+ SSC | 302.6±6.4$^{a*}$ | 0.513 | | |
| | RT+ SSC | 303.9±17.1$^{a*}$ | 0.765 | | |
| | HIIT+ SSC | 305.7±11.7$^{a*}$ | 0.928 | | |
| | C | 325.2±12.4 | 0.569 | | |
| Insulin (µU/ml) | AT | 7.6±1.2 $^{a*h*g*}$ | 0.327 | 9.37 | 0.001* |
| | RT | 7.5±1.4$^{a*d*b*}$ | 0.521 | | |
| | HIIT | 7.7±2.2$^{a*e*c*}$ | 0.972 | | |
| | AT+ SSC | 6.9±2.5$^{a*}$ | 0.361 | | |
| | RT+ SSC | 6.8±2.7$^{a*}$ | 0.307 | | |
| | HIIT+ SSC | 6.4±3.2$^{a*}$ | 0.959 | | |
| | C | 8.3±3.1 | 0.481 | | |
| HOMA-IR | AT | 0.97±0.16$^{a*h*}$ | 0.689 | 5.71 | 0.001* |
| | RT | 0.96±0.14$^{a*d*}$ | 0.741 | | |
| | HIIT | 0.98±0.11$^{a*c*}$ | 0.505 | | |
| | AT+ SSC | 0.92±0.11$^{a*}$ | 0.637 | | |
| | RT+ SSC | 0.91±0.17$^{a*}$ | 0.467 | | |

Table 6. The comparison of between groups in UCP-1, TRPV1 and HOMA-IR (continued)

| Variables | Groups | | | ANOVA F | P Value |
|---|---|---|---|---|---|
| | Groups | Mean ± S.D | Shapiro-Wilk Test | | |
| HOMA-IR | HIIT+ SSC | 0.94±0.12[a*] | 0.943 | 5.71 | 0.001* |
| | C | 1.2±0.18 | 0.949 | | |

Values are expressed as Mean±SD (n = 6 per group).
a, vs. Control; b, vs. RT- HIIT+ SSC; c, vs. HIIT- RT+ SSC; d, vs. RT- RT+ SSC; e, vs. HIIT- HIIT+ SSC;
f, vs. HIIT- RT+ SSC; g, vs. AT- HIIT+ SSC; h, vs. AT- RT+ SSC
I, vs. AT+ SSC- HIIT+ SSC; j, vs. RT+ SSC- HIIT+ SSC
*P<0.05 was considered the significance level.
Abbreviations: AT =Aerobic Training; RT =Resistance Training; HIIT= High-Intensity Interval Training; AT+ SSC= Aerobic Training + Spirulina supplement consumption; RT+ SSC= Resistance Training + Spirulina supplement consumption; HIIT+ SSC= High-Intensity Interval Training + Spirulina supplement consumption; C= Control SD= Standard Deviation; UCP-1 = uncoupling protein 1; TRPV1 = Transient receptor potential vanilloid 1; FBS= Fasting Blood Sugar; HOMA-IR = Homeostatic Model Assessment-Insulin Resistance.

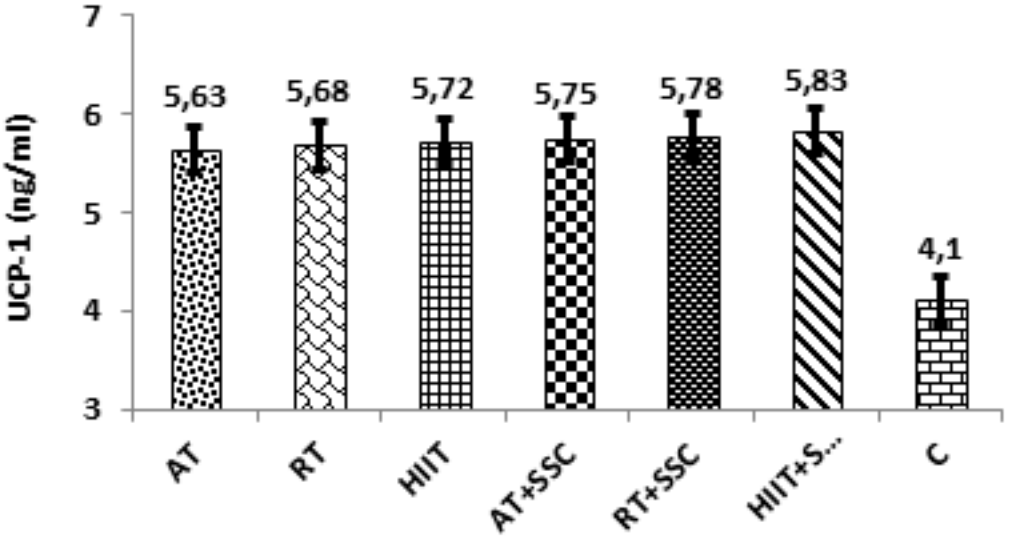


Fig. 3. Changes of UCP-1 in diabetic rats (UCP-1 = uncoupling protein 1; AT =Aerobic Training; RT =Resistance Training; HIIT= High-Intensity Interval Training; AT+ SSC= Aerobic Training + Spirulina supplement consumption; RT+ SSC= Resistance Training + Spirulina supplement consumption; HIIT+ SSC= High-Intensity Interval Training + Spirulina supplement consumption; C= Control)

aerobic training (5.63±0.18 ng/ml) and interval training with supplementation (5.83±0.65 ng/ml)

resistance training (5.68±0.42 ng/ml) and interval training with supplementation (5.83±0.65 ng/ml)

interval training (5.72±0.15 ng/ml) and resistance training with supplementation (5.78±0.22 ng/ml)

aerobic training with supplementation (5.75±0.32 ng/ml) and interval training with supplementation (5.83±0.65 ng/ml)

As shown by Table 6, the intergroup comparisons indicated a significant increase in the mean TRPV1 concentrations after 8 weeks of aerobic training with/without supplementation, resistance training with/without supplementation, and interval training with/without supplementation, compared to the control group.

As seen in Table 6, the results of the post hoc Tukey test showed that there were significant differences between the mean TRPV1 concentrations of the following groups:

resistance training (2.45±0.32 ng/l) and resistance training with supplementation (2.47±0.17 ng/l) (Figure 4)

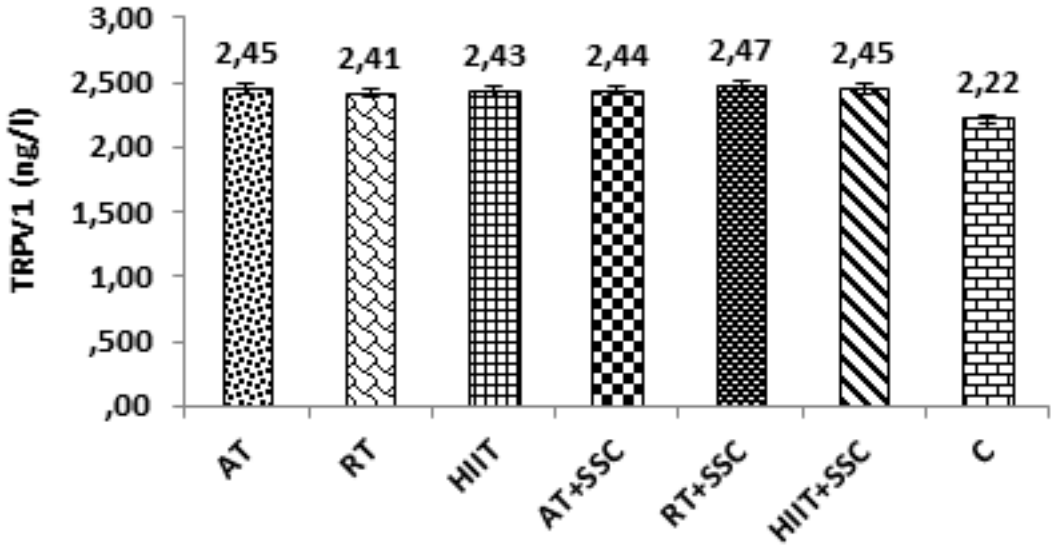


Fig. 4. Changes of TRPV1 in diabetic rats (TRPV1 = Transient receptor potential vanilloid 1; AT =Aerobic Training; RT =Resistance Training; HIIT= High-Intensity Interval Training; AT+ SSC= Aerobic Training + Spirulina supplement consumption; RT+ SSC= Resistance Training + Spirulina supplement consumption; HIIT+ SSC= High-Intensity Interval Training + Spirulina supplement consumption; C= Control)

*Serum changes in glucose, insulin, and insulin resistance index.* As seen in Table 6, the intergroup comparisons indicated a significant decrease in the mean glucose, insulin, and insulin-resistance index after 8 weeks of aerobic training with/without supplementation, resistance training with/without supplementation, and interval training with/without supplementation, compared to the control group.

As seen in Table 6, the results of the post hoc Tukey test showed that there were significant differences between the mean glucose and insulin concentrations of the following groups:

aerobic training (glucose: 318.5±16.43 mg/dl and insulin: 7.6±1.2 μU/ml) and resistance training with supplementation (glucose: 303.9±17.1 mg/dl and insulin: 6.8±2.7 μU/ml)

aerobic training (glucose: 318.5±16.43 mg/dl and insulin: 7.6±1.2 μU/ml) and interval training with supplementation (glucose: 305.7±11.7 mg/dl and insulin: 6.4±3.2 μU/ml)

resistance training (glucose: 318.1±18.3 mg/dl and insulin: 7.5±1.4 μU/ml) and interval training with supplementation (glucose: 305.7±11.7 mg/dl and insulin: 6.4±3.2 μU/ml)

resistance training (glucose: 318.1±18.3 mg/dl and insulin: 7.5±1.4 μU/ml) and resistance training with supplementation (glucose: 303.9±17.1 mg/dl and insulin: 6.8±2.7 μU/ml (

interval training (glucose: 314.5±15.8 mg/dl and insulin: 7.7±2.2 μU/ml) and interval training with supplementation (glucose: 305.7±11.7 mg/dl and insulin: 6.4±3.2 μU/ml)

interval training (glucose: 314.5±15.8 mg/dl and insulin: 7.7±2.2 μU/ml) and resistance training with supplementation (glucose: 303.9±17.1 mg/dl and insulin: 6.8±2.7 μU/ml)

As seen in Table 6, the results of the post hoc Tukey test showed that there were significant differences between the insulin-resistance means of the following groups (Figure 5):

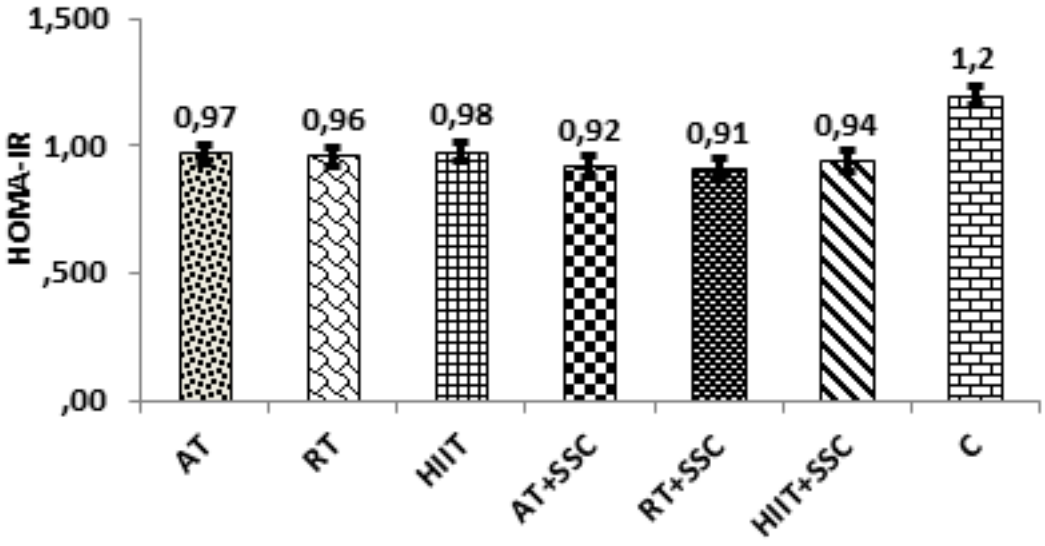


Fig. 5. Changes of HOMA-IR in diabetic rats (HOMA-IR = Homeostatic Model Assessment-Insulin Resistance; AT =Aerobic Training; RT =Resistance Training; HIIT= High-Intensity Interval Training; AT+ SSC= Aerobic Training + Spirulina supplement consumption; RT+ SSC= Resistance Training + Spirulina supplement consumption; HIIT+ SSC= High-Intensity Interval Training + Spirulina supplement consumption; C= Control)

aerobic training (0.97±0.16) and resistance training with supplementation (0.91±0.17)

resistance training (0.96±0.14) and resistance training with supplementation (0.91±0.17)

interval training (0.98±0.11) and resistance training with supplementation (0.91±0.17)

## Discussion

The findings of the present study show that, compared to the control group, significant decreases in the mean glucose, insulin, and insulin-resistance indexes were observed after 8 weeks of aerobic training with/without supplementation, resistance training with/without supplementation, and interval training with/without supplementation. Some studies show that, similar to aerobic training, resistance training can enhance insulin resistance (POEHLMAN et al., 2000). Some researchers attribute the improvement in the mechanism of action of insulin to the positive regulation of insulin receptor components (such as insulin receptor protein concentration, protein kinase B, and glycogen synthase) and glucose transporter type 4 (GLUT-4) (POEHLMAN et al., 2000). Among the

mechanisms leading to the enhancement of insulin functioning after aerobic training are the signaling enhancement of insulin receptors (HOLTEN et al., 2004), expression enhancement of the GLUT-4 protein, activation enhancement of glycogen synthase and hexokinase (POEHLMAN et al., 2000), reduction of release and rise of free fatty acid clearance, increase in blood-muscle glucose release due to the quantity of muscular capillaries, and a change in muscle composition towards increasing glucose uptake (ERIKSSON et al., 1997). Thus, aerobic training is one of the methods for reducing insulin resistance and diabetes type II infection risk, especially in obese people (DAS, 2010). The mechanisms responsible for reducing insulin resistance due to resistance training are similar to aerobic training (SIGAL et al., 2007). Therefore, we can claim that all three training types, including aerobic, resistance, and interval, effectively improve insulin resistance in diabetic people and can be employed for diabetes prevention. Another reason for such contradictory results can probably be the differences in the duration, intensity, and level of training. The number of training sessions per week, not training intensity, is associated with the improvement in insulin sensitivity. Both training intensity and duration are effective to the extent that an improvement in insulin sensitivity occurs when the training volume is maximal (KODAMA et al., 2006).The sugar in Spirulina is absorbed by minimum insulin interference and minimum pressure on the pancreas. Furthermore, Spirulina lessens the human tendency for food, which lowers the need for insulin (BANJI et al., 2013). In diabetes-suffering patients, the body's vitamin rate is minimal, increasing nerve damage. Some supplements such as Spirulina that contain vitamins, ameliorate glucose resistance. The Magnesium and Chromium in Spirulina improve glucose consumption in diabetic patients. The Gamm-Linolenic present in Spirulina is useful for these individuals since it not only reduces cardiac problems in these vulnerable people, but also helps with body metabolism (KHAN et al., 2005). Seemingly, due to its antioxidant properties, the Spirulina supplement is crucial in diabetes treatment since its role in decreasing blood sugar and oxidative stress has been proved. Concerning nutritional specifications, its concentration as a full supplement is preferred to unnatural nutrients (VITALONE et al., 2011). By increasing specific enzymes (Hexokinase) in the liver, Spirulina enhances blood glucose uptake by the liver. Likewise, the activation of this enzyme generally boosts carbohydrate metabolism, and breaks down the endogenous synthesis of lipids. Eventually, Spirulina consumption can reduce blood sugar (PANDEY et al., 2011). Therefore, one of the reasons for the amelioration of the variables in the present study can be attributed to the effect of the Spirulina supplement on reducing the expression of transcription factors impacting the enhancement of white adipose tissue accumulation in the supplement-consuming groups compared to the non-consuming ones.

The findings of the present study relating to male diabetes-induced rats showed that 8 weeks of aerobic, resistance, and interval training, with and without supplementation, significantly increased UCP-1 and TRPV1 concentrations compared to the control group. However, the significant increase in the UCP-1 levels in the group provided with interval training with supplementation was larger than those of the other groups. Furthermore, the resistance training with supplementation group had higher levels of TRPV1 than the other independent training groups. This result is consonant with the results of AFSHARI et al. (2017), however, it is inconsonant with the results of KHALAFI et al. (2016), HUH et al. (2014) and PEKKALA et al., (2013). By comparing the effects of moderate and high volume aerobic training on the gene expression of UCP-1 in subcutaneous white adipose tissue in 24 Wistar rats, AFSHARI et al. (2017) concluded that the gene expression of UCP-1 in moderate-volume aerobic training was statistically higher than in the control group; however, the gene expression of UCP-1 in the high-volume aerobic training group was not statistically higher compared to the control group. In contrast, KHALAFI et al. (2016) compared the effects of two sports activities, including high-intensity interval training, with an intensity of 90-95%VO2max in 12, 1-minute intervals, with 1-minute rest periods, on a treadmill, and moderate-

intensity continuous training with an intensity of 35-60%VO2max for 40 minutes, on irisin serum levels and UCP-1 of subcutaneous fats in male rats. The author concluded that the irisin and UCP-1 levels of subcutaneous fat were significantly higher in the interval group as compared with the control group. Nonetheless, there were no significant differences between the moderate-intense continuous training group and the control group. Mediated by physical activities, the norepinephrine hormone enhances the expressions of UCP-1 and PGC-1a in adipose tissue by stimulating the pathways linked to the cAMP and Beta-adrenergic receptors (NEDERGAARD and CANNON, 2014). The rise in norepinephrine could probably be one of the effective factors in the UCP-1 upsurge of subcutaneous adipose. On the other hand, the irisin hormone is the main stimulator increasing the UCP-1 expression and the thermogenesis capacity of adipose tissue. Hence, due to physical activities, the irisin levels, causing the expression of mRNA UCP-1 in the white adipose cells, turn white adipose tissue brown by activating PPAR-a and increase energy expenditure through thermogenesis (BOSTRÖM et al., 2012). Another effective factor in UCP-1 expression is the expression of PGC-1a (RINGHOLM et al., 2013). The activation of brown adipose tissue and, consequently, the activation of UCP-1, are controlled by the Hypothalamus. This is accomplished through the release of norepinephrine by the sympathetic neural system, which stimulates the function of brown adipose tissue (JIMÉNEZ et al., 2016). This process triggers the hydrolysis of glyceride stored in small fat droplets, as well as the activation of the free fatty acids of UCP-1 (FULMER, 2010). SEO et al. (2018) showed that the consumption of Spirulina in rats using a high-fat diet could stimulate the expressions of PRDM16 and UCP-1, besides activating the AMPK-PGC1 pathway. Thus, it seems that through incrementally regulating the expression of thermogenesis-involved proteins, such as PRDM16, PGC-1a, UCP-1, and TRPV1, the use of Spirulina can lead to the differentiation of brown adipose from white adipose tissue. Hence, the Spirulina supplement reduces fat accumulation by decreasing the expression of the main proteins involved in the adipogenic process, such as PPARγ, C/EBPα, and aP2, and the proteins involved in the lipogenic process, such as PAATβ, FAS, ACC, SREBP1, and lipin1. Likewise, the components of the Spirulina supplement index, such as chlorophyll and phycocyanin C, can inhibit adipogenesis and lipogenesis. However, studies reveal that the consumption of the Spirulina supplement can reduce mass, body fat mass, triglyceride content, and cholesterol levels in rats using high-fat diets (SEO et al., 2018). Spirulina increases the expressions of thermogenic factors, such as PRDM16, PGC-1α, UCP-1, and TRPV1, due to AMPK activation (SEO et al., 2018). With respect to these subjects and compared to the control group, the role of the Spirulina supplement in stimulating the proteins involved in the thermogenic process, reducing mass, and improving insulin resistance, is the reason for the further enhancement of the UCP-1 and TPRV1 levels, and more improvement in the insulin-resistance index in the three aerobic, resistance, and interval training groups with Spirulina supplementation. One of the limitations of this study was the impossibility of examining gene expression. Moreover, the UCP-1 and TPRV1 values did not develop in the adipose tissues of the rats' different body parts which would have enabled the researcher to compare these values in different adipose tissues. Likewise, lateral factors impacting the cascade signaling of the white-to-brown conversion of adipose tissue were not measured. It seems that enlarging the sample size and lengthening the duration of physical activities would allow us to witness the significant variations of this key gene in diabetes treatment. However, further studies are required to uncover the molecular pathways and mechanisms involved.

## Conclusion

The results of the present study on diabetes-induced rats showed that eight weeks of aerobic, resistance, and interval training with Spirulina supplementation reduced mass, glucose, insulin, and insulin resistance and gave rise to significant increases in UCP-1 and TPRV1 concentrations. The use of these supplement-combined training interventions is recommended due to the heightened

levels of the UCP-1 and TPRV1 concentrations, which mainly contribute to reducing diabetes sequels, such as obesity and hyperinsulinemia, in the target tissue.

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**Disclosure statement**
No potential conflict of interest was reported by the author(s).

**Authors' Contribution**
All authors read and approved the final version of the manuscript. Study concept and design: MF, AR, S KH and KH (100%), acquisition of data: KH and MF (50-50%); analysis and interpretation of data: KH (100%) and drafting of the manuscript: KH (100%), critical revision of the manuscript for important intellectual content: MF and KH (50-50%), administrative, technical, and material support: KH (100%); study supervision: MF (100%).

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**FATHI, M., A. RASHIDLAMIR, S. KHAIRABADI, K. HEJAZI: Učinak intervalnog treninga, aerobnog treninga i treninga otpornosti te konzumiranog dodatka spiruline na razine UCP-1, TRPV1 i HOMA-IR u bijelom masnom tkivu štakora sa šećernom bolesti. Vet.arhiv 93, 97-116, 2023.**

**SAŽETAK**

Svrha istraživanja bila je utvrditi učinak treninga s intervalom visokog intenziteta, anaerobnog treninga, treninga otpornosti i konzumiranja dodatka spiruline na razine nevezanog proteina-1 (UCP1), vaniloidnog potencijalnog prijelaznog receptora (TRPV1) te na procjenu homeostatskog modela za rezistenciju na inzulin (HOMA-IR) u bijelom masnom tkivu štakora sa šećernom bolesti. Ukupno 42 muška štakora sa šećernom bolesti tipa II nasumično su raspoređena u sedam skupina kako slijedi: aerobni trening (n = 6), trening otpornosti (n = 6), intervalni trening (n = 6), aerobni trening kombiniran s dodatkom spiruline (n = 6), trening otpornosti kombiniran s dodatkom spiruline (n = 6), intervalni trening kombiniran s dodatkom spirulina (n = 6) i kontrolna skupina (n = 6). Trening (8 tjedana/5 dana) je proveden na pomičnoj traci i ljestvama za glodavce. Za analizu podataka korišteni su t-test za parne uzorke i jednosmjerna ANOVA. Tri vrste treninga, sa i bez dodataka spiruline, znakovito su smanjile masu, glukozu, inzulin i rezistenciju na inzulin. U tri skupine s kombiniranim treningom i dodatkom spiruline razlika u indeksu HOMA-IR bila je znakovita, međutim pad je bio veći u skupini treninga otpornosti s dodatkom spiruline. Koncentracija proteina UCP-1 i TRPV1 znakovito se povećala u svim skupinama s treningom, sa i bez dodataka spiruline. Pri tome, povećanje razina UCP-1 u skupini koja je imala trening s intervalnom visokog intenziteta i dodatak spiruline bilo je znakovito više nego u drugim skupinama. Nadalje, razine proteina u TRPV1 bile su više u skupini s treningom otpornosti i dodatkom spiruline. Osam tjedana treninga, sa i bez dodatka spiruline, smanjilo je inzulinsku rezistenciju i dovelo do znakovitih promjena u koncentracijama UCP-1 i TPRV1.

**Ključne riječi:** dijabetes; aerobni trening; trening otpornosti; intervalni trening; dodatak spiruline