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Interactive Effects of Endurance Training and Crocin on Aerobic Capacity, Dietary Intake and Weight of High-Fat Diet- Induced Type 2 Diabetic Rats

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ABSTRACT

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Keywords:
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Aerobic capacity;
Diet;
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Background: Diabetes and obesity are closely related with each other. The purpose of this study was to investigate the interactive effects of endurance training and crocin on aerobic capacity, weight and diet in high fat diet and streptozotocin induced diabetic rats.

Methods: In this experimental study, 49 adult diabetic rats (induced by high-fat diet and venous injection of streptozotocin) were randomly assigned to (1) high intensity interval training (HIIT), (2) low intensity continuous training(LICT), (3) HIIT and crocin consumption, (4) LICT and crocin consumption, (5) crocin consumption, (6) sham and (7) control. HIIT and LICT groups exercised on a rodent treadmill for eight weeks, three sessions per week, with an intensity of 80- 85, and 50- 55 percent of maximum speed, respectively, and crocin consumption groups received peritoneally 25 mg/kg of crocin each day for eight weeks. The data were analyzed by independent sample t-test, dependent sample t-test, two-way analysis of variance and Bonferroni's post hoc tests ($p \leq 0.05$).

Results: Eight weeks of endurance training had significant effect on weight loss, dietary intake and increased aerobic capacity ($p \leq 0.05$). Crocin consumption and endurance training have interactive effects on weight loss ($p \leq 0.05$) However, this interaction is not significant in increasing aerobic capacity and dietary intake. ($p \geq 0.05$).

Conclusion: It seems that endurance training and crocin consumption have interactive effects on weight loss in high fat diet and streptozotocin induced diabetic rats.

Introduction

Diabetes and obesity have a very close relationship. Type 2 diabetes often occurs with obesity. Obesity is therefore a major cause of type 2 diabetes [1]. Abnormal weight gain is one of the most important risk factors for type 2 diabetes, which leads to increased systemic inflammation and insulin resistance [2]. This prevalent and non-contagious disease is increasing worldwide, with respect to the lifestyle and dietary habits of the people [3]. Studies show that the incidence of cardiovascular disease in type 2 diabetic patients is four times higher than other people in the community. Hence, research evidence suggests a close relationship between fat percentage, body mass index (BMI), and insulin concentration. With increasing fat weight, the amount of leptin secretion increases, but the resistance to leptin increases gradually, and thus the weight of the fat is not reduced [4]. Many scholars around the world are working to reduce the incidence of diabetes by using various methods. Therefore, performing sports activities is one of the most important basic strategies for controlling and treating blood glucose and hyperlipidemia, and decreases the incidence of diabetes and cardiovascular complications. Also, having regular exercise programs in diabetics will eliminate strong insulin-responsive reaction, as muscles and liver get accustomed to saving more glycogen. Proper exercise improves muscle mass and decreases fat mass and insulin resistance [5]. It has also been shown that the energy received regulates the leptin gene expression positively or negatively, thus changing energy costs,

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respiratory rate, and the oxygen consumed through exercise may also affect the individuals' body weight [4,6]. Researchers believe that effective exercise (intensity, duration, type and number of sessions per week) for diabetic patients can be an important factor in reducing complications of diabetes;nevertheless, the proposed energy consumed for three training sessions per week was 400 kilocalories per session and 4 or 5 training sessions per week was 500 kilocalories per session. Still, the guidelines for exercise therapy effect need to be reviewed and more information is needed to maximize the benefits of exercise for different types of diabetic type 2 patients [7]. A lot of studies have been done about the mechanism of the effect of various sports activities such as aerobic training [4], swimming training [3], moderate intensity endurance training [5], resistance training [8] and moderate intensity interval training [9] on the general health (improved the lipid profile, decreased insulin resistance and fasting glucose) of diabetic patients which report different outcomes. On the other hand, the use of medicinal herbs due to less complications than synthetic drugs, in addition to sports activities, has attracted the attention of most sports science researchers [10]. One of the most effective medicinal herbs used to control blood glucose, lipids and risk factors for cardiovascular disease in diabetic patients is saffron [8, 10-12]. Crocin is the main compound known in saffron, and the color of saffron is due to this compound. Regarding the contradiction in the effect of intensity, duration and type of exercise as well as energy costs on weight loss and general health of diabetics, as well as the potential effects of saffron on the improvement of diabetes-related risks, finding the best practice and simultaneous consumption of medicinal herbs the importance of doing the present study is doubled. In this regard, the aim of this study was to investigate the effect of low intensity continuous training (LICT) and high intensity interval training(HIIT) with crocin consumption on aerobic capacity, weight and dietary intake in high fat diet and streptozotocin induced diabetic rats.

Subjects and methods

In this experimental and fundamental research, 49 eight-week-oldmale Sprague-Dawley rats, weighing 150±30 grams, were purchased from the Animals Breeding Center at Islamic Azad University, Marvdasht Branch. Rats were then transferred to the animal physiology lab, under standard conditions at an ambient temperature of 22 to 27° C, relative humidity of 50%, and controlled light (12-hour cycle of light and darkness), followed by a seven-day adaptation period. Animals were exposed to ad libitum access to water and food during the period. To induce type 2 diabetes, a combination of high-fat diet and STZ (made by Sigma company) injection was used in the present study. For this purpose, all rats over a period of eight weekswere exposed to a high fat diet of 45% total energy (derived from animal fat) containing 24 grams of fat, 24 grams of protein and 41 grams of carbohydrate per 100 grams [13]. After eight weeks, diabetes was intraperitoneally induced with a single dose of 30 mg/kg STZ (Sigma-Aldrich, Germany) dissolved in sodium citrate buffer with PH=5.4 [13]. For confirmation of diabetes, 96 hours after injection, blood samples gathered from tail of rats by punching methods and glucose measured by glucometer device; then rats with glucose levels higher than 300 mg/dL were selected as the sample [13]. All rats base on blood glucose were divided into seven groups of 7 rats, including: (1) HIIT, (2) LICT, (3) HIIT and crocin consumption, (4) LICT and crocin consumption, (5) crocin consumption, (6) sham and (7) control.

To evaluate the maximum running speed, the graded sport performance test with a zero gradient was performed. To perform this test, the rats started at a speed of 10 m/min, and then the speed of the treadmill was increased to 1 m/min for each one minute. This process continued until the rats were not able to run anymore (exhaustion) [13].

After estimating the speed, groups 1 and 3 did training for 8 weeks, three sessions per week with intensity of 80 to 85% of maximum speed for 2 minutes and one-minute active resting periods. HIIT from six bouts in the first week of training reached 12 bouts in the last week. It means in first and second weeks 6 bouts, in third week 7 bouts, in fourth week 8 bouts, in fifth week 9 bouts, in sixth week 10 bouts, in seventh week 11 bouts and in eighth week 12 bouts.

Groups 2 and 4 also did training for eight weeks, three sessions a week, with intensity ranging from 50 to 55 percent of the maximum running speed. LICT began in the first week from 25 minutes and reached 50 minutes in the last week.

It should be noted that the total volume of exercise activity (intensity, duration and repetition) was matched between the two groups of low intensity continuous training and high intensity interval training [13]. Also, groups 3, 4 and 5 received daily 25 mg/kg of intraperitoneal crocin (dissolved in normal saline) [14]. In present study crocin purchased from Sigma company of Germany. It should be noted that considering that in most studies the effect of 25 mg/kg crocin confirmed; in present study researchers prescribed 25 mg/kg crocin. In order to control the effects of injection on the variables of the study, the sham group received crocin solvent intraperitoneally each day. Before the beginning of the training and 24 hours after the last training session at the end of the eighth week, the parameters of weight (using the Kern scale,made in Germany with a precision of 0.01 g), dietary intake [15] and aerobic capacity [13] of the rats were measured.Dependentsample t-test, independent sample t-test and two-way ANOVA with Bonferroni's post hoc tests were used for analyzing the findings of the research ($p \leq 0.05$).

Results

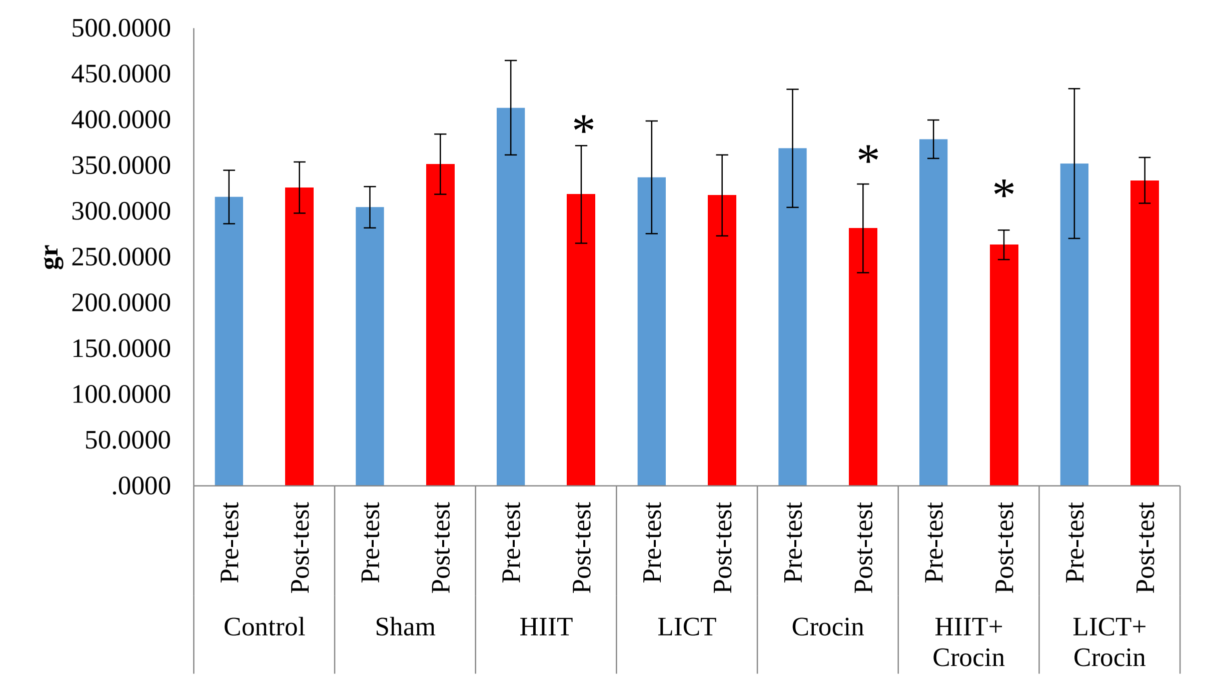
The mean and standard deviations of the variables of the research are presented in (Table 1). The results of independent t-test showed that weight levels in the crocin consumption group were significantly decreased compared to the sham group (t= 3.10, p= 0.001).However, there was no significant difference between the sham and crocin groups in the levels of aerobic capacity (t= 1.96, p= 0.07) and dietary intake (t= -0.04, p= 0.96).The results of dependent t-test showed that in the crocin consumption group, the levels of weight in the post-test were significantly decreased compared to the pretest (t=-3.13 and p= 0.01). In the HIIT group, the levels of dietary intake (t= -6.24 and p= 0.001) and weight (t=-12.71 and p= 0.001) in the post-test, decreased significantly compared to the pre-test; also, aerobic capacity levels (t= 13.27 and p= 0.001) were significantly increased in the post-test compared to the pre-test. In the HIIT and crocin consumption group, the levels of weight (t=-29.82, p= 0.001) and dietary intake (t=-7.41 and p= 0.001) in the post-test were significantly decreased compared to the pretest, and aerobic capacity (t= 12.47 and p= 0.001) were significantly increased. In the LICT group, the levels of dietary intake (t= -6.49 and p= 0.001) were significantly reduced in the post-test compared to the pre-test. In the LICTwith crocin consumption of, the levels of dietary intake (t=-5.43 and p= 0.001) decreased significantly in the post-test compared to the pre-test; also in the LICT with crocin consumption, aerobic capacity levels (t= 15.42 and p= 0.001) increased in the post-test compared to the pre-test (Figure 1-3).

The results of two-way ANOVA showed that crocin consumption (F= 7.00, p= 0.01, and effect size=0.14) and training (F= 12.73, p= 0.001, and effect size=0.37) alone have a significant effect on weight loss in high fat diet and streptozotocin induced diabetic rats. Also, training along with crocin consumption has interactive effect on weight loss in high fat diet and streptozotocin induced diabetic rats (F= 4.11, p= 0.02, and effect size=0.16). Moreover, the results of Bonferroni’s post-hoc test indicated that HIIThad a significant effect on weight loss in high fat diet and streptozotocin induced diabetic rats (M= 66.37 and p= 0.002). However, LICT did not significantly affect the weight of high fat diet and streptozotocin induced diabetic rats (M= -19.53 and p= 0.85) (Figure 4).

The results of two-way ANOVA showed that eight weeks ofcrocin consumption had no significant effect on increasing the aerobic capacityin high fat diet and streptozotocin induced diabetic rats (F= 1.03, p= 0.31 and effect size= 0.02). However, training had a significant effect on the increase inaerobic capacity of rats (F= 20.85,p= 0.001, and the effect size=0.49).On the other hand, the interactive effect of training and crocin consumption was not significant on the increase ofaerobic capacityin high fat diet and streptozotocin induced diabetic rats(F= 3.04, p= 0.06, and effect size=0.12).The results of Bonferroni’s post-hoc test showed that HIIT (M= -10.62 and p= 0.001) and LICT (M= -7.39 and p= 0.001) had a significant effect on the increase in aerobic capacity of high fat diet and streptozotocin induced diabetic rats.However, HIIT and LICThave the same effects on increasing the aerobic capacity of high fat diet and streptozotocin induced diabetic rats (M= -3.20, p= 0.18) (Figure 5).

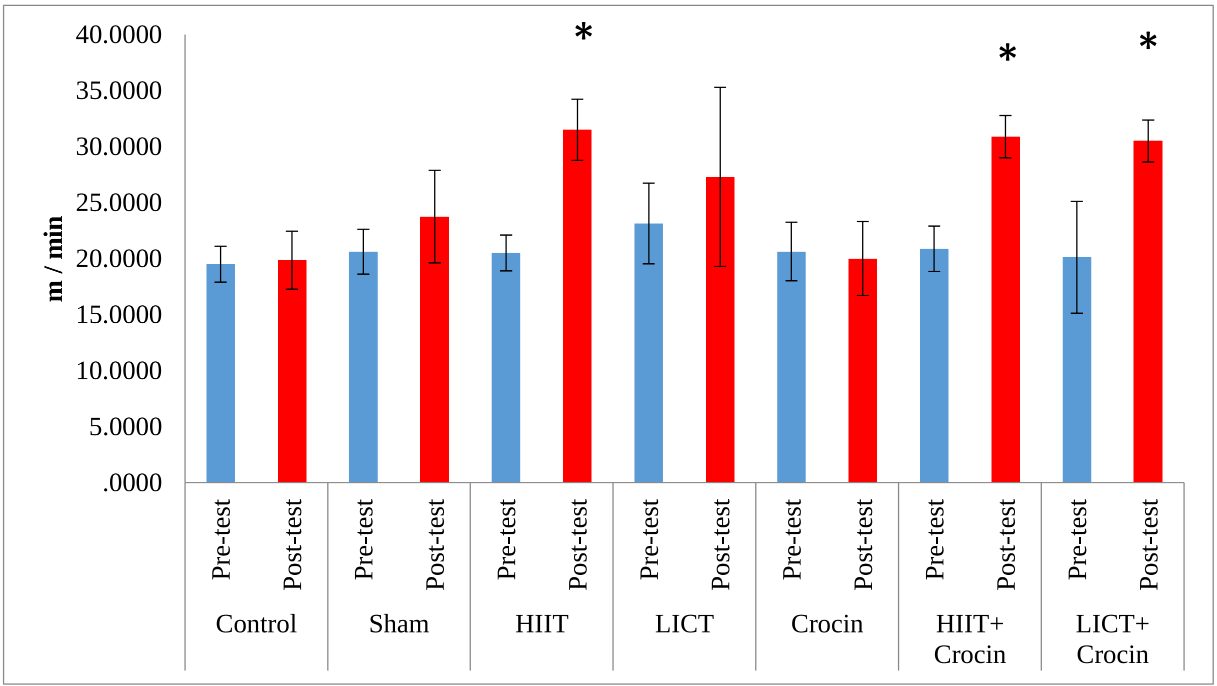
The results of two-way ANOVA showed that eight weeks of crocin consumption had no significant effect on the dietary intake of high fat diet and streptozotocin induced diabetic rats (F= 0.46, p= 0.49, and effect size= 0.01). However, training had a significant effect on decreasing the amount of dietary intake of high fat diet and streptozotocin induced diabetic rats (F= 18.97, p=

0.001, and effect size=0.47). In addition, the interaction of crocin and training was not significant in reducing the amount of dietary intake in high fat diet and streptozotocin induced diabetic rats (F= 1.84, p= 0.17, and effect size= 0.08). The results of Bonferroni's post-hoc test indicated that HIIT (M= 6.99 and p= 0.009) and LIIT (M= 13.72 and p= 0.001) had a significant effect on reducing the amount of dietary intake in high fat diet and streptozotocin induced diabetic rats; however, HIIT had a greater effect on reducing the amount of dietary intake compared to LICT (M= -6.72 and p= 0.01) (Figure 6).



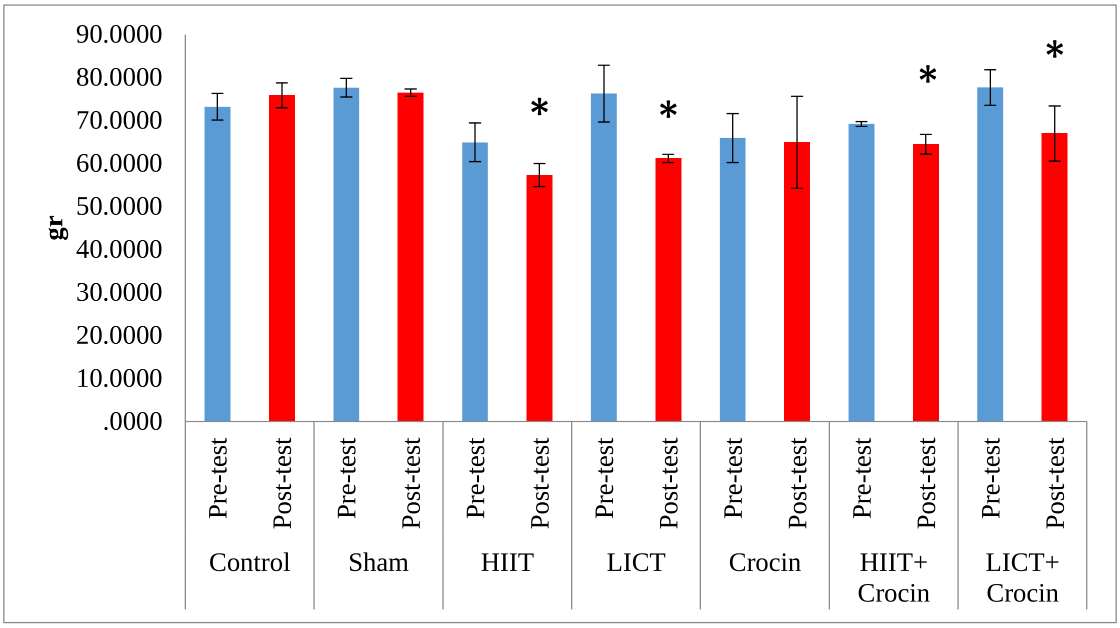
* Significant reduction rather than pre- test

Figure 1. Weights of rats in research groups



* Significant increase rather than pre- test

Figure 2. Aerobic capacity of rats in research groups



* Significant decrease rather than pre- test

Figure 3. Dietary intake of rats in research groups

Table 1. Mean and standard deviation of research variables in the pre-test and post-test

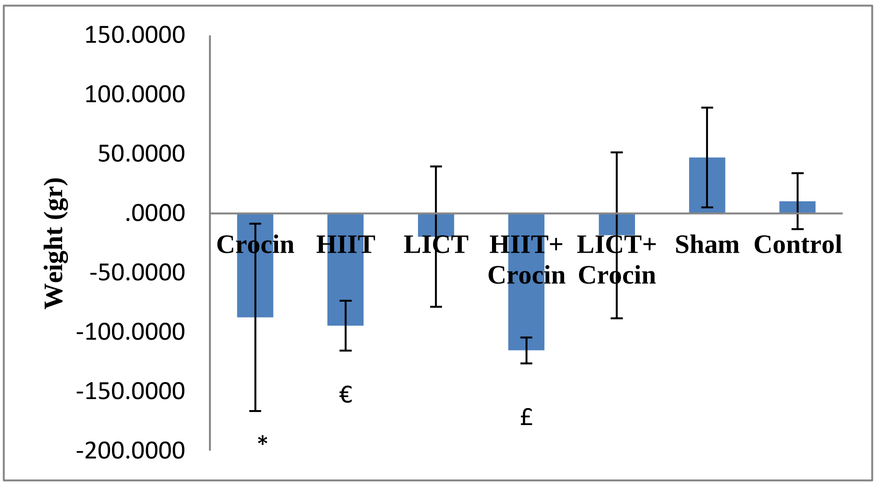
Variable	Weight (gr)		Aerobic capacity (m / min)		Dietary intake (gr)	
Group	Pre- test	Post- test	Pre- test	Post- test	Pre- test	Post- test
Crocin	368.87±64.58	281.37±48.45	20.62± 2.61	20.00±3.29	65.88±5.67	64.90±10.69
HIIT	413.12±51.55	318.50±53.33	20.50±1.60	31.50±2.72	64.89±4.50	57.27±2.69
LICT	337.00±61.54	317.37±44.12	23.12±3.60	27.29±7.99	76.26±6.59	61.15±1.00
HIIT+ Crocin	378.75±21.07	263.37±16.06	20.87±2.03	30.87±1.88	69.16±0.56	64.45±2.27
LICT+ Crocin	352.25±81.86	333.75±25.11	20.12±4.99	30.50±1.87	77.66±4.13	66.98±6.41
Sham	304.50±22.47	351.50±32.81	20.62±1.99	23.75±4.13	77.62±2.18	76.44±0.85
Control	315.62±29.07	325.87±27.87	19.50±1.60	19.87±2.58	73.17±3.06	75.81±2.92

Table 2. Results of two-way ANOVA test to examine the interactions of crocin and training on research variables

Variable	Source	Sum of Squares	df	F	Sig.	Partial Eta Squared
weight	Crocin	18369.18	1	7.00	0.01	0.14
	Training	64925.79	2	12.37	0.001	0.37
	Interaction of training and crocin	21578.37	2	4.11	0.02	0.16
Aerobic capacity	Crocin	23.60	1	1.03	0.31	0.02
	Training	949.62	2	20.85	0.001	0.49
	Interaction of training and crocin	138.67	2	3.04	0.058	0.12
Dietary intake	Crocin	18.47	1	0.46	0.49	0.01
	Training	1507.05	2	18.97	0.001	0.47
	Interaction of training and crocin	146.30	2	1.84	0.17	0.08

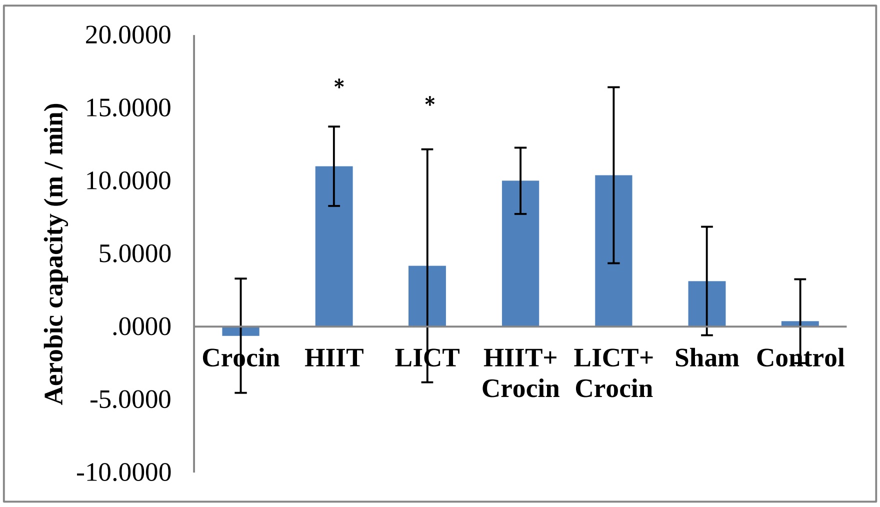
Table 3. Results of Bonferroni's post-hoc test to compare the effects of HIIT and LICT on weight, aerobic capacity and dietary intake inhigh fat diet and streptozotocin induced diabetic rats

	Factor	No exercise	LICT
Weight	HIIT	M=66.37, p=0.002	M=85.93, p=0.001
	LICT	M=-19.56, p=0.85	------
Aerobic capacity	HIIT	M=-10.62, p=0.001	M=-3.23, p=0.18
	LICT	M=-7.39, p=0.001	------
Dietary intake	HIIT	M=6.96, p=0.009	M=-6.72, p=0.01
	LICT	M=13.72, p=0.001	------



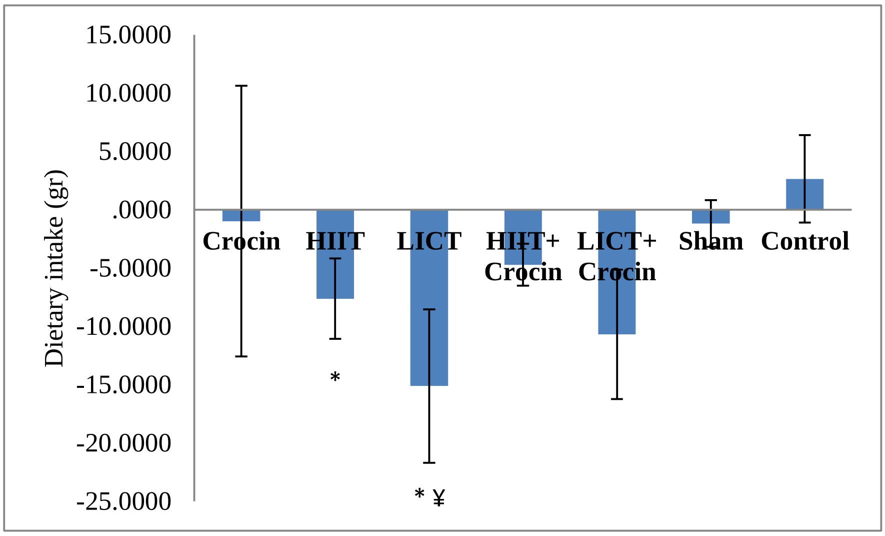
* Significant effect of crocin consumption on reduction of weight in diabetic rats
€ Significant effect of HIIT on reduction of weight in diabetic rats
£ Interactional effect of HIIT and crocin consumption on reduction of weight in diabetic rats

Figure 4. Changes of weight after HIIT and LICT training with crocin consumption



* Significant effect of HIIT and LICT on increase of aerobic capacity in diabetic rats

Figure 5. Changes of aerobic capacity after HIIT and LICT training with crocin consumption



* Significant effect of HIIT and LICT on reduction of dietary intake in diabetic rats
¥ Significant reduction of dietary intake in LICT group rather than HIIT group

Figure 6. Changes of dietary intake after HIIT and LICT training with crocin consumption

Discussion

The results of this study showed that eight weeks of crocin consumption had a significant effect on weight loss in high fat diet and streptozotocin induced diabetic rats. However, it has no significant effect on the increase of aerobic capacity and decrease the amount of dietary intake in high fat diet and streptozotocin induced diabetic rats. In insulin resistant patients, decreasing the sensitivity of fat cells to insulin hormone increases the amount of free fatty acid in the blood, which is a hallmark of type 2 diabetes and gradually increases insulin resistance and ineffectiveness of pancreatic beta cells [1]. Molecular and genetic studies in visceral fat and subcutaneous fat also indicate that 20 percent of genes associated with subcutaneous fat storage and 30 percent of genes associated with visceral fat storage have been identified. Researchers have argued that metabolic genes in obesity and metabolic disturbances may be affected by mutations and result in changes in calories storage and consumption in the body, and induce changes in the process of fat storage and decomposition, in the pattern of insulin release, in dietary intake and body weight, and in the amount of sugar required by the brain [1]. Consequently, in today's societies, some of the metabolic genes are affected by high calorie and sedentary and inactive life, which they either seriously change or are removed in this process, and ultimately it causes a change in metabolism and respiratory exchange [1]. Farshid et al (2016) showed that 10 and 20 mg/kg crocin consumption significantly reduced the weights of diabetic rats (nevertheless 5 mg/kg crocin consumption did not reduce the weight of diabetic rats) [16] which these findings are parallel with findings of present study. Also in parallel with present study Hazman et al (2016) reported that 150 mg/kg for 6 weeks significantly reduced the weights of obes and diabetic rats [17].

Research on saffron has shown that saffron consumption has beneficial effects on lipids and lipoproteins plasma levels.By examining the compounds in different parts of the saffron plant, it was found that the main components of this plant are crocin, crocetin, safranal and linoleic acid.

The mechanism of the effect of crocin on adipose tissue has not yet been completely determined, but one of the possible mechanisms of the effect of crocin is preventing the activity of the pancreatic lipase and reducing the absorption of fats, as well as increasing disposaland metabolism of fats, which reduce visceral fat [18-20]. Researchers have argued that crocin can be absorbed into serotonin by synergistic activities with neurotransmitter pathways, and possibly by affecting the appetite control centers, will restrict dietary intake. Studies have also shown that crocin can prevent excessive accumulation of fat at serum levels and some tissues [21]. Studies on the effects of crocin on weight loss and fat mass are limited, however, the researchers concluded that receiving crocin for 4 weeks at 25, 50 and 100 mg/kg doses resulted in a significant decrease in serum lipids and increase in lipolysis [20]; 40 and 80 mg/kg of saffron and crocin had a significant effect on the reduction of serum triglyceride levels in high-fat diet adult rats [22]. Six weeks, 25 mg/kg body weight consumption of saffron aqueous extracthas a significant effect on glycemic indices of male diabetic rats [8]; three weeks receiving 50 and 100 mg/kg crocin per day resulted in a significant decrease in triglyceride, glucose and cholesterol, and a significant increase in serum adiponectin levels in diabetic rats [23], on the other hand, eight weeks receiving 30 mg/kg saffron per day significantly improved lipid profiles, visceral fat and decreased dietary intake in patients with coronary artery stenosis [21]. This study was not consistent with the present study regarding the amount of dietary intake. The reasons for the inconsistency of this study with the present study can be explained by the difference in the statistical population and different dosage.

The results of the current study showed that eight weeks ofHIITand LICT have a significant effect on weight loss, dietary intake and increased aerobic capacity of high fat diet and streptozotocin induced diabetic rats. It is well known that the level energy intake increases linearly with increasing physical activity. Usually the amount of changes in the amount of dietary intake is precisely aligned with the amount of energy disturbances that results in maintaining body weight, especially fat content.

Studies have also shown that changes in body temperature and pressure caused by high intensity activity, an increase in lactic acid and catecholamine levels, a decrease in the secretion of acylated ghrelinin high intensity exercise, the subjective expectation of receiving post-exercise rewards and the individual's nature in the response to hunger compared to exercise can be considered as factors in changing the individual’s appetite compared to exercise [24].

Also, low aerobic capacity in diabetic patients is associated with certain pathological mechanisms such as hyperglycemia, low capillary density, and adverse changes in oxygenation. Aerobic activity can increase the sensitivity of cells to insulin by increasing aerobic capacity. Along with increasing aerobic capacity, physiological changes such as changes in heart rate, increased blood volume and hemoglobin, low blood pressure, blood distribution, and lactic acid depletion are caused. Therefore, such a possible mechanism has been reported that endurance training increases the aerobic capacity and lipolysis, and eventually leads to weight loss [25].

Two weeks, three sessions per week of high intensity interval training with 4 bouts of one-minute activity with 4 minutes of rest with a maximum speed of 20 meters resulted in a significant reduction in fat mass and weight loss of inactive overweight young men [26]. The findings of this study were consistent with the present study. Concerning the reasons for this, the same type of training can be mentioned. Eight weeks, three sessions per week of aerobic training led to a significant decrease in insulin resistance and lipid profiles in obese people [27]; among the possible reasons for consistency of this study with the present study can be the prolonged time for both studies. Twelve weeks, three sessions per week of yoga training for 60 minutes, had a significant effect on aerobic capacity and skin fat percentage in 35 to 55 years old men [28];one of the possible reasons for consistency of this research with the present research is the prolonged period of both research. Eight weeks, three sessions per week, each session 60 minutes running at speeds of 10 to 17 m/min had a significant effect on the improvement of fat profile in rats with diabetes [5]. Also, eight weeks, three sessions per week, each session including 10 one-minute bouts with 50% intensity and 2 minutes of rest [29] had no significant effect on serum cholesterol and triglyceride in postmenopausal female rats. In addition, eight weeks, three sessions per week with an intensity of 60-70 heart rate reserve did not have a significant effect on the total caloric intake of women with metabolic syndrome [24]; among the reasons for inconsistency of this study with the present study, we can refer todifferent statistical population and sample.

Concerning the interactive effects, the results of this study showed that crocin consumption and training have interactive effects on weight loss in high fat diet and streptozotocin induced diabetic rats. However, they do not have interactive effects on increasing aerobic capacity and dietary intake. By examining the results of previous studies, it can be argued that saffron and its active ingredients, including crocin and various sports exercises, affect lipid profile and weight as well as calorie intake. However, due to the mechanisms of these two factors, it can be concluded that crocin prevents excessive accumulation of fat at serum levels and some tissues [21] and by inhibiting the activity of pancreatic lipase and reducing the absorption of fats, increased fat disposal and metabolism, reduces visceral fat. Also, crocin, through neurotransmitter pathways, may be absorbed to specially serotonin, and may possibly restrict dietary intake by affecting appetite control centers [18-20]. Nontheless, the results of this study showed that crocin had no significant effect on aerobic capacity and dietary intake. The mechanism of crocin effect onaerobic capacity is unknown, a study was not found to evaluate the effect of crocin on the maximum oxygen consumption. Also, the dose and duration of crocin consumption have an effect on the amount of appetite and changes in caloric intake and consumption. Therefore, the non-significance of the reducing effect of crocin on the amount of dietary intake in the present study can be attributed to these two factors (dose and duration).

On the other hand, endurance training along with the above-mentioned mechanism can be considered as factors for changing one's appetite in comparison with exercise [24]. Also, aerobic activity can increase the sensitivity of cells to insulin by increasing aerobic capacity. Along with increasing aerobic capacity, physiological changes such as changes in heart rate, increased blood volume and hemoglobin, low blood pressure, blood distribution, and lactic acid depletion take place. Therefore, endurance training with this possible mechanism increase the lipolysisand aerobic capacity and eventually lead to weight loss [25]. In this regard, 12 weeks aerobic training and saffron consumption improved the fat profile of inactive women, but did not have a significant effect on triglyceride [11]. Six weeks, three sessions of resistance training and simultaneous consumption of 25 mg/kg 25 aqueous extracts of saffron had an interactive effect on reducing insulin resistance, glycolizedhemoglobin and glucose in diabetic rats [8]; however, no study was found to examine the interactive effects of different exercises and simultaneous use of crocin on fat mass, dietary intake and aerobic capacity. Therefore, the present study failed to compare the results of this study with the results of similar studies. Regarding the effects of endurance training and crocin on weight of rats and the lack of measurement of visceral fat mass in rats in this study, it is recommended that in the future studies weights of visceral fat in rats should be measured and the results be compared with this study. Considering the importance of body mass index, one of the limitations of the present research was the lack of measurement of body mass index in rats. Therefore, it is suggested that in the future studies, BMI in rats should be measured to judge more assertively about the effect of endurance training and crocin consumption on weight loss in rats. Among other limitations of the present study, considering the effect of different doses on aerobic capacity and reducing the dietary intake and appetite, we can refer to not taking further or lesser doses to ensure the veracity of this hypothesis; therefore, it is suggested in the future studies to make use of other crocin doses and investigate their effects on these two variables.

Conclusion

Regarding the findings of this study, it seems that endurance training and crocin consumption have interactive effects on weight loss in high fat diet and streptozotocin induced diabetic rats.

Conflict of interest

None of authors have conflict of interests.

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None.

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