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Neurotrophic Effects of Swimming and Crocin Consumption on the Rats with Obesity Induced by a High-fat Diet

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ABSTRACT

Introduction: Obesity is a multifactorial physical disorder. Regular physical exercise and crocin consumption have been reported to have beneficial effects on obesity. The present study aimed to investigate the effects of eight weeks of swimming training with crocin consumption on the neurotrophic factors in the cardiac tissues of obese rats.

Methods: This experimental study was conducted on 28 obese Sprague-Dawley rats (fat: 24 g, protein: 24 g, carbohydrates: 41 g/100 g), which were selected randomly and divided into five groups of seven, including: control (normal saline), swimming training, crocin consumption, and swimming with crocin consumption. Groups two and four performed swimming training for eight weeks (three sessions per week, each session: 30-60 minutes). Groups three and four were intraperitoneally administered with crocin on a daily basis (25 mg/kg).

Results: Eight weeks of swimming and swimming with crocin consumption increased the expression of brain-derived neurotrophic factor (BDNF) in the cardiac tissues of the obese rats ($P \leq 0.05$). In addition, crocin consumption significantly increased BDNF and nerve growth factor (NGF) in the cardiac tissues of the obese rats ($P \leq 0.05$). However, no significant differences were observed in the gene expression levels of BDNF and NGF in the cardiac tissues of the animals with swimming and crocin consumption compared to the crocin consumption group ($P \geq 0.05$).

Conclusion: According to the results, swimming alone, swimming with crocin consumption, and crocin consumption alone had similar effects on the increased level of BDNF in the cardiac tissues of obese rats. Furthermore, crocin consumption could significantly increase the expression of NGF in the cardiac tissues of obese rats.

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Introduction

Over the past 40 years, the prevalence of obesity has increased dramatically across the world (1). The dietary regimen of individuals and adherence to high-fat diets along with the mechanism of increased insulin resistance in various organs, chronic and acute changes in hyperglycemia, and increased plasma triglyceride levels lead to type II diabetes, gradually causing structural changes and cardiac failure (2). Obesity-induced injury is associated with cardial neuroendocrine disorders and increased oxidative stress in the cardiac tissues (3). Researchers have argued that neuroendocrine disorders may increase the parasympathetic function, thereby increasing cardiac risks. However, genetic studies have indicated that sympathovagal balance is directly correlated with neurotrophins, such as the brain-derived neurotrophic factor (BDNF) (4) and nerve growth factor (NGF) (5, 6). The studies in this regard have denoted that increased levels of BDNF are associated with reduced heart rate due to anxiety (5). In addition, neutrophins such as BDNF and NGF have been reported to exert modulating effects on the sympathetic nervous system, thereby improving parasympathetic activity (6).

Numerous studies have confirmed that cardiovascular diseases impose severe heath costs and physical and mental damages on obese individuals. However, current findings have not been able to conclusively determine the optimal approaches for the prevention and treatment of various diseases in obese individuals. Nevertheless, researchers have confirmed the

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beneficial effects of proper dietary regimens and physical activity on cardiac health and homeostasis (3). The combination of caloric restriction and physical exercise has been reported to enhance metabolism and mitochondrial function more effectively than each intervention alone in the cardiac tissues of rats (3).

In this regard, physical exercise has been reported to increase the BDNF levels in diabetic rats (7), while preventing the reduced expression of BDNF (8). However, the BDNF protein levels of the skeletal and cardiac muscles have been reported to increase in the healthy areas of the heart after four weeks of physical exercise, while no changes have been denoted in the gene expression levels in the cardiac muscle in cases with left ventricular ischemia (9). Furthermore, endurance training has been shown to significantly increase the BDNF levels in obese and healthy men, while these changes have not been considered significant in the levels of NGF (10). Considering these contradictory results, use of supplementary medicines and medicinal plants, along with regular physical activity, has recently attracted the attention of medical researchers (7) owing to the fewer complications.

Crocin is a carotenoid-made glycoside, which is responsible for the color of saffron (4). This substance could induce metabolic effects through increasing antioxidant levels (11). In this regard, researchers have reported that the consumption of crocin could significantly enhance the cardiac waves and serum antioxidants in diabetic rats (11). Moreover, crocin consumption has been shown to increase the expression of BDNF and VGF in the cerebellar tissues of healthy rats (12), as well as rats with Malathion toxicity (13).

With this background in mind, it seems that limited research has been focused on the effects of various physical exercises on the levels of neurotrophins as an influential factor in the sympathetic nervous system in cardiac tissues. Since few studies have specifically addressed the effects of crocin consumption and swimming on cardiac tissues, the present study aimed to investigate the effects of crocin consumption and swimming on the gene expression of BDNF and NGF in the cardiac tissues of rats with obesity induced by a high-fat diet. The main research hypothesis was that crocin consumption and swimming have interactive effects on the increasing of BDNF and NGF levels in the cardiac tissues of rats with obesity induced by a high-fat diet.

Materials and Methods

This experimental study was conducted on 28 male Sprague-Dawley rats aged eight weeks and weighing 150±30 grams. The animals were purchased from the Animal House and Reproduction Center of Islamic Azad University, Marvdasht Branch, Iran. After transferring the animals to the Animal Physiological Laboratory, they went through a seven-day adaptation period in standard conditions with the temperature of 22-27°C, relative humidity of 50%, 12-hour light/dark cycle, and free access to sufficient food and water.

Obesity Induction and Grouping

To induce obesity in the animals, a high-fat diet (45% of total energy supplemented by animal fats) containing 24 grams of fat, 24 grams of protein, and 41 grams of carbohydrates per 100 grams was prescribed for eight weeks (14). Following that, the obese rats were divided into four groups of seven, including control (normal saline or crocin solvent), swimming, swimming with crocin consumption, and crocin consumption.

Swimming Protocol

For the familiarization of the animals with the training program, the rats in groups two and three initially performed training for one week (15-30 minutes each day) at the temperature of 30-32°C in a special swimming tank with the height of 50 centimeters and diameter of 30 centimeters. To perform the training program, the rats carried out the training for eight weeks three sessions pre week (30-60 minutes).

Crocin Consumption

The animals in groups three and four were intraperitoneally administered with 25 mg/kg of crocin daily, which was dissolved in normal saline (16).

Measurement of Research Variables

By the end of the eighth week of training and 24 hours after the last training session, the rats were surgically treated to measure the research parameters. The animals were anesthetized with 10% ketamine (50 mg/kg of body weight) and 2% xylazine (10 mg/kg of body weight) after approximately five minutes. Afterwards, the cardiac tissues of the animals were extracted by the experts, placed in a cryotube in liquid nitrogen, and stored at the temperature of -70°C for further analysis. After DNA extraction, BDNF and NGF gene expression was measured using real-time polymerase chain reaction (RT-PCR). The sequence of the used primers is shown in Table 1.

Table 1. Sequence of Forward-Reverse Primers of Genes in RT-PCR Reaction

Gene	Forward (3'-5')	Reverse (3-5')	Product Size (bp)
B2m	CGTGCTTGCCATTCAGAAA	ATATACATCGGTCTCGGTGG	244
BDNF	CTGCAGAGGATGATTGCTGA	GATCAGCTCGGGCACTTTAG	174
NGF	ATCGCTCTGTGGATGACTGAGTAC	AGAGACAGCCAGGAGAAATCAAAC	134

Statistical Analysis

Data analysis was performed in SPSS version 21 using the Shapiro-Wilk test to determine the normal distribution of the data. In addition, one-way analysis of variance (ANOVA) was used for the analysis of the findings, along with Tukey's post-hoc test at the significance level of P≤0.05.

Results

BDNF and NGF gene expression in the cardiac tissues of obese rats is depicted in Figures 1 and 2, respectively. According to the results of one-way ANOVA to examine the differences in the research variables in the study groups (Table 2), there were significant differences in body weight (P=0.001), BDNF (P=0.001), and NGF (P=0.001) in the cardiac tissues of the obese rats.

Changes in BDNF

The results of Tukey's post-hoc test to determine the differences between the study groups indicated that eight weeks of swimming alone (P=0.001) and swimming with crocin consumption (P=0.001) significantly affected the weight loss of the obese rats. On the other hand, crocin consumption alone (P=0.31) had no significant effect on the weight loss of the obese rats. Moreover, no significant difference was observed between the animals in the groups of swimming alone and swimming with crocin consumption (P=0.98). However, the weight of the animals in the groups of swimming with crocin consumption was significantly lower compared to the rats in the crocin consumption group (P=0.03).

Table 2. Results of One-way ANOVA to Examine Variables in Study Groups

Variable	Factor	Sum of Squares	df	Mean Square	F	Sig.
Weight	Inter-group	21097.295	4	5274.324	8.419	0.001
	Intra-group	18793.602	30	626.453		
	Total	39890.897	34			
BDNF	Inter-group	3.383	4	0.846	19.964	0.001
	Intra-group	1.271	30	0.042		
	Total	4.653	34			
NGF	Inter-group	5.032	4	1.258	957.372	0.001
	Intra-group	0.039	30	0.001		
	Total	5.072	34			

According to the obtained results, eight weeks of swimming alone (P=0.003), swimming with crocin consumption (P=0.001), and crocin consumption alone (P=0.001) significantly increased BDNF gene expression in the cardiac tissues of the obese rats. However, no significant difference was observed in the BDNF gene expression in the animals in the swimming group compared to the swimming and crocin consumption group (P=0.94) and crocin consumption group (P=0.71). Furthermore, no significant difference was denoted in BDNF gene expression in the cardiac tissues of the animals in the swimming and crocin consumption group compared to the crocin consumption group (P=0.98) (Figure 1).

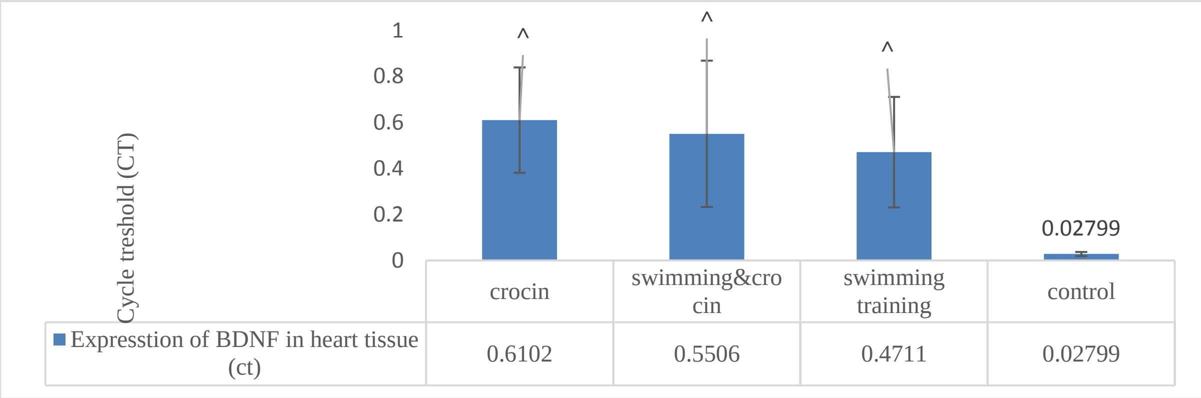

Figure 1. BDNF Gene Expression in Cardiac Tissues of Obese Rats in Study Groups
(Increased BDNF gene expression in swimming, swimming with crocin consumption, and crocin consumption groups compared to control group [P≤0.05])

Changes in NGF

According to the findings, eight weeks of swimming (P=0.59) and eight weeks of swimming with crocin consumption (P=0.13) had no significant effects on the increased levels of NGF gene expression in the cardiac tissues of the rats. On the other hand, eight weeks of crocin consumption significantly increased NGF gene expression in the cardiac tissues of the obese rats (P=0.01).

The obtained results indicated no significant difference between the animals in the swimming group compared to the groups of swimming and crocin consumption (P=0.24) and crocin consumption (P=0.85). In addition, no significant difference was denoted in the NGF gene expression in the cardiac tissues of the rats in the swimming with crocin consumption group compared to the crocin consumption group (P=0.80) (Figure 2).

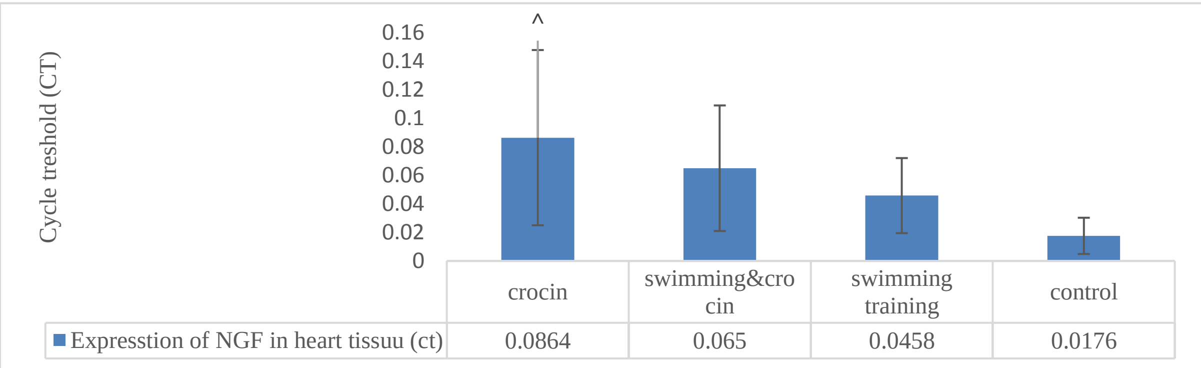

Figure 2. NGF Gene Expression in Cardiac Tissues of Obese Rats in Study Groups
(Increased NGF gene expression in crocin consumption group compared to control group [P≤0.05])

Discussion

According to the results of the present study, eight weeks of swimming increased BDNF gene expression, while no significant increase was observed in NGF gene expressions in the cardiac tissues of obese rats. Obesity is considered to be a major metabolic disorder, which is associated with inflammation. The constant control of blood lipids and energy balance, as well as the development of cardiovascular hemostasis, has been reported to depend on metabotropic factors, such as BDNF and NGF. These neurotrophins not only participate in the regeneration of neuronal cells, but they are also significantly involved in the metabolism and survival of immune cells, fat cells, and endothelial cells (17).

Previous studies have demonstrated the anti-apoptotic effects of BDNF on the myocardial tissues of ischemic rats (18). Furthermore, recent studies have examined the semi-insulin effects of

NGF neurotrophin on some tissues as metabolic modulators (19). It seems that obesity inhibits TrkB (BDNF receptor) and activates exon IV (inhibitor of BDNF protein synthesis) through increasing the cAMP receptor phosphorylation and enhancing the macrophages in the proximity of the endothelial cells (8, 19, 20).

Evidence suggests that the increased expression of BDNF in the cardiac tissues following physical activities is associated with the mechanism of change in pro-inflammatory factors (e.g., TNF-α, IL-1β, and IL-1β) and anti-inflammatory cytokines. Physical exercise deteriorates the activity of pro-inflammatory factors (e.g., TNF-α, IL-1β, and IL-6) and increases anti-inflammatory cytokines (e.g., IL-10) in the left ventricle, thereby resulting in the appropriate mechanism for increasing the mBDNF levels in the cardiac tissues (9). However, few studies have investigated the effects of neurotrophins on the cardiac tissues following physical exercise. The mechanism of the effects of physical exercise on the neurotrophins in the cardiac tissues remains unknown, while the increased receptors of BDNF and NGF (Trk-B and Trk-A) via the MAPK and reversed antisense signaling pathways following physical exercise seems to the main mechanism of physical exercise in case of these neurotrophins (7).

In line with the results of the present study, endurance training has been reported to increase BDNF in obese and healthy men with no significant effects on NGF (10). Moreover, endurance training using a treadmill has been shown to significantly affect the levels of BDNF protein in the skeletal muscles and healthy regions of the cardiac tissues in the animal model of myocardial infarction (8) and spinal cord of diabetic rats (21). High-intensity training has also been reported to significantly increase BDNF and NGF in obese men (22), while endurance training has been shown to significantly increase BDNF and NGF in the brain tissues of the rats with obesity induced by a high-fat diet (23). The findings of the mentioned studies regarding the effects of physical exercise on NGF are inconsistent with the results of the present study. This discrepancy could be due to the differences in the sample population of the first study, type of the assessed tissues in the second study, and type of physical exercise in these studies with the current research.

According to the findings of the current research, crocin consumption could significantly increase the expression of BDNF and NGF in the cardiac tissues of obese rats. However, the mechanism of the neurotrophic effects of crocin on the cardiac tissues remains unclear. In this regard, no prior research has investigated the effects of crocin consumption on BDNF and NGF in the cardiac tissues. The findings in this respect have suggested that the short-term consumption of crocin at various concentrations could significantly increase the expression of BDNF micro-RNAs and enhance the antioxidant factors in the rats treated with Malathion (35). In another study, administration of crocin (25 mg/kg) was reported to significantly increase the expression of BDNF and cyclic-AMP response element-binding (CREB) protein in the central nervous system of morphine-treated rats (24). Furthermore, the consumption of 30 mg/kg of crocin has been shown to improve the expression of antioxidant enzymes and reduce the expression of IL-6 and TNF-α in rats with osteoarthritis (25). It is believed that crocin could also reduce malondialdehyde, oxidative stress, and inflammatory factors (e.g., TNF-α and triglyceride) and increase antioxidants (e.g., glutathione), thereby contributing to the increased expression of nerve neutrophins, especially BDNF.

Saffron and its products have been shown to increase BDNF and NGF through the interactive increasing of the expression of CREB and the neuropeptide VGF. In addition, crocin could inhibit total cholesterol and low-density lipoprotein, increase the thickness of the intima-aortic layer and nitric oxide levels, and inhibit the conventional activation of the nucleus factor kappa-B in the aorta and expression of the intercellular adhesion molecule (26).

According to the results of the present study, swimming with crocin consumption, swimming alone, and crocin consumption alone had similar effects on the increasing of BDNF gene expression in the cardiac tissues of the obese rats. However, swimming with crocin consumption and swimming alone had no significant effects on the increased levels of NGF gene expression in the cardiac tissues of the obese rats.

In the current research, swimming was observed to have interactive effects on the increased BDNF gene expression, as well as the non-significant increase of NGF, in the cardiac tissues of the obese rats with crocin consumption through reducing inflammation and macrophages, cAMP phosphorylation, Ras/MAPK receptors, and enhancing the neurotrophic receptors and antioxidant system (7, 13, 24, 26).

Few studies have been focused on the effects of the concurrent use of crocin and physical exercise on cardiac tissues. However, use of the aqueous extract of saffron and aerobic training has been reported to have significant effects on the modulation of increased cardiac troponin T and serum keratin kinase compared to eight weeks of aerobic training and use of saffron extract alone (27) in rats following a session of exhaustive exercise. Moreover, continuous and interval training with crocin consumption have been shown to have interactive effects on increased *Bcl-2* and decreased *Bax* and *p53* in the cardiac tissues of the rats with diabetes induced by a high-fat diet and streptozotocin (28).

One of the limitations of the current research was the scarce data regarding the effects of crocin and physical exercise and the function of neurotrophins in the cardiac tissues; therefore, further research is required in this regard. Considering the factors involved in the pathway for the increasing of BDNF and NGF, the measurement of factors such as CREB, pro-inflammatory factors, and anti-inflammatory factors is recommended in further investigations. Among the other limitations of the present study were the daily received calorie control and measurement of daily consumed calories to calculate the input and output energy. Therefore, it is recommended that in the further investigations in this regard, researchers control the energy intake and consumption and review the effects of swimming with various intensities.

Conclusion

According to the results, swimming alone, swimming with crocin consumption, and crocin consumption alone had similar effects on the increasing of BDNF in the cardiac tissues of the obese rats. In addition, crocin consumption could significantly increase NGF gene expression in the cardiac tissues of the obese rats.

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