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The Effect of High-Intensity Interval Training (HIIT) and Caffeine Supplementation on Brain-derived Neurotrophic Factor and Glial Line-derived Neurotrophic Factor in Streptozotocin-Induced Diabetic Rats

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Abstract

Background: Diabetes mellitus is a widespread disease disrupting cognitive function. We investigated the effect of eight-week high-intensity interval training (HIIT) and caffeine supplementation on Brain-derived neurotrophic factor (BDNF) and glial line-derived neurotrophic factor (GDNF) in a rat diabetic model.

Methods: In this experimental study, streptozotocin-induced diabetic rats were randomly divided into: control (C), diabetic (D), diabetic+caffeine (D+CA), diabetic+training (D+T) and diabetic+training+caffeine (D+T+CA) groups. Training groups underwent a high-intensity interval training program (5 sessions a week over 8 weeks). The supplement groups were administered with 7mg caffeine/100gr body weight for 5 days a week before each exercise session throughout the experimental period. The rat hippocampus and brainstem were removed 48 h after the last training session and blood samples were taken from left ventricle. The levels of glucose, BDNF and GDNF were measured by ELISA assay. Data were analyzed using two-way ANOVA test.

Results: Streptozotocin-induced diabetes increased blood glucose ($P<0.01$) whereas decreased BDNF and GDNF levels ($P=0.002$). The results showed that HIIT decreased blood glucose ($P=0.002$) but increased BDNF and GDNF levels in diabetic rats ($P=0.003$ and $P=0.001$, respectively). Even though caffeine supplementation significantly reduced blood glucose concentration ($P=0.0001$), it had no significant effect on BDNF and GDNF levels in diabetic rats ($P>0.05$). We also observed a significant interaction between treatments regarding GDNF changes ($P=0.024$); yet, the interaction between caffeine and HIIT on BDNF did not reach the significance level ($P=0.074$).

Conclusion: Based on the findings, HIIT increased BDNF and GDNF levels in rat diabetic model, but caffeine ingestion had no significant effect on neurotrophic factors. However, caffeine seems to blunt HIIT-induced increase in neurotrophic factors which remains to be further investigated.

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Introduction

Diabetes mellitus (DM) is a widespread disease often associated with long-term complications, including retinopathy, nephropathy, heart disease, and neuropathy. Neural complications of diabetes includes peripheral and autonomic neuropathy, stroke, epilepsy, depression, and cognitive dysfunction (1). Recently, a surge of interest has been developed about the effect of diabetes on the brain and cognitive function. Strachan, *et al*., (2009) estimated that 20–70% of patients with DM show cognitive decline, and 60% are at higher risk of dementia (2). Thus, diabetes can lead to cognitive disorders as well as abnormal brain structures, and consequently hippocampus can be especially affected (3). Although the mechanisms of diabetes effects on cognitive dysfunction have not clearly been understood, the role of neurotrophins, including Brain-derived neurotrophic factor (BDNF) and glial line-derived neurotrophic factor (GDNF) seems to be prominent. BDNF plays an essential role in neural growth, synaptic connections, nerve cells repair, and CNS plasticity (4, 5). Besides, GDNF is a prominent factor for neuronal survival (6), and plays a critical role in the development and differentiation of gastrointestinal sensory nerves (7). Numerous studies indicated that circulatory and hippocampus levels of BDNF and GDNF are reduced by diabetes (4, 7-9). Decreased neurotrophin levels in diabetes may have a role in a broad category of brain diseases including depression, Alzheimer, cognitive dysfunction, abnormal brain structure, central and peripheral neurological disorders, as well as gastrointestinal disorders (7, 8, 10). Thus, any strategy to hinder the development of cognitive dysfunction and brain-related diseases could be of high importance, particularly to people at high risk such as diabetic patients.

Recently, evidence is growing regarding the benefits of applied and non-pharmacological interventions, such as various exercise training programs and dietary supplements, on neurotrophic factors in diabetic patients. It has been established that diabetic patients can benefit from metabolic and cardiovascular effects of regular exercise training. High intensity interval training (HIIT) is a form of exercise that has recently attracted attention as a time-efficient exercise modality (11). A number of studies have declared feasibility and glucose lowering effect of HIIT in diabetes (12). Thus, HIIT has been recently recommended to this population (12); however, little is known about the effect of HIIT on neurotrophic factors in this population. Besides, the potential of caffeine on glycemic control have been reported in the literature (13). Caffeine has also been suggested to exert protective effect on nerve system (14). Duarte et al., reported that in diabetic rats, caffeine affects neurochemical adaptations to hyperglycemia and exerts neuroprotective effects (14). However, the potential and interaction of these two interventions on neurotrophic factors in diabetic patients remains to be explored. In the present study, we investigated the effect of HIIT training and caffeine intake on BDNF and GDNF in streptozotocin-induced diabetic rats.

Materials and Methods

Animals

An experimental design was employed for this study and all experimental procedures were performed according to the guidelines of Helsinki declaration and approved by the Regional Research Ethics Committee of Tabriz University of Medical Sciences. Fifty male Wistar rats (weight: 225-300 gr) were purchased from the animal institute of Tabriz University of Medical Sciences. Over 2 weeks of acclimatization, animals

were housed 3-5 per cages in a room with the temperature of 20 ± 2 ° C, relative humidity of 50 %± 5 %, minimum noise, and 12 h light-dark cycle, and were fed ad libitum. They were then randomized to 5 groups (10 rats per each) including healthy control (C), diabetic (D), diabetic + caffeine (D+CA), diabetic + training (D+T), and diabetic + training + caffeine (D+ T + CA).

Streptozotocin-induced diabetes

Following two weeks of adaption to the laboratory environment, in order to induce type 2 diabetes, a diet with high-fat foods were provided to the animals for 2 weeks. Then, streptozotocin (Sigma Aldrich Co. USA) intraperitoneal injection (IP) in a single dose of 35 mg / kg dissolved in citrate buffer pH = 0.1 molar, was applied after 6 h fasting. A week following diabetes induction, blood was collected from the caudate vein and analyzed for glucose level using enzyme-linked glucose oxidase assay and blood glucose concentrations above 250 mg /dL were recognized as diabetic (15).

Caffeine supplementation

Evidence shows that caffeine uptake and bioavailability based on the area under the curves (AUC) exhibit a similar trend in human and rats. Thus, 99% of caffeine is absorbed within 45 min post-ingestion in a dose-dependent manner (16). Hence, in order to enhance the plasma caffeine level during exercise, caffeine was injected 60 min before the exercise protocol. The intraperitoneal (IP) injection of 7 mg hydrated caffeine /100 gr of body weight was applied 5 days a week before each exercise session over 8 weeks (17).

Training Method

Animals were first familiarized with exercise procedure on a motor-driven treadmill (0%, 10–15 m min^{-1}, 5–10 min d^{-1}) for 7 days. Then, an incremental exhaustive exercise test was implemented to assess maximal speed, commenced with 10 m/min, with an increase of 3 m/min every 2 min until failure. Each exercise session started with 5 min warm up and followed by a brief cool down. The main exercise training included 6-12 repetitions of 2 min high intensity exercise with the speed of 85-90% of maximum speed interspersed with 1 min low intensity exercise at 10 m/min. The running speed was increased to the average of 10% every week throughout the study (18). Since handling animals on the treadmill may cause non-exercise stresses, sedentary control animals were trained on the treadmill once a week to familiarize with handling and treadmill environment.

Tissue removal

All animals were intraperitoneally (IP) anesthetized with ketamine (90 mg kg^{-1}) and xylazine (10 mg kg^{-1}) and sacrificed 48 h after the last training session. Sufficient amount of blood was taken from the left ventricle by syringe. In order to provide serum, the blood samples were centrifuged at 3000rpm for 15 minutes and their serum was separated and then stored in a refrigerator at -80 ° C. The rat hippocampus and brainstem were carefully removed and frozen immediately in liquid nitrogen and stored at -80 °C until use.

Blood glucose, BDNF, and GDNF assay

Fasting glucose levels were measured by enzymatic calorimetric method using glucose oxidase technology handling with glucose kit (Padgin Teb Co, Iran;

sensitivity=1mg/dl). To assay BDNF and GDNF proteins, first, the extracted tissues were homogenized and centrifuged. Then, the levels of BDNF and GDNF proteins were measured by ELISA assay using a lab kit (CUSABIO, China) as pg/mg of protein according to the manufacturer structure (BDNF: sensitivity=0.078 ng/ml, detection range: 0.20-0.312 ng/ml; GDNF: sensitivity=0.06 ng/ml, detection range: 0.16-0.25 ng/ml).

Statistical analysis

Normal distribution of the data was assessed using Shapiro Wilk test. Then, they were analyzed by the independent *t*-test and two-way ANOVA. All statistical analyses were performed using SPSS software version 19 and statistical significant level was $P < 0.05$.

Results

Shapiro Wilk test showed that data were normally distributed. The Mean ± SD of variables are presented in Table 2. The results obtained in the present study showed that streptozotocin- induced diabetes in rats resulted in a significant increase of blood glucose (P<0.01); however, the levels of neurotrophins, BDNF (P=0.002) and GDNF (P=0.002), were significantly decreased (Table 2).

Table 1. The high-intensity interval training (HIIT) protocol

	Week of training							
	1	2	3	4	5	6	7	8
No. of running intervals (2 min)	6	7	8	9	10	11	12	12
Mean speed/wk, (m/min)	24	27	30	33	36	39	44	44
Speed of active recovery (m/min)	10	10	10	10	10	10	10	10
Treadmill grade (%)	0	0	0	0	0	0	0	0

Table 2. Characteristics of rats

	C	D	D+CA	D+T	D+CA+T
Initial body weight (g)	299.12±15.88	308.85±27.61	301.8±27.15	292.15±54.3	299.14±62.21
Final body weight (g)	329.25±24.04	296.14±18.49	312.85±39.03	279.42±83.59	283.28±19.66
Blood glucose (mg/dl)	80.6±5.23	373.9±89.78*	141.8±50.18	235.80±48.95	150.6±29.45
BDNF (pg/mg protein)	1.3±0.28	0.62±0.19*	0.74±0.04	1.48±0.58	0.78±0.34
GDNF (pg/mg protein)	1.98±0.46	0.86±0.27*	0.89±0.08	2.2±1.11	1.06±0.45

Control(C), Diabetic (D), Diabetic + Caffeine (D+CA), Diabetic + Training(D+T) and Diabetic + Training + Caffeine(D+T+CA)

*P<0.01, Significant differences compared to the control group

Regarding glucose levels, the results of the present study indicated that HIIT and the caffeine consumption significantly reduced blood glucose level in diabetic rats (P=0.002 and P=0.0001, respectively). However, no significant interaction was observed between caffeine and HIIT treatments (P=0.18) (Table 3).

Table 3. ANOVA reports and effect size

		F	P	Eta squared
Blood glucose (mg/dl)	Training	11.19	0.002	0.47
	Caffeine	16.75	0.0001	0.63
	Training× Caffeine	2.64	0.14	0.18
BDNF (pg/mg protein)	Training	12.72	0.003	0.44
	Caffeine	6.49	0.22	0.13
	Training× Caffeine	3.65	0.074	0.21
GDNF (pg/mg protein)	Training	15.91	0.001	0.49
	Caffeine	9.97	0.34	0.11
	Training× Caffeine	6.21	0.024	0.28

Furthermore, HIIT significantly increased BDNF and GDNF levels (P=0.003 and P=0.001, respectively) but the caffeine consumption did not induce any significant alteration in BDNF and GDNF levels (P=0.22 and P=0.34, respectively). We also observed a significant interaction between treatments regarding GDNF changes (P=0.024), yet, the interaction between caffeine and HIIT on BDNF changes did not reach the significant level (P=0.074) (Figure 1 & 2, Table 3).

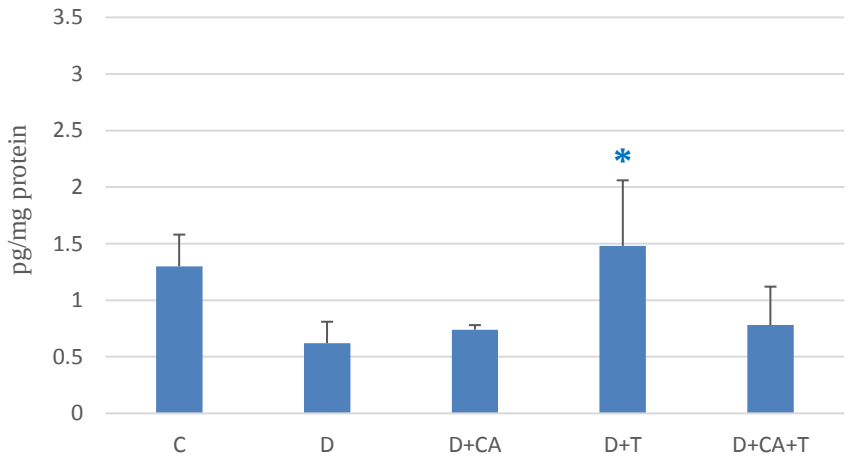

Figure 1. BDNF levels in diabetic rats after eight-week high intensity interval training and caffeine supplementation;

Control (C), Diabetic (D), Diabetic + Caffeine (D+CA), Diabetic + Training (D+T) and Diabetic + Training + Caffeine (D+T+CA)

*P<0.01, Significant differences compared to other diabetic group

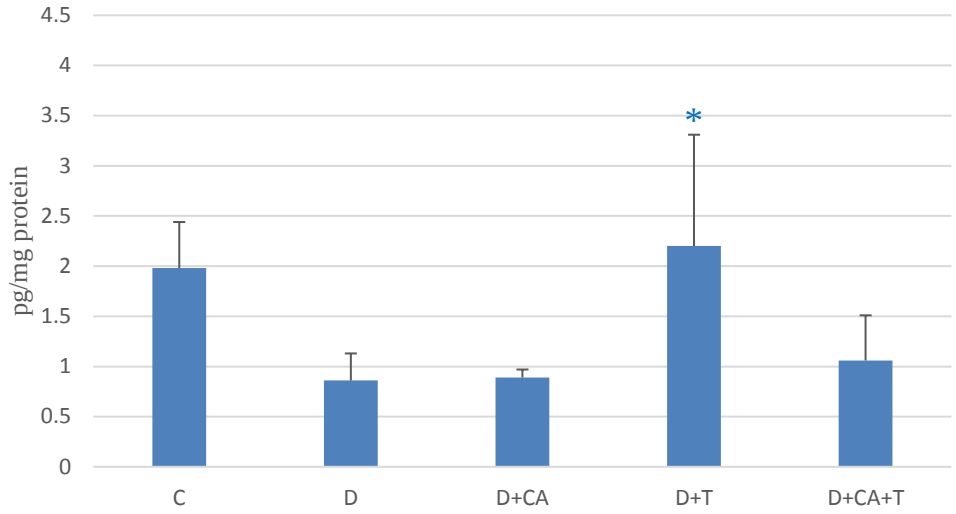

Figure 2. GDNF levels in diabetic rats after eight-week high intensity interval training and caffeine supplementation;

Control (C), Diabetic (D), Diabetic + Caffeine (D+CA), Diabetic + Training (D+T) and Diabetic + Training + Caffeine (D+T+CA)

*P<0.01, Significant differences compared to other diabetic group

Discussion

The present study was conducted to investigate the effect of HIIT and caffeine supplementation on BDNF and GDNF and blood glucose level in male diabetic rats. The results showed that 8-week HIIT along with caffeine consumption significantly reduced blood glucose level with no significant interaction between treatments; HIIT and caffeine resulted in 36.93% and 62% reduction of blood glucose level, respectively. Numerous studies have reported the effects of aerobic and resistance exercise trainings on glycemic indices in diabetic patients (19, 20); yet, a few researches have been carried out examining the effect of HIIT on glucose control and neurotrophin levels. In line with our results, Alvarez, *et al.* (2016) indicated that 16-week HIIT elicits some remarkable improvements in fasting glucose level, HbA1c, and other cardio-metabolic risk factors in diabetic patients (21). According to the literature, exercise intensity plays a critical role in the management of diabetes. It has been reported that HIIT exercise protocols seems to effectively improve HbA1c (22), fasting insulin (22, 23), and blood glucose levels (23) in diabetic patients compared to continuous training programs. This could be explained by an improvement in hepatic glucose output, elevated muscle glycogen depletion (12), and increased insulin sensitivity following intensive exercise training. Moreover, the effect of HIIT on insulin sensitivity has been established to be long-lasting compared to continuous training protocols (12). In addition, our results indicated that caffeine administration reduced blood glucose by 62%. Urzua *et al.* (2012) reported that long-term caffeine administration with a dose of 31 mg/kg/day decreased blood glucose levels and improved glucose tolerance (24). Caffeine is known to increase the rate of glucose transport independent of insulin in rodent skeletal muscle (25) and to enhance the expression of glucose transporter-4 (GlUT-4) in cultured myotubes (26). Egawa *et al.*, (2009) suggested that caffeine is involved in the regulation of

glucose transport by protein kinase (cyclic AMP)-alpha1 activation through insulin-independent mechanisms (25).

In the present study, HIIT, alone, increased BDNF (139%) and GDNF (156%) protein content in male diabetic rats and caffeine ingestion alone could not impose a significant change; whereas, HIIT plus caffeine intake increased BDNF and GDNF levels by 26% and 23%, respectively. Additionally, caffeine intake alone had a trivial effect on neurotrophins and raised BDNF and GDNF levels by 19% and 3%, respectively. Therefore, there seems to be an interaction between HIIT and caffeine administration on BDNF and GDNF proteins. It means that caffeine ingestion may blunt HIIT effect on BDNF and GDNF proteins in diabetic rats. It was a surprising finding and since this is the first study investigating simultaneous effect of HIIT and caffeine on neurotropic factors, it is hard to compare this finding with literature and interpret the findings. Regarding the effect of exercise on neurotrophic factors, Saucedo Marquez *et al.*, (2015) reported that both HIIT and continuous training increased serum BDNF levels but the magnitude of changes was higher following HIIT protocol (27). Freitas *et al*., (2018) also reported that 6-week HIIT increased BDNF protein content in hippocampus of rat brains (28). Although the underlying mechanisms of the effect of exercise on neurotrophic factors has not been clearly understood, few explanations may be raised. Exercise up-regulates BDNF gene expression in the brain, especially in hippocampus, via tyrosine kinase B receptor. Gomez Pinilla *et al.*, (2011) reported that exercise increases BDNF gene expression via acetylation of histone H3 in promoter IV of BDNF gene (29). In addition, higher muscle contraction by HIIT protocol can result in overproduction of BDNF (27). In contrast to our results, Vosadi *et al*., (2013) reported that exercise training had no remarkable effect on BDNF levels (30). The main reason for this discrepancy can be training protocol, duration, experimental design, and the baseline levels of BDNF. In this study, the diabetic subjects had lower BDNF values at baseline which may be more affected by HIIT program. GDNF protein showed a similar trend to that of BDNF following exercise intervention. Zigmond *et al.*, (2009) showed the similar findings and suggested that exercise training simultaneously increased antioxidant capacity and increased the expression of neutrophins, like GDNF. GDNF protein prevents dopaminergic neurons from damage and increases dopamine secretion (31). However, caffeine administration had no significant effect on BDNF and GDNF protein levels in male diabetic rats. We noticed that caffeine supplementation along with HIIT blunted the effect of exercise on neurotrophic proteins, especially BDNF. Despite the results of the present study, most previous studies suggested a significant positive effect of caffeine on neurotrophic proteins, in diabetic and non-diabetic population (14, 17, 32, 33). Recently, Lao-Peregrin *et al*., (2017) indicated that caffeine can release BDNF protein and regulate synaptic plasticity via signaling pathway of IRS2 (insulin receptor substrate-2) (17). The underlying mechanism of caffeine-associated effects on neuronal function and neurotrophic proteins is not clear. Diabetes is accompanied by inflammation in central nervous system and caffeine, on the other side, can inhibit pre-inflammatory cytokines (34) and increase BDNF and signaling function (35). Accordingly, Vila-Luna *et al*., (2012) indicted that chronic caffeine intake can result in higher hippocampal CA1 dendritic connections and prevention of age-related cognitive decline (36). In contrast, Bairam *et al*., (2010) reported that long-term caffeine consumption (15 mg/kg/day) from birth to adulthood in rats

could not show any effect on the expression of BDNF and tyrosine kinas B receptors (TrkBR) (37). There seems to be a discrepancy in the literature regarding the effect of caffeine on neurotrophic factors which may be explained, to some extent, with dose, duration and methods of supplementation as well as the selected subjects (healthy or non-healthy). The findings of the present study raise the question that whether exercise training has any interaction with caffeine administration in diabetic patients. Moreover, longer duration of caffeine administration may be required to observe positive effects on neurotrophic factors in diabetic patients. These questions need to be answered later. The present study had certain limitations, which need to be addressed. The expression levels of neurotrophic genes and proteins could not be assayed using Western blotting and RT-PCR. Another study limitation was related to the lack of different dozes and different protocols for caffeine supplement administration in diabetic laboratory animals. Also, the other indicators, including insulin and insulin sensitivity were not assayed.

Conclusion

Overall, based on the results, HIIT along with caffeine potentially reduced blood glucose in diabetic rats. However, only HIIT intervention could increase neurotrophic proteins and caffeine ingestion had no influence on these variables. Furthermore, caffeine administration ameliorated the effects of HIIT program on neurotrophic proteins including GDNF in diabetic rats. However, this finding and underlying mechanisms need to be confirmed in future studies.

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Conflict of Interest Statements

The authors declare that there is no conflict of interest.

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