Brief Report

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The Effect of High-Intensity Interval Training on Apoptotic-Related Genes in Skeletal Muscle and Serumic TNF-Alpha of Diabetic Rats

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Abstract

Objective: Diabetes is characterized by a comprehensive increase in apoptosis, mitochondrial dysfunction, and inflammation in skeletal muscle. Impaired mitochondrial function in skeletal muscle leads to an increase in Cytochrome C and Caspase-9, and muscle performance is reduced consequently. Exercise training through decreasing inflammatory factors and increasing anti-inflammatory elements prevents apoptosis pathways.

Materials and Methods: Forty male Wistar rats (150±10 g, 8 weeks age) were assigned to 4 groups: control (C), diabetes (D), high-intensity interval training (HIIT), and diabetes high-intensity interval training (DHIIT). Diabetes was induced with intraperitoneal injections of Streptozotocin (STZ) and blood sugar higher than 250 was considered diabetic. The effects of six weeks of HIIT on soleus muscles, Cytochrome C, and Caspase-9 gene expression, as well as evaluation of tumor necrosis factor-alpha (TNF-α) in serum were evaluated using Real-Time PCR and ELISA techniques respectively.

Results: In comparison with C group Diabetes has significantly increased the Cytochrome C (P= 0.001) and caspase-9 (P= 0.003). However, HIIT training in diabetic rats significantly decreased the Cytochrome C (P= 0.001) and caspase-9 (P= 0.008) in comparison of D group. Also, TNFα (P= 0.01) increased in the D group in comparison with C and DHIIT group (P= 0.001). In comparison between DHIIT groups in HIIT groups, has a significant increase in time to exhaustion post test than pretest (P= 0.001).

Conclusion: It seems that HIIT training decreases intrinsic factors of the apoptosis pathway by decreasing inflammatory factors which leads to significant improvement in skeletal muscle function and overall health in diabetic rats.

Keywords: Apoptosis, High-intensity interval training, Cytochrome C, Diabetes

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Introduction

The elevated glucose level is associated with mitochondrial dysfunction, skeletal muscle inflammation, and DNA damage which may contribute to skeletal muscle loss, unweighing, atrophy (1). Generally, exercise training can reduce the risk of hyperglycemia, insulin resistance, mitochondrial dysfunction, and inflammatory signaling leading to activating intrinsic apoptotic factors (2). Recently, many studies demonstrated different high-intensity interval training (HIIT) are equally effective in lowering blood haemoglobin and increasing whole body and skeletal muscle oxidative capacity in diabetes, and these changes are related to fiber types in skeletal muscle (3). One of the important factors to stimulate the inflammatory cascade in response to hyperglycemia is elevated level of the proinflammatory cytokine TNF-α in skeletal muscle, adipose tissue, and plasma (4). A meta-analysis study showed that HIIT improves level of serum TNF-α , adiponectin and leptin, and the result shows that HIIT may be an effective and time-efficient intervention to reduce low-grade inflammation in metabolic diseases such as diabetes (5).

Apoptosis has been identified as a mechanism of programmed cell death, that initiates physiological and biological process through several mechanisms which notabley is represented by DNA fragmentation (6). Mitochondria are major regulatory centers for apoptosis and there is plenty of evidence to prove that apoptosis plays a major role in skeletal muscle cell loss, including muscle unweighing and skeletal muscle denervation, and muscl function (7). Cytochrome C is an essential protein for energy production and is located inside the mitochondria, so it has a very important role in activating apoptosis, and by hyperglycemia and insulin resistance. Consequently, the apoptosis pathway begins by activating initiator (in particular, Caspase-9) and effector caspase respectively. Althought the authours of the study demonstrated that performing HIIT by diabetic rat can lead to an increase in Caspase-9 in cardiac muscle (8), the evidence showed that both single bout of exercises and chronic exercise are efficient in prevention of Cytochrome C release from mitochondria and Caspase-9 activation (9). However, the effects of HIIT on the modulation of apoptosis of skeletal muscle is not confirmed yet in diabetes, further investigations are ongoing.

We hypothesized that induce diabetes with STZ in rats followed by six-weeks HIIT would increase the expression of the mitochondrial and nuclear apoptotic gene, Cytochrome C and Caspase-9, in rat soleus muscle.

Materials and Methods

Animal models

40 male Wistar rats (8weeks age) with an average weight of 150±10 g randomly divided into 4 groups containing 10 rats, including control (C), diabetes (D), high intensity interval training (HIIT), and diabetes high intensity interval training (DHIIT) (10). HIIT and C groups received standard rats chow (60 % carbohydrate, 30% protein, 10 % fat), while D and DHIIT groups received a special diet for the first 4 weeks (22% fat, 48% carbohydrates and 20% protein), for 10 weeks to rise insulin resistance (11). The D and DHIIT groups were induced by intraperitoneal (IP) injection of Streptozotocin (SIGMA Company) 55 mg/kg of animals body weight (12). After 24 hours, the blood sugar range was above 250 mg/dl was considered diabetes in rats. Familiarization with a treadmill (Danesh Salar Iranian company) was performed for 1 week. HIIT training involved running on treadmill 3 days per week for six-weeks, The training method is reported in the table 1 (13).

Tissue collection

After 24 hours of the last session of training, a glucose tolerance test was taken, then all rats were injected with ketamine 10% (10 mg/kh),

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and xylozine 2% (1.5 mg/kg) to anesthetize. The Soleus muscle and serum samples were snap-frozen in the liquid nitrogen and then stored at a freezer (-80 °C).

Real time-PCR for Cytochrome C and Caspase-9

Extraction of total RNA from the muscle tissue with the RNaeasy Mini Kit (Qiagen, GmbH and Hilden, Germany). The relative expression levels of Cytochrome C and Caspase-9 via Real-time & PCR (real-time&PCR kit: Maxima SYBR / ROX Qpcr) was used with the specific PCR primers (Table 2) run in 10µ l volumes on a Rotor-Gene TM 6000 real-time analyzer (Corbett Research, Qiagen, GmbH and Hilden, Germany) for 40 cycles.

TNFα analysis

In addition, blood was conducted to determine TNF analysis with ELISA laboratory method. We used a commercial kit (ZellBio, GmbH, and Veltlinerweg, Germany) with a sensitivity of less than 15 pg/ml and TNF Intra-assay CVs were 6.1%.

Statistical analysis

The $2^{-\Delta\Delta CT}$ method was used to analysed genes expression. Tukey's post hoc test and M-ANOVA with significance level ($P \leq 0.01$) were used to determine significance.

Ethical considerations

In this experimental study (Ethic Approved Code IR.SSRI.REC.1397.255 by SSRC),

Results

Table 1. HIIT protocol

Protocol	Wk 1st	Wk 2nd	Wk 3rd	Wk 4th	Wk 5th	Wk 6th
Familiarizations	50-60 % Vo2max Incline 2 Speed 18-20 m/min	65-75% Vo2max Incline 4 Speed 22-24m/min	75-85% Vo2max Incline 6 Speed 24-26m/min	85-90% Vo2max Incline 8 Speed 26-27m/min	90-100% %Vo2max Incline 10 Speed 27-29m/min	100-110% Vo2max Incline 10 Speed 29-31m/min
Fasting Blood Sugar						
Tissue Collection						
Fasting Blood Sugar						

- **Rest to active ratio 1:2 consist of 10 Rep plus 5 min warm up (Vo2max 45-50%) per session and three times per week of high-intensity interval training.**

Table 1. Primer Sequences for Real-time PCR

Gene name	Accession No.	Primers	Sequence from 5' to 3'	TM (°C)	Amplicon size (bp)
Casapase-9	NM_031632	Forward	TCATCATCAACAACGTGAACTTCTG	60.02	150
		Reverse	TGACCATTTTCTTAGCAGTCAGGT	60.2	
Cytochrome C	NM_012839	Forward	ATGCCAACAAGAACAAAGGTATCAC	60.28	120
		Reverse	CTCCCTTCTTCTTAATTCCAGCGAA	60.86	
Glyceraldehyde 3-phosphate dehydrogenase (Gapdh)	NM_017008	Forward	AACTCCCATTCTTCCACCTTTGAT	60.22	94
		Reverse	AGCCATATTCATTGTCATACCAGGA	59.93	

Table 3. Caspase-9 and Cytochrome C gene expression and tnf-a

Groups	2-ΔΔCT	Cytochrome C *P*	2-ΔΔCT	Caspose9 *P*	TNF-α	TNF-α *P*
D	30.55	0.001	10.51	0.0088	20.60	0.0001
DIIT	8.50		5.55		10.70	
HIIT	2.82	0.0047	2.70	0.066	6.13	0.005
DIIT	8.50		5.55		10.70	
C	1.00	0.0001	1.00	0.0003	12.52	0.365
D	30.55		10.51		10.60	
C	1.00	0.06	1.00	0.06	12.52	0.317
HIIT	2.82		2.70		6.13	

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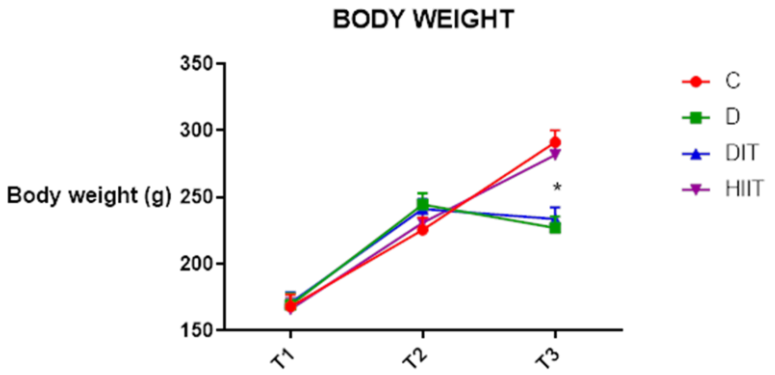

Figure 1. Body weight mean ± standard. C: control; D: diabetes; DHIIT: diabetes + HIIT training; HIIT: high intensity interval training; T1: initial body weight; T2: after 4 weeks high caloric diet, before STZ injection; T3: final body weight. As data showed, there were no differences in body weight of all rats (*P*> 0.01). However, in T1 time, body weight in DHIIT and D groups was significant (*P*= 0.036). After six-weeks of HIIT there were significant decrease in the D Compared with C group; and in DHIIT group comparision to the HIIT group (*P*= 0.001).

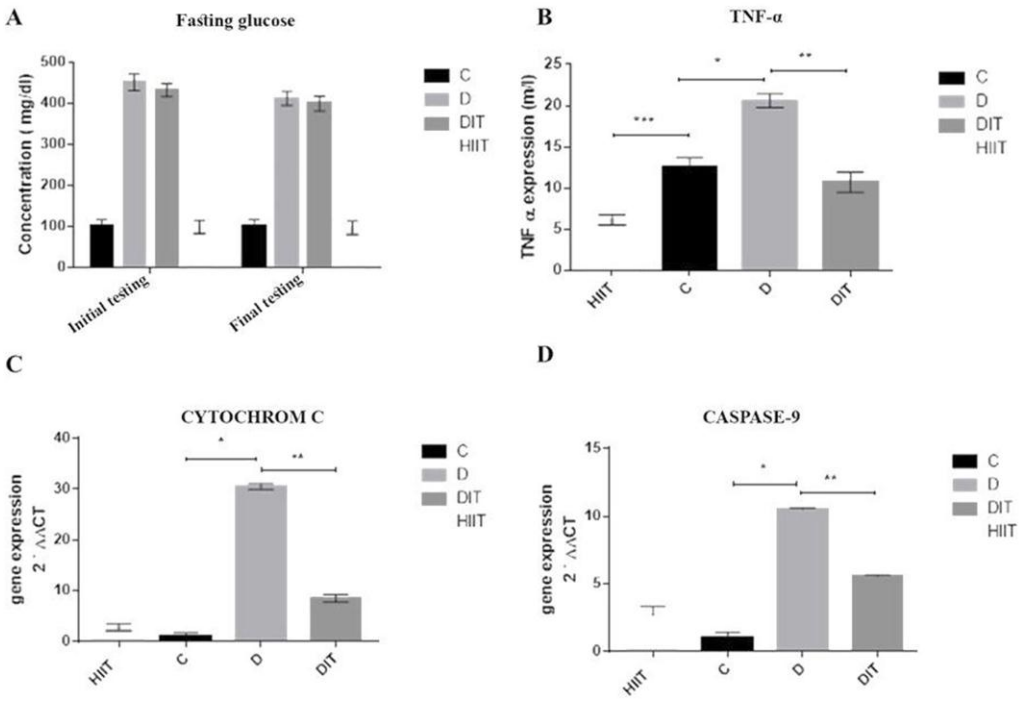

Figure 2. The caspase-9 and cytochrome C genes, tnf-a and fasting glucose mean ± standard deviation. C: control; D: diabetes; DHIIT: diabetes + HIIT training; HIIT: high intensity interval training. Part A: fast blood glucose (FBG) in the initial and final test in all groups, the D group high FBG (*P*= 0.001). There was decrease significant between DHIIT with D group (*P*= 0.064). Part B: analysis showed that TNF-α increased significantly in the D group with C group (*P*= 0.004). Moreover, TNF-α were decreased significantly in the DHIIT group compare to D group (*P*= 0.001), and HIIT group with C group and the result was significant (= 0.0125). Part C: Cytochrome C gene expression was a significant increase in D group than C group (*P*< 0.01). But, a significant decrease demonstrated in DHIIT group in comparision to D group (*P*< 0.01). Part D: Caspase-9 gene expression was a significant increase in D group compare to C group (*P*= 0.0003). But it decreased in DHIIT group when compared to D group (*P*= 0.0088).

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Discussion

Diabetes is a common metabolic disorder worldwide and apoptosis is one of the consequences of diabetes in most organs and has an underlying molecular mechanism in skeletal muscle, but has not been elucidated completely. Both types of diabetes rat models can be obtained through different doses of STZ injection in adult rats or neonatal rats. Also, a high-fat diet is used to induce insulin resistance, that this is characteristic of type 2 diabetes, in STZ Rat Model diabetic (14). In this study, despite an increase in initial body weight after diet intervention, the final body weight was not changed considerably in the diabetic group in comparison to healthy groups, but the body weight decreased in diabetic groups after six weeks of HIIT(not significantly). The possible mechanism to explain the reduced body weight, might be related to the effects of high dosage of STZ on the disregulation of some neural centers such as hippotalamus, and decrement of glucocorticoid or glucocorticoid receptors activities, which are affected by hyperglycemia (15). In a recent study, Qing Lv et al (2022) investigated an association between Parkinson's disease (PD) and diabetes and demonstrated that long-term hyperglycemia induced through STZ, progresses PD and remarkably decreases body weight in mice model of type 2 diabetes (16). However, multiple results showed that regular physical activity can modulate insulin resistance in different tissues especially in insulin target tissues, eventually the effects are independent of body weight or fat mass reduction by exercise (17,18). Probably, in our research, due to the change in the activity of glucocorticoid receptors and increased metabolism caused by HIIT, the body weight of diabetic rats was unexpectedly reduced.

Our study provided several important findings related to the effects of STZ-diabetes with a high caloric diet, and increased expressions of apoptosis-related genes, Cytochrome C and Caspase-9 in skeletal muscle. First, these findings imply that skeletal muscle inflammation may be influenced by diabetes with a high-fat diet. However, the negative effect of diabetes on systemic inflammation, indicated by serum TNF-α levels and has reversed after six weeks of HIIT in diabetic rats. Other studies showed that 12 weeks of combined training can decrease the pro-inflammatory cytokines and increased anti-inflammatory cytokines through the effects on TNF-α in diabetic female subjects (19).

Second, six weeks of treadmill HIIT significantly decreased the diabetes-induced apoptosis in rat soleus muscle, and it was indicated that induction of diabetes causes a high amount of Caspase-9 and Cytochrome C gene expression, while six weeks of HIIT significantly reduced expression of Cytochrome C and Caspase-9 in diabetic rats. The release of Cytochrome C into the cytosol is the first stage for begining apoptosis (20). After the release of Cytochrome C from the mitochondria, an apoptosis-initiating complex is formed which results in the activation of pro-caspase-9 (20). Therefore, mitochondrial dysfunction can activate B-cell lymphoma protein–associated X (Bax) which promotes apoptosis via activation of pro-Caspase-3 and then Caspase-9 (9). Jun-Won Heo et al (2017) investigated that obesity increase Cythocrome C and Caspase-9 level and cause muscle atrophy while exercise decreases these complications (21). Also, Balindiwe Sishi et al (2011) indicated that diet-induced obesity resulted in significant atrophy of the gastrocnemius muscle, and also illustrated a significant increase in apoptosis and caspase 3 and 9 activations in the skeletal muscle in obese objects (11). However, the effect of different exercise training on apoptosis in skeletal muscle is controversial, and the diet interventions with and without exercise have shown that in diabetes high Caloric diet-induced changes can alter apoptosis levels in various tissues. Moreover, the mechanism underlying the effect of HIIT on skeletal muscle apoptosis in diabetes has remained unknown.

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The role of skeletal muscle to control hyperglycemia in diabetes following HIIT is related to the up-regulation of GLUT4 and increased insulin sensitivity. Although some studies have reported improvement in glucose homeostasis by reduction in hyperglycemia following HIIT programs. in other studies the hyperglycemia has not improved significantly, and it might be controlled by increasing the oxidative capacity of skeletal muscle and higher glucose transportation (22).

Conclusions

We provided new insights into the function of high intensity treadmill exercise on diminishing skeletal muscle apoptosis and atrophy. Altogether, our data may be an important evidence that could increase the application of high-intensity interval training to improve overall health and muscle function in diabetes.

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Conflict of Interest

No conflict of interest has been declared by the authors.

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