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Effect of Endurance Training on Peripheral Neuropathic Pain and Inflammatory Mediators in Diabetic Rats

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Abstract

Introduction: The pain caused by peripheral nervous neuropathy is one of the most important factors affecting the quality of life of people with diabetes mellitus. In this vein, considering the possible anti-inflammatory effect of regular exercise on reducing pain associated with diabetes, the present study intends to evaluate the effect of six weeks of endurance training on peripheral neuropathic pain, interleukin- 6 (IL-6) and C- reactive protein (CRP) in diabetic neuropathic rats.

Methods: 40 male Wistar rats (age: 8 weeks old; weight 200-250g) were randomly divided into 4 equal groups of 10, including: diabetic neuropathy training (DNT), diabetic neuropathy control (DNC), healthy training (HT) and healthy control (HC). The diabetic groups were induced by intraperitoneal injection of streptozotocin (STZ) and two weeks after induction of diabetes, behavioral pain tests were administered and after verifying the neuropathic pain, the endurance training protocol was performed for 6 weeks and 5 sessions per week. For analysis of data paired sample t- test, ANOVA and Bonferroni's post hoc test were used ($p \leq 0.05$).

Results: In different groups before and after the training program, the mean reduction in glucose in the training groups was significantly higher than the control groups ($P \leq 0.05$); Serum levels of IL-6 and CPR in the diabetic groups was significantly higher than the training groups ($P \leq 0.05$); The mean pain of the diabetic neuropathy group were significantly higher than the control rats ($P \leq 0.05$); Also, the correlation between IL6, CRP and glucose with the threshold of pain was significantly positive ($P \leq 0.05$).

Conclusion: It seems that endurance exercise in diabetic neuropathic rats can affect the reduction of pain and inflammatory factors, and aerobic training is a suitable method for preventing, controlling and treating pain caused by diabetes.

Keywords: Training, Peripheral Neuropathic Pain, Diabetes, IL-6, CRP

Introduction

Peripheral neuropathy is one of the major and largely untreated causes of pain and suffering in diabetic patients, the most common cause of which is diabetes mellitus, which imposes a great deal of treatment costs on communities (1). In the long-term, diabetes also causes some diseases such as neuropathy, cardiovascular diseases, immune system deficiencies, vascular injuries and it can have significant effects on patient as well as society. According to a demographic study, over 50% of patients with type 1 and type 2 diabetes develop diabetes neuropathy, of which 25% suffer from neuropathic pain. Drug treatment has not been effective for this patent just it has side effects and dependence, and has also caused some problems (1,2). Although tremendous progress is being made in treatment, neuropathy still remains unresponsive to all therapies. According to reports, approximately 66 % of patients with neuropathic pain during the course of treatment do not experience pain relief and up to the present, no treatment has prevented the development or control of neuropathic pain (3). One of the factors can be causes neuropathic pain is inflammatory factors.

Thus, the methods of reducing inflammation will provide a new horizon for the improvement of neuropathic diseases. Studies have shown that an increase in the expression of inflammatory cytokines in the peripheral nervous system can alter the sense of pain in streptozotocin (STZ)- induced diabetic rats. Inflammatory factors such as tumor necrosis factor- alpha (TNF-α), nuclear factor kB (NF-kB) and interleukin- 6 (IL-6) in the systemic blood circulation and neural tissues, which interfere with inflammatory processes caused by diabetes; represent a condition for nerve inflammation. Thus studies have shown that an important role in the pathogenesis of neuronal damage played by inflammatory factors, also thermal hyperalgesia and mechanical allodynia develop as signs of neuropathic pain in STZ-diabetic rats (4,5). The measurement of C-reactive protein (CRP) due to its rapid increase in the onset of tissue lesion and its rapid reduction after recovery is the best way to detect tissue lesions (6). Also activating the immune response of the nervous system and the subsequent production of pro-inflammatory factors, such as IL- 6, can change the synaptic activity in a stable way and cause neuropathic pain (7). Recent studies also show that some signaling pathways are very important for the development of neuropathic pain and the production of inflammatory mediators and inflammation. According to the findings of some researchers, physical activity can reduce inflammatory factors, although some of them have not reported changes in inflammatory factors after exercise activities. In fact, by exercising and subsequently control of blood glucose, damage to the nerves can be decreased; also it can reduce the complications of sensory motor neuropathy and pain that occur due to the destruction of nerve cells (8). Ghodsbin *et al.,* (2017) investigated the correlation between CRP and IL-6 serum levels, after induction of diabetes and eight weeks of resistance training in rats and they showed that there is no correlation between CRP and IL-6 serum levels, after the induction of diabetes and eight weeks of resistance training in rats (9). Nemati *et al.,* (2017) showed that high intensity training (for 6 weeks) caused reduction in TNF-α levels in type 2 diabetic women (7); Molinari Shamsi *et al.,* (2014) showed that eight-weeks of resistance training (3 days per week) did not have significant effects on the TNF-α and IL- 6 levels in fast-twitch muscle tissue, but, there was a significant and positive correlation between the levels of the TNF-α and IL-6 levels (10). The overall goal of reducing pain is to return patients to a normal life. This is only possible if the relationship between symptoms, mechanisms and symptoms of neuropathic pain is understood (11). In this regard, due to the increasing number of people with diabetes, the cost of treatment and the high mortality rate of diabetic neuropathic patients, and considering that so far, few studies have investigated the association of inflammatory factors with pain; the present study aimed to investigate the effect of six weeks of endurance training on peripheral neuropathic pain and inflammatory factors of IL-6 and CRP in diabetic neuropathic rats.

Methods

The protocols and procedures of this experimental study had previously been approved by the Research Ethic Committee of Lorestan University. A total of 40 Westar male rats at the age of 8 weeks with a mean weight of 200- 250 gr were taken from the research center of Ahwaz Jundishapur University of Medical Sciences. They were divided into 4 groups and maintained in standard cages of polycarbonate at temperature conditions 22 ± 2 ºC and under 12:12 hours cycle of darkness-light. Rat food (Pellet, Pars food company in Tehran) and water were sufficiently accessible to animals, with the exception of the time of the tests. All experiments were carried out at the Animal Laboratory after at least two weeks of establishment of the animals in order to create an adaptive environment. The rats were

randomly divided into 4 groups with equal number (n=10) including diabetic neuropathy training (DNT), diabetic neuropathy control (DNC), healthy training (HT), healthy control (HC), and eventually entered the next steps of study. After completing the anonymization protocol, following 12 hours of food deprivation, two groups of diabetes-induced received 50 mg per kg body weight of the intraperitoneal injection of streptozotocin dissolved in a citrate buffer of M=0.05 with pH=4.5 (12). In two other non-diabetic groups of rats, the equivalent volume of citrate buffer (M=0.05, pH=4.5) was injected peritoneally. 48 hours after injection, by creating a small injury by the lancet on the tail vein, a drop of blood was placed on the glucometer strip, and the strip was measured by a glucometer device (Glucotrend 2, Roche Germany) and rats with blood glucose above 300 mg/dL, were considered as diabetic. To ensure that blood glucose did not return, blood glucose levels were measured at the end of the 4th and 8th weeks (13). A glucometer (Glucotrend 2, Roche Germany) was used to measure blood glucose levels. The concentration of serum IL-6 was measured using French IL-6 kit (Diaclone) and serum CPR concentration by immuno-fluorescence method using South Korean penta-kit and i-chroma device. The sensitive of the kits were %6.5 and %5 ng /ml for CRP and %5.6 and 20 pg/ml for IL6. After confirmation of the development of sensory neuropathic pain in rats, the endurance training protocol was performed for 6 weeks. In the present study, the endurance training protocol was of moderate intensity that was used with progressive intensity and duration and with the principle of overloading, so that the diabetic neuropathy and healthy training groups were exposed to 5-session trainings per week for 6 weeks. All training sessions were held at the end of the animal's sleep cycle between 16:00 and 18:00 (14). All of the variables were kept constant at the ending week (week 6) to achieve homogeneity in the adaptations (Table 1). For Formalin test, two weeks after induction of diabetes (before the onset of endurance protocol) and 48 hours after the last training session, neuropathic pain behavioral (formalin) tests were performed using the standard Dennis and Dubuisson (15) methods for all groups. The animal was placed in a Plexiglas container (25×25×30 cm) located on the surface of the formalin machine, and after 15 minutes, 50 µl of formalin solution 2.5% (diluted with 0.9% sodium chloride solution) was subcutaneously injected into the back toe of the rear and right foot of the mouse with a micro syringe 30 gauge and then the animal was immediately returned to the container. A mirror was located 45 degrees below the glass surface so that the animal's foot could be seen accurately. Following the injection of formalin, painful behaviors occurred to the animal. Considering the intensity of pain and the type of behavior observed, four separated levels were assigned to the animal, as the following: (1) The animal sits or goes on the injected foot: score 0; (2) The animal does not put the injected toe easily on the contact surface, but puts its weight on its healthy foot: score 1; (3) The animal separates the formalin injected toe from the contact surface: score 2; (4) The animal licks, bites or shakes the injected toe: score 3. Behavioral responses were recorded at 15-second intervals immediately after formalin injection and calculated up to minute 60 (test time). Using this method, numbers 0 to 3 were obtained for pain score at different times per interval. T 0, T 1, T 2 and T 3 are respectively 15 seconds in which the animal shows 0, 1, 2 and 3 behaviors over a 5-minute period. The mean score of pain for 0- 10 and 15- 60 minutes after formalin injection was considered as a standard measurement of acute and chronic pain, respectively. The time interval between10- 15 minutes is considered to be a short-term offset period, with no response from the injected toe. Data analysis was performed using SPSS software version 19. In order to compare the mean of the parameters measured in the experimental groups, paired

sample t- test, ANOVA and Bonferroni's post hoc test were used. The significant level was considered as P <0.05.

Results

Changes in the body weight, glucose levels, pain level in the acute phase and pain level in the chronic phase are presented in Table 2. Also, levels of IL- 6 and CPR in the research groups are presented in Table 3. The results of one-way ANOVA test showed that at the beginning of the study, there was no significant difference between the weight of animals in different groups (p= 0.96); but paired samples t- test showed that there was a significant difference between the weight of animals in different groups (p≤0.05). Indeed, the weight of control and training healthy groups increased, but in the control and training neuropathy groups decreased. The results of one-way ANOVA test showed that at the end of the training program, there was a significant difference between the weight of animals in different groups (p≤0.05). The Bonferroni's post-hoc test showed that there was a significant difference between the weight of health control and diabetic neuropathy control groups and between healthy training and diabetic neuropathy training groups (p≤0.05) (Table 2). The results of one-way ANOVA test showed that at the beginning of the study, there was a significant difference between glucose levels in different groups (p≤0.05). Bonferroni's post-hoc test showed that there was a significant different in glucose levels between health control and diabetic neuropathy control groups and between healthy training and diabetic neuropathy training groups (p≤0.05). The results of paired sample t- test showed that there was no significant difference between pre-test and post-test levels of glucose in health control, healthy training and diabetic neuropathy control groups (p≥0.05); nevertheless, there was a significant difference between pre-test and post-test levels of glucose in diabetic neuropathy training group (p≤0.05) (Table 2). The results of one-way ANOVA test in Table 2 showed that at the beginning of the study, there was a significant difference between the acute pain phase of animals in different groups (p≤0.05). The results of Bonferroni's post-hoc test showed that there was a significant difference between the acute pain phase of health control group and diabetic neuropathy control group (p≤0.05). There was a significant difference between the acute pain phase of healthy training group and diabetic neuropathy training group (p≤0.05). The results of paired-samples t-test showed that there was a significant difference between the acute pain of animals in the pre-test and post-test in different groups (p≤0.05) except the control group (p≥0.05); indeed, the acute pain of health control and healthy training groups significantly decreased (p≤0.05), but in the diabetic neuropathy control and diabetic neuropathy training groups significantly increased (p≤0.05). The results of one-way ANOVA test in Table 2 showed that at the beginning of the study, there was a significant difference between the chronic pain phase of animals in different groups (p≤0.05).

Table 1. Numeric display of the training program in different weeks

Week	Speed (m/m)	Time (m)
First	10	10
Second	10	10
Third	14-15	20
Fourth	14-15	30
Fifth	17-18	30
Sixth	17-18	30

Table 2. The results of paired sample t- test and one way ANOVA test for compare the body weight, glucose, of rats before and after six weeks of research

Variable	Group	Time	Mean ± Standard Deviation	Paired sample t- test	One way ANOVA
Weight (g)	Healthy Control	Pre- test	238±8.56	t=-42.14, *	F= 124.885, * P=0.001
		Post - test	306.6±8.31	p=0.01	
	Healthy Training	Pre- test	238 ±7.14	t=-69.81, *	
		Post - test	295 ±9.42	p=0.01	
	Diabetic Neuropathy Control	Pre- test	239.5±10.39	t=18.20,*	
		Post - test	192±10.32	p=0.01	
	Diabetic Neuropathy Training	Pre- test	237.5±10.06	t=2.87, *	
		Post - test	210±29.05	p=0.02	
Glucose (mg/dl)	Healthy Control	Pre- test	118.6±6.73	t=1.26,	F= 1.479, P=.237
		Post - test	121±7.77	p=0.21	
	Healthy Training	Pre- test	118.8±5.30	t=1.32,	
		Post - test	116.6±5.14	p=0.219	
	Diabetic Neuropathy Control	Pre- test	409±51.73	t=0.06,	
		Post - test	407±61.83	p=0.94	
	Diabetic Neuropathy Training	Pre- test	405.5±41.39	t=4.17, *	
		Post - test	366.6±38.72	p=0.02	
Pain level in acute phase (min)	Healthy Control	Pre- test	1.69±.09	t=-1.75,	F= 51.952, P=.001
		Post - test	1.73±.06	p=0.113	
	Healthy Training	Pre- test	1.70±.09	t=5.69,*	
		Post - test	1.45±.18	p=0.01	
	Diabetic Neuropathy Control	Pre- test	2.03±.11	t=-6.02, *	
		Post - test	2.44±.16	p=0.01	
	Diabetic Neuropathy Training	Pre- test	1.94±.08	t=9.10,*	
		Post - test	1.67±.07	p=0.01	
Pain level in chronic phase(min)	Healthy Control	Pre- test	1.67±.07	t=1.02,	F= 68.457, * P=.001
		Post - test	1.62±.16	p=0.33	
	Healthy Training	Pre- test	1.67±.10	t=9.76, *	
		Post - test	1.22±.09	p=0.01	
	Diabetic Neuropathy Control	Pre- test	2.03±.09	t=-12.08, *	
		Post - test	2.40±.13	p=0.01	
	Diabetic Neuropathy Training	Pre- test	2.08±.18	t=7.44, *	
		Post - test	1.55±.126	p=0.01	

* Indicates difference at significant level of P≤ 0.05.

The results of Bonferroni's post-hoc test showed that there was a significant difference between the chronic pain phase of health control group and diabetic neuropathy control group (p≤0.05). There was a significant difference between the chronic pain phase of healthy training group and diabetic neuropathy training group (p≤0.05). The results of paired-samples t-test showed that there was a significant difference between the chronic pain of animals in the pre-test and post-test in different groups (p≤0.05) except the control group (p≥ 0.05);

Table 3. The results of one way ANOVA test to compare the levels of IL- 6 and CRP between rats of research groups

Variable	Group	Mean±	Standard Deviation	F	P
IL6 (pg/ml)	Healthy Control	50.6	3.23	41.57*	0.001
	Healthy Training	42.2	3.73		
	Diabetic Neuropathy Control	56	5.24		
	Diabetic Neuropathy Training	39.6	2.71		
CRP (ng/ml)	Healthy Control	1.58	0.23	9.21*	0.001
	Healthy Training	0.88	0.18		
	Diabetic Neuropathy Control	2.62	0.60		
	Diabetic Neuropathy Training	1.26	0.28		

* Indicates difference at significant level of P≤ 0.05.

Table 4. The results of Bonferroni's post hoc test to compare the changes of IL- 6 and CRP in rats of research groups

Variable	Groups		Mean Difference	P
IL6	Healthy Control	Training Healthy	8.4*	.001
		Diabetic Neuropathy Control	-5.4*	.021
		Diabetic Neuropathy Training	11*	.001
	Healthy Training	Diabetic Neuropathy Control	-13.8*	.001
		Diabetic Neuropathy Training	2.6	.840
	Diabetic Neuropathy Control	Diabetic Neuropathy Training	16.4*	.001
CRP	Healthy Control	Training Healthy	.7*	.001
		Diabetic Neuropathy Control	-1.04*	.001
		Diabetic Neuropathy Training	.32	.352
	Healthy Training	Diabetic Neuropathy Control	-1.74*	.001
		Diabetic Neuropathy Training	-.38	.157
	Diabetic Neuropathy Control	Diabetic Neuropathy Training	1.36*	.001

* Indicates difference at significant level of P≤ 0.05.

Table 5. The results of Pearson correlation test for review the relationship between IL- 6, CRP, Glucose and the threshold of pain in rats after six weeks of aerobic training

Gropes	Correlation Analyze	Glucose	IL6	CRP
Acute Pain	Pearson Correlation	0.659*	0.635*	0.807*
	Sig. (2-tailed)	0.001	0.001	0.001
Chronic Pain	Pearson Correlation	0.674*	0.700*	0.867*
	Sig. (2-tailed)	0.001	0.001	0.001

* Indicates correlation at significant level of p≤0.05.

indeed, the chronic pain of health control and healthy training groups significantly decreased ($p \leq 0.05$), but in the diabetic neuropathy control and diabetic neuropathy training groups significantly increased ($p \leq 0.05$). The results of one-way ANOVA test showed that there was a significant difference in IL- 6 ($p=0.001$) and CRP ($p=0.001$) of rats in the research groups (Table 3). The results of Bonferroni's post-hoc in Table 4 showed that training in the healthy and diabetic neuropathy significantly reduced IL- 6 ($p=0.001$) and CRP ($p=0.001$). The results of Pearson correlation test in Table 5 showed that there was a significant correlation between IL- 6, CRP, glucose and the threshold of pain after 6 weeks of aerobic training ($p \leq 0.05$). Also, there was a significant correlation between pain levels in the acute and chronic phases with IL- 6, CRP and glucose after 6 weeks of aerobic training ($p \leq 0.05$).

Discussion

The purpose of this study was to investigate the effect of endurance training on peripheral neuropathic pain and inflammatory mediators in diabetic rats. Because diabetic peripheral neuropathy (DPN) is one of the side effects of diabetes, more than half of diabetic patients are affected by this condition, which leads to a decrease in the quality of life of these people. One of the symptoms of DPN is pain, numbness and tingling. Various theories have been made for the mechanisms for the development of neuropathic pain, among which we can referred to blood glucose levels (16). Based on the results of this study, glucose level in diabetic rats was significantly different from the healthy group before training. Also, after six weeks of training, despite the decrease in glucose in the training groups compared to the control groups, there was no significant difference; however, the difference between healthy and diabetic groups was significant in blood glucose levels. This could be seen as a symptom of pain, so that researchers declared that glucose control in 60% of patients reduced the progression of diabetic neuropathy. Therefore, it can be said that the goal of accurately controlling blood glucose is to prevent diabetic neuropathy (17). An increase in blood glucose levels leads to oxidative stress, diabetes neuropathy and, in turn, activates NF-κB, which is associated with an increase in the level of pro-inflammatory cytokines in the bloodstream (18, 19). Among other factors affecting the amount of pain, inflammatory factors can be noted. Based on the results of this study, the levels of IL- 6 and CRP inflammatory factors in the healthy training group showed a significant difference compared to the healthy control group. Although diabetes significantly increased the IL6 and CRP levels compared to the healthy group, six weeks of aerobic training showed a significant decrease in inflammatory factors. These findings, are inconsistent with the results of previous studies which showed no effect of resistance training and endurance training on IL- 6 (20). The reason for this contradiction may be due to differences in training programs and type of subjects, but they are consistent with the studies of Nicklas *et al.,* 2008 and Prestes *et al.,* 2009*,* which show significant reduction in IL- 6 following different training protocols (21, 22). Also, some studies have shown that the levels of IL6 and CRP inflammatory factors increase as a result of high intensity training (23, 24), which is not consistent with the results of this study. The main reason for this is the intensity of training; however, the probability of pain reduction in diabetic rats can be seen as the result of training effect on reducing these inflammatory factors. Several studies have shown a reduction in CRP due to exercise activity. The findings of Touvra *et al.,* (2011) and Yu Z *et al,*. (2009) are consistent with the findings of this study (25, 26). Exercise activity can reduce blood CRP levels directly by decreasing the production of cytokines in fatty tissue, muscle and mononuclear cells, and indirectly by increasing insulin sensitivity and improving

endothelial function (27, 28). Balducci *et al*., (2010), investigated the effect of exercise on inflammatory and anti-inflammatory levels of cytokines in patients with type 2 diabetes (29). Results showed that exercise reduced the concentration of CRP, IL- 6, IL- 1 and TNF- α and increased IL- 10 and IL- 4 anti-inflammatory cytokines levels. Therefore, exercise by increasing the production of anti-inflammatory factors and reducing the inflammatory factors, reduces pain, which matches the present results. In this study, formalin test was used to evaluate the pain caused by environmental neuronal damage. The presence of mechanical allodyna has already been proven to be the first sign of diabetes neuropathy, which has partly been attributed to the high toxic effects of glucose on the peripheral nervous system (30, 31) Diabetes mellitus also stimulates pain signals in the spinal cord (32). On the other hand, recent research suggests that streptozotocin-induced diabetic rats can be considered as a chronic pain model, in which hyperalgesia and allodynia are well observed in the short term of 1 to 2 months (33). Based on the results obtained before the training, the severity of pain in both acute and chronic phases in the diabetic neuropathic group was significantly different from that of healthy groups. In the present study, diabetic rats showed an increase in pain response in both acute and chronic phase in the formalin test, which after training, the pain response decreased in both phases. Regarding the correlation between the findings of the neuropathic pain test with glucose, IL- 6 and CRP, and the effect of exercise activity on reducing these factors, as well as the level of pain sensation in the diabetic and healthy training rats, it can be stated that endurance activity has been able to relieve diabetic neuropathic pain. Visser *et al.,* (2006) demonstrated the correlation between formalin test and neuropathic pain in different animal species in experimental animals (34). Romero *et al*., (2012) studied the effect of exercise on feelings of pain and reported the impact of exercise on reducing pain, which was consistent with the results of the study (35). Exercise by affecting the performance of peripheral nerves can reduce neuropathic pain in diabetic patients. Also, aerobic exercise can have beneficial effects on neuropathic complications by increased vascular dilatation, decreased oxidative stress and increased neurotropic factors and decreased inflammatory factors (36).

Conclusion

In general, according to the results of this study, it seems that six weeks of endurance training on diabetic neuropathic rats can affect the reduction of pain and inflammatory mediators. Also, prescribing regular mild to moderate aerobic exercises for diabetes can be a good way to prevent, control, and treat pain caused by it.

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Ethical issues

Not applicable.

Authors' contributions

Authors equally contributed to the writing and revision of this paper.

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