The effect of aerobic exercise and a synthesized insulin nanocomposite hydrogel on TNF-α and IL-6 in plasma of type 1 diabetic rats

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Abstract

Introduction: Diabetes is one of the leading causes of mortality in the world. The present study aimed to investigate the effect of aerobic exercise training and a synthesized insulin nanocomposite hydrogel on TNF-α and IL-6 in type 1 diabetic rats.
Materials and methods: Twenty-five rats were divided into five equal groups of animals each containing five animals, control - healthy, Control-diabetes, Nano-insulin Diabetes, Exercise Diabetes and Nano-insulin Diabetes-Exercise. The exercise training program lasted eight weeks. After the five days of familiarization, exercise time for the exercise groups were as follows: 20 m/min for the first and second weeks, 25 m/min for the third and fourth weeks and 30 m / min for the fifth and sixth weeks. The rats were also given the Nano-insulin supplement. Rats were killed 48 hours after the last training session. Their plasma was taken and used for the analysis of markers.
Results: There were significant differences in IL-6 ($P<0.001$) and TNF-α ($P<0.001$) between the groups.
Conclusion: The results of this study showed that aerobic exercise training along with Nano-insulin supplementation significantly reduced TNF-α and IL-6 in rats with type 1 diabetes and these changes were more in the Nano-insulin Diabetes group.

Keywords: Aerobic training, Synthesized insulin nanocomposite hydrogel, TNF-α, IL-6, Type 1 diabetic rat

Introduction

Type 1 diabetes is an autoimmune disease that can be affected by genetics or the environment. (1). This type of diabetes usually affects children and young adults, although it can occur at any age (2). In adults, type 1 diabetes accounts for about 5% of diabetics, and there is no way to prevent it (1). To survive, these patients need to be repeatedly ingested by insulin (2). Diabetes has been reported to be a chronic inflammatory condition. Under these conditions, insulin resistance and levels of inflammatory adipokines increase and levels of anti-inflammatory adipokines decrease (3). Mild chronic inflammation is manifested by a 2 to 3-fold increase in systemic inflammatory concentrations of inflammatory cytokines. These inflammatory cytokines include tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) (4). IL-6 is produced by vascular endothelial cells, adipocytes, and fibroblasts in response to microbes and other cytokines, especially IL-1 and TNF-α (5). Increased IL-6 levels are associated with insulin resistance and increased risk of

type 2 diabetes. Serum IL-6 concentration has been shown to be positively correlated with obesity levels and BMI (6). Some researchers have found IL-6 to be an inducer of insulin secretion from pancreatic beta cells (7). TNF-α is mainly produced in monocytes, macrophages and adipose tissue and to a minimal extent in lymphocytes and killer cells. It is one of the most important mediators of inflammation in the body, which is more produced in individuals with obesity (8). TNF-α impairs insulin signaling pathway, including phosphorylation of insulin receptor substrate protein (IRS) in pathological conditions such as chronic inflammation and initiation of acute phase reactions by enhancing the survival of inflammatory signaling and stimulating cell death. (9). An inactive lifestyle is associated with a high risk of developing insulin resistance and cardiovascular diseases. According to available research reports, active lifestyle is associated with decreased levels of inflammatory cytokines and improved insulin sensitivity. Regular exercise training, as a non-invasive strategy for reducing obesity and related diseases, improves the inflammation and insulin sensitivity in healthy obese or type 2 diabetic patients. In a study on older women conducted by eight weeks of low-intensity resistance training, a significant decrease in IL-6 and TNF-α levels was reported (10). In another study, obese men with type 2 diabetes performed circular aerobic and resistance training for eight weeks, five sessions per week, and 30–50 minutes each session. The results of this study showed no significant change in IL-6 and TNF-α levels. In this study, the reason for the lack of significant change in the levels of these cytokines was the lack of significant change in fat mass and also bodyweight of the subjects (11). In a study on overweight young women, two endurance and resistance training protocols were evaluated. One group performed moderate-intensity endurance training, and the other group performed resistance training with 50-60% intensity of a maximal repetition with 12 repetitions and four sets. The training duration was 12 weeks and three sessions per week. The results showed a significant decrease in IL-6, TNF-α levels and fat percentage but no significant changes in BMI. Besides, there was also no significant difference between the two groups (12). External insulin supplementation is one of the treatments for type 1 diabetes. Despite the overuse of synthetic insulin, there are several problems with long-term use of insulin for the treatment of diabetes, including the time of using, preparation, dosage, and its injection before or after exercise (1). However, the use of Nano-system can reduce repeated injections per day (13). The remarkable process in the development of biodegradable Nanoparticles is a suitable method for the transfer of peptides and proteins (14). This polymeric drug delivery system has the advantages of transferring drugs to the target tissue, the ability to transfer proteins, peptides, genes, and enhancing therapeutic benefits with fewer drug side effects (15). The results of studies indicate that Nano-nonsulin may be a desirable strategy for the treatment of diabetes that may target several mechanisms involved in the development of diabetes (1). Nanoculin administration is associated with an increase in the expression of insulin receptor substrates 1 and 2, indicating its ability to bind to insulin receptors. Therefore, it may increase the level of phosphorylation and be used to restore insulin secretion (16). It also requires ten times fewer doses to treat diabetes than other insulin therapies (16, 17). In this regard, Samadar et al. (2013) observed that Nano-insulin could modulate the expression level of several glucose transporters and return them to near-normal levels (16, 17). According to the new Nano-insulin technology, studies have not determined the effect of this type of diabetes treatment on IL-6 and TNF-α in diabetic patients yet.

On the other hand, aerobic exercise has been shown to play a pivotal role in the treatment of type 1 and type 2 diabetes. Still, since no study is available on the combination of Nano-insulin supplementation with aerobic exercise, therefore the present study tries to answer these questions: Do Nano-insulin supplements affect these cytokines? Will exercise alone has a positive impact on type 1 diabetics by reducing these cytokines? Will the combination of exercise and Nano-insulin supplementation be more effective? The present study aims to investigate the effect of aerobic exercise, together with Nano-insulin supplementation on IL-6 and TNF-α in type 1 diabetic mice.

Materials and methods

The rats were purchased from Tehran Pasteur Institute at the age of 6-8 weeks and with the mean weight of 180 ±10. In vitro, rats were fed a high-fat diet (70 g/kg daily) for four weeks. After 12 weeks, they weighed 240 to 250, and then, they were divided into five groups based on their body weight: 1) healthy control group, 2) type 1 diabetic group, 3) diabetic group type 1 + nano-insulin, 4) type 1 diabetic group + aerobic exercise, 5) type 1 diabetic group + nano-insulin + aerobic exercise.

Subjects were kept in transparent polycarbonate cages measuring 30 × 15 × 15 cm, with an ambient temperature of 20 ± 2° C and 50 ± 5% humidity with proper ventilation and light/dark cycle of 12:12. Their required food was provided from one of the companies of animal feeding. Besides, their water was given to them in 500 ml bottle special for laboratory animals.

The rats were fasting for 18 hours before injection of streptozotocin solution. Water consumption was unacceptable. Citrate buffer solution with 4.5% acidity and 20 mm concentration was used. The amount of injectable streptozotocin was 55 mg in proportion to each kilogram of the animal body. It was injected in the peritoneal form. After 72 hours, blood glucose was measured, and mice with a blood glucose level greater than 300 mg/dl were determined as type 1 diabetic.

We first dissolved carboxymethyl cellulose in the water at 60° C for 120 minutes, then diethanolamine, PEG, PLGA and finally vanadium was added to them. We then froze the prepared drug and subsequently measured the drug release at different HP and compared them with the pre-prepared drug calibration chart. Carboxymethylcellulose (CMC), Polyethylene Glycol (PEG), Polylactic Glycolic Acid (PLGA), alginate, ethanol, diamine, vanadium, dimethyl sulfoxide (DMSO) and dichloromethane were purchased from Merck Company of Germany. Insulin was also obtained from Exir Company of Birjand.

Since gavage action may cause ulceration and injury to the mouth ceiling of diabetic rats, we dissolved a dose of Nano-insulin in 50 ml of water, and each rat had its dish. Until the 60 ml solution was ingested entirely, the water was not poured into the dishes (the rat were given soluble at night, and they ate it until morning). The amount of Nano-insulin was two units of insulin, contained in the synthesized insulin hydrogel Nanocomposite, per kg of body weight.

In the present study, a 5-band treadmill was used; and for the first time, five rats were placed in it for training. The rats ran for 56 days. After the 5-day period of familiarization, exercise time for the exercise groups were as follows: 20 m/min for the first and second weeks, 25 m/min for the third and fourth weeks and 30 m / min for the fifth and sixth weeks (Table 1).

Forty-eight hours after the last training session and 10-12 hours of fasting, the subjects were anesthetised by intraperitoneal injection of a combination of ketamine (60 mg/kg) and xylzine (5 mg/kg) with a ratio of 5 to 2. Blood samples were obtained and then the blood was poured into EDTA tubes then centrifuged at 3000 rpm for 15 minutes, and then

laboratory measurements were performed. Interleukin-6 and TNF-α were measured using ELISA and mouse-specific ELISA kit (R and D Systems, USA) using the manufacturer's recommended test method.

Table 1. Aerobic exercise protocol.

Time	First Week	Second Week	Third Week	Fourth Week	Fifth Week	Sixth Week	Seventh Week	Eighth Week
Speed (m/min)	15	20	20	25	25	30	30	30
Duration (min)	20	20	25	25	30	30	35	35

Statistical analysis

All statistical operations were analyzed by SPSS software version 23. Descriptive statistics section used indices of standard deviation, mean and graph. Inferential statistics section was used to determine the normality of data distribution using the Kolmogorov-Smirnov test. The similarity of variances was also assessed by the Levin test. Then, intra-group variations and one-way analysis of variance for intra-group changes and Tukey post hoc test were used to examine differences between groups. $P<0.05$ was considered to be significant.

Results

Analysis of inter-group variations for TNF-α values showed a significant difference between various research groups ($P> 0.001$) (Figure 1). Bonferroni post hoc test revealed a significant difference between the group of healthy-control group and groups of diabetes ($P>0.001$), exercise-diabetes ($P>0.001$), nano-diabetes ($P>0.001$) and Diabetes-Nano-exercise ($P>0.001$), between the group of "diabetes" and groups of diabetes-exercise ($P>0.001$), diabetes -nano ($P>0.001$), between the group of diabetes-nano and groups of diabetes-exercise ($P = 0.001$) and diabetes-nano-exercise ($P = 0.001$) and between the group of diabetes -exercise group and the group of diabetes-nano-exercise ($P = 0.001$).

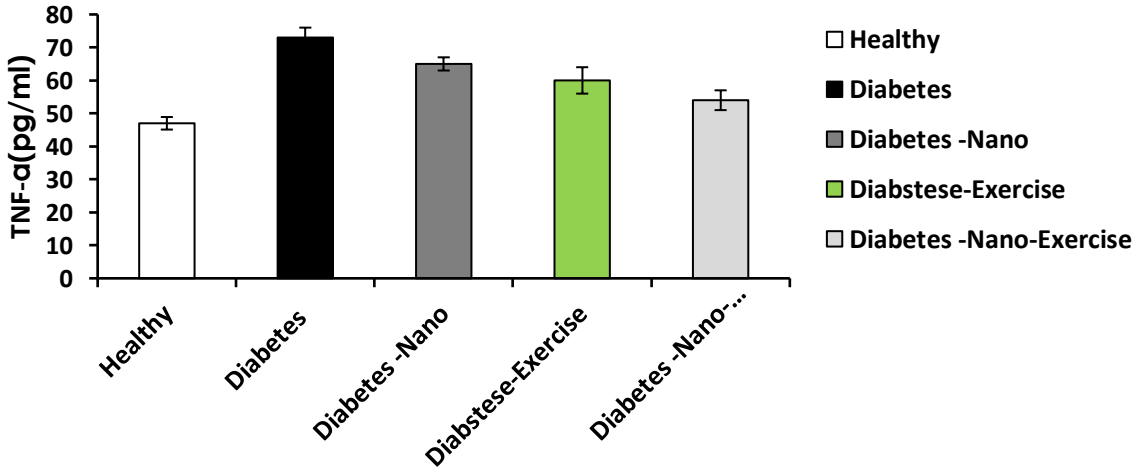

Figure 1. The levels of TNF-α pre- and post-exercise training. The amount of TNF-α has been decreased profoundly in the diabetes -Nano-exercise group

One-way analysis of variance (ANOVA) for investigating inter-group variations for IL-6 values showed a significant difference between the different research groups (P>0.001) (Figure 2). Bonferroni post hoc test revealed a significant difference between the group of "healthy-control" group and groups of diabetes (P>0.001), exercise -diabetes (P>0.001), nano-diabetes (P>0.001) and diabetes-nano-exercise (P>0.001), between the group of "diabetes" and groups of diabetes-exercise (P>0.001), diabetes -nano (P>0.001), between the group of diabetes -nano and groups of diabetes -exercise (P = 0.029) and diabetes-nano-exercise (P = 0.001) and between the group of diabetes -exercise group and the group of diabetes -nano-exercise (P = 0.034).

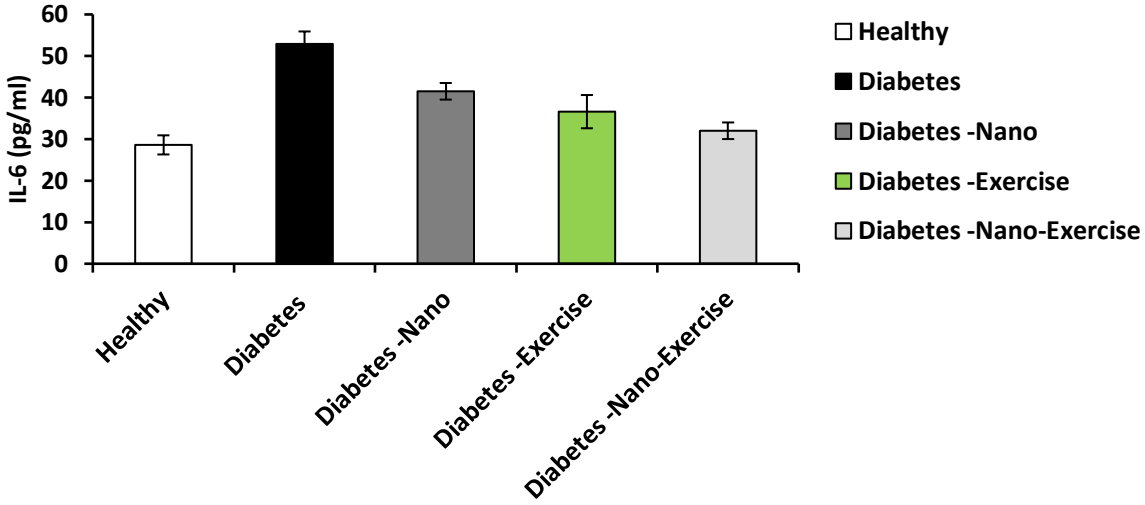

Figure 2. The levels of of IL-6 pre- and post-exercise training. The amount of IL-6 has been decreased profoundly in the diabetes -Nano-exercise group.

Discussion

One of the most important findings of our study was a decrease in TNF-α levels of cardiac tissue in the three groups of Diabetes-Nano, Nano-Diabetes and Nano-Diabetes- Exercise. This decrease was more in the Nano-Diabetic group. According to our findings, research on the evaluation of inflammatory cytokines in cardiac tissue was minimal. Consistent with our findings, 8-week-old female rats were divided into four groups: normal diet, high fat diet, normal diet, and aerobic exercise protocol (swimming for 1 hour in each day for five days a week for eight weeks) with a high-fat diet and aerobic exercise protocol. The results of this study showed high levels of TNF-α in high-fat diet rats. Also, TNF-α decreased significantly in the high-fat diet and exercise groups (18). TNF-α has been shown to be secreted in the heart by myocardial macrophages and cardiac myocytes and contributes to myocardial dysfunction. TNF-α induces pathological changes in the heart by inducing hypertrophy and fibrosis in the heart (19). In diabetic conditions, high blood sugar and free fatty acids cause inflammation in cardiac tissues and increase TNF-α levels (20). Increased TNF-α leads to increased cardiac fibrosis and apoptosis (18). It has been reported that TNF-α is a pro-inflammatory and increases lipid profile by increasing levels of triglycerides and LDL and also leads to obesity (21). TNF-α induces the expression of matrix metalloproteinase, which is involved in the activation of TGFβ1 secreted by myocytes and macrophages. TGFβ1 enhances

collagen I and III synthesis and regulates collagen turnover. Increased collagen alters the stiffness of the ventricular walls and disrupts the normal function of the heart, leading to increased fibrosis and heart failure (18, 22). During exercise, muscles release myokines that are involved in growth, repair, and anti-inflammatory responses (23). IL-6 is the major myokine released in response to exercise, which increases the level of anti-inflammatory IL-10 and decreases the levels of TNF-α (18). IL-10 has been shown to improve cardiac functions by reducing cardiac fibrosis (24). Aerobic exercise training significantly increases IL-10 in cardiac tissues, and previous reports have shown that IL-10 inhibits TNF-α (18, 25). Another finding was a decrease in il-6 levels of cardiac tissue in the three nano-diabetes, nano-diabetes and diabetes -nano- exercise groups. Consistent with our findings, adult male Wistar rats were divided into five groups: Healthy control, diabetic control, healthy with stevia consumption, diabetic group with stevia consumption and diabetic group with metformin consumption. The results showed that Stevia consumption in the diabetic group significantly decreased IL-6 levels in cardiac tissue (26). IL-6 is secreted by stimuli such as viral and bacterial infection, inflammatory cytokines (TNF-α and IL-1), angiotensin II, oxidative stress, and exercise training (27). High concentrations of IL-6 in patients with type 1 diabetes cause a persistent chronic inflammatory process and might negatively affect cardiovascular endothelial cells (28). IL-6 is involved in the pathogenesis of endothelial dysfunction by stimulating cell adhesion cells in diabetics. In diabetic subjects, it has been reported to stimulate IL-6 mRNA expression through TNF-α receptor and p38 mitogen-activated protein kinase, PI3K/Akt pathway, and NF-κB pathway (28). To our knowledge, no study examined the effect of exercise training on IL-6 levels in cardiac tissue. A decrease in TNF-α secretion as a result of aerobic exercise may decrease the IL-6 of cardiac tissue. Another treatment for type 1 diabetes is the use of a Nano-system for insulin administration. Several studies have suggested that in the management of type 1 diabetes, increasing doses of insulin administration are often associated with the risk of sudden hypoglycemia. Therefore, insulin dosages are mainly an important factor in the effective management of the disease. Therefore, one of the main goals of using Nano-insulin is to reduce the dosage of insulin and to help release insulin from the Nanocapsule to make it more effective. Concerning the positive effects of Nano-insulin on the control of diabetes, Nanoparticles containing tablets led to post-meal blood glucose control in diabetic pigs in a study (29). Also, in another study, the muscle cells were exposed to insulin or Nano-insulin for 30 minutes after exposure to sodium articide. The results showed that both insulin and Nano-insulin improved mitochondrial functions, but Nano-insulin, at a dosage of ten times less than insulin, showed a more significant effect (17). According to several studies, the main goals of insulin Nanolin are mainly on the different glucose carriers (GLUTs) present in the pancreas, muscles, brain, etc. which primarily transport glucose to several organs to maintain glucose homeostasis in the body. (1). Samadder et al. (2013) reported that Nano-insulin could modulate GLUT expressions levels more effective than insulin in diabetic patients and also increase their expressions levels to the normal condition. Even the mitochondrial signaling pathway that is adversely influenced by diabetic conditions was affected by Nano-insulin compared with insulin (16, 17). Nanoseculin administration is associated with an increase in the expression of insulin receptor substrates 1 and 2, indicating its ability to bind to insulin receptors. Therefore, it may increase the level of phosphorylation and be used to restore insulin secretion (16). Concerning other effects of Nano-insulin capsules, these tablets have been reported to protect insulin

from entering the stomach and keep blood sugar stable for 10 hours. The Nanoparticles absorb water and, when they reach the bloodstream, break down in response to blood PH and then release insulin (29). It is unclear, however, whether long-term Nano-insulin can prevent cardiac cardiomyopathy in type 1 diabetes.

Conclusion

The results of this study showed that a period of aerobic exercise training and synthesized insulin Nanocomposite hydrogels significantly decreased the levels of TNF-α and IL-6 in the heart tissue of type 1 diabetic rats and this reduction was more significant in the Nano-diabetic group. According to studies that have suggested changes in blood glucose levels as one of the triggers of these cytokines, it may be concluded that Nano-insulin may have an important role in these mechanisms and it can be proposed as a novel method for the treatment of type 1 diabetes.

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