Effects of Eight Weeks Aerobic Exercise on Plasma Levels of Orexin A, Leptin, Glucose, Insulin, and Insulin Resistance in Males with Type 2 Diabetes

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Abstract

Objective: The recent study aimed to investigate the effects of chronic aerobic activity on the plasma levels of orexin A, leptin, glucose, insulin, and insulin resistance in males with type 2 diabetes.

Materials and Methods: Twenty subjects randomly assigned into control and experimental groups, involving 10 people in each group. Exercise protocol consisted of eight session aerobic exercise. Each session was done between 15 to 30 minutes so that in earlier weeks started with 50% of maximum heart rate for 15 minutes per day and continued with 80% of maximum heart rate for 30 minutes per day in last week. The heart rate was measured using heart rate monitor. Before the start of training and 48 hours after the last training session, blood samples were taken from both groups.

Results: the mean age of participants was 45.40 ± 5.42 year, mean weight was 80.91 ± 6.35 kg, the mean of body mass index was 25.41 ± 2.76 kg/m^2.The analysis of findings in the $P \leq 0.05$ indicated that chronic aerobic exercise caused a significant increase in plasma leves of orexin A and significant decrease in insulin and insulin resistance, and leptin.

Conclusion: Chronic aerobic exercise can decrease the insulin resistance in people with type 2 diabetes via increasing the plasma levels of orexin A and decreasing in plasma level of glucose and insulin.

Keywords: Aerobic activity, Orexin A, Leptin, Insulin, Insulin resistance, Type 2 diabetes

Introduction

The body's hormone system disruptions are the main factors in metabolic disorders such as insulin resistance and type 2 diabetes that is due to lifestyle changes, obesity, and aging process (1). Leptin is one of these hormones that is secreted from adipose tissue and play an important role in regulating of energy consumption and metabolism. Different studies showed that the lack of leptin causes obesity and its injection increases the energy consumption and decreases desire to take food (2). Leptin reduces glucose levels in the blood. It is reported that leptin concentration in serum is associated to insulin concentration and insulin sensitivity. Accordingly, it is thought that leptin has a role

in the etiology of insulin resistance and type 2 diabetes (3).

In addition to well-knowned regulating role of leptin in energy homeostasis, there are growing evidence indicated leptin rolesin regulation of glucose homeostasis, particularly in control of peripheral tissues insulin sensitivity (4). The earliest evidence comes from studies in mice with genetic leptin deficiency or leptin receptor deficiency. These animals displayedinsulin resistance and diabetes in addition tohyperphagia and obesity (5). Several observations suggest that leptin regulates glucose metabolism, independently of its effect on energy balance. It is found that leptin administration to leptin-deficient mice or humans improves hyperglycemia and hyperinsulinemia (4). Interactive effects of leptin and insulin resulted from changes in blood glucose are expressed as adipoinsular axis. This means that enhanced glucose levels increases insulin secretion and increases the secretion of leptin, in turn. In this axis, leptin directly and indirectly affects insulin secretion and reduces it (6).

Other effective hormone that affect the body's metabolic process is Orexin or Hypocretin. This hormone is produced by a very small population of neurons located in and around the hypothalamic lateral nuclei (LH), however the axons from these neurons extend throughout the brain and spinal cord (7). Orexin found in two types; OrexinA (OXA) and Orexin B (OXB) that are 33 and 28 amino acid residues long, respectively, and are made by the activity of prepro-orexin (PPO) genes. Researches showed that OXA may be of greater biological importance than OXB (5). OXA has inevitable role in glucose metabolism so that decreasing in OXA lead to dysfunction, glucose tolerance and insulin resistance in non-obese male mice and almost obese female mice (8). Skrzypski et al. suggested that OXA increased glucose uptake by elevated translocating of glucose transporter GLUT4 from cytoplasm into the plasma membrane (9). Recent evidence suggests that sufficient amounts of orexin in mice protects the body against the development of insulin resistance caused by aging. Also, it is known that OX2R signaling resists against diet-induced obesity and reduced insulin sensitivity by improving leptin sensitivity (10). Miyasaka et al. (2002) reported that OXA intracerebral injection stimulates insulin secretion in the rat (11). Some studies suggested that reduction in blood glucose resulting from insulin injectionsin brain increases the mRNA of OXA precursor in the lateral hypothalamic area. In addition, it is known that stimulation of the OXA secretion and its role in increased desire to take food is because of reduction in glucose levels. On the other hand, increased levels of plasma leptin, which is a sign of increasing energy reserves of adipose tissue, inhibits the secretion of OXA. Probably the effect of insulin on the hypothalamic expression or secretion of orexin is dependent on glucose concentration (6,12). Since OXA is an effective factor in integrating peripheral metabolism, central regulation of behaviors and maintenance of energy homeostasis (13), it can affect by factors that change energy homeostasis. Given that physical activity increased the activity of cardiovascular, breathing and the energy-generating systems, affecting OXA secretion (14). This has been observed in several studies in non-human samples in order to evaluate the role of physical activity in OXA secretion (15-17). Recent evidence indicated that the disruption in production of OXA is one of the effective factors in aging-induced glucose homeostasis and diabetes (1). Since, there are a few studies that investigate the effect of physical activity on OXA changes in human samples.In addition, we found no research that demonstrated the effect of physical activity on OXA and adipoinsular axis. Accordingly, it is necessary to investigate the effect of physical activity on these hormones. Therefore the present study was done to evaluate the effect of chronic aerobic exercise on plasma levels of OXA, leptin, insulin, glucose, and insulin resistance in males with type 2 diabetes.

Materials and Methods

Twenty patients with type 2 diabetes among the males admitted to Ahwaz Golestan hospital were selected by random sampling. They were between 40 and 50 years old. The inclusion criteria were, having blood sugar in the range of 126 - 200 mg/dl, No history of diabetes over five years, not smoking and any other drugs addiction or medication, not having any particular disease such as cardiovascular, respiratory, kidney, and hypertension diseases, as well as do not use insulin and having no diabetes complications such as peripheral vascular disease and diabetic foot ulcers. The subjects were randomly assigned into experimental and control groups, involving 10 people in each group. In a briefing participants called and preparations such as completing a questionnaire on demographic information, explaining about the investigation, collecting their consent, cooperation schedule, understanding how to complete the dietary recalls questionnaire during the investigation and so on was done. Before and after the main activity, anthropometric measurements (weight and height) and body composition (BMI, body fat percentage) were done for each subject in the laboratory. Subject's fat percentage measured with bioelectrical impedances (BIA) made in South Korea using bioelectrical method. Maximum oxygen consumption (VO_{2max}) were measured using a modified Bruce test (18).

Each session was done between 15 to 30 minutes so that in earlier weeks started with 50 % of maximum heart rate for 15 minutes per day and continued with 80 % of maximum heart rate for 30 minutes per day in eighth week. The heart rate was measured using heart rate monitor. Since nutrition is a factor affecting the hormonal changes, the participants were asked not to change their nutrition program and avoid taking nutritional supplements during the study. In this study, all patients received the same nutritional advices by nutritionists. they were asked to refrain from doing other exercise activities, and do not change their routine diet. The nutritional status were measured through 24-hour dietary recall forms in a week before activity, fourth, and last week of activity, in terms of intake energy levels and energy expenditure and required feedbacks was provided to them.

First sampling was done in fasting at 8 a.m. from all participants to measure the plasma levels of OXA, leptin, glucose, insulin and insulin resistance. Blood samples was obtained from capital vein of participants by 5 cc. In addition, 48 hours after last session of chronic physical activity second sampling was donein fasting to measure the plasma levels of OXA, leptin, glucose, insulin, and insulin resistance. Blood samples were withdrawn into heparinized tubes containing anticoagulant citrate or EDTA 5% and gently shake in order to prevent clotting. Heparinized whole blood samples were immediately centrifuged at 200 to 300 rpm in 4 °C for 20 minutes. Immediately after isolation, plasma samples were stored at -80°C until measuring variables. Glucose concentration were measured using the enzymatic colorimetric method (glucose oxidase, Pars test, Tehran, Iran) and the Auto analyzer Selectra 2. Leptin was measured using double-antibody technique by a kit from Boster company. Insulin was measured by ELISA using Monobind kits. OXA also measured by ELISA using Phoenix Pharmaceutical, INC kits. Insulin resistance was calculated using the following HOMA-IR formula (19).

HOMA-IR = [fasting glucose (mmol/dL) × fasting insulin (mU/L) / 22.5]

Statistical analysis

The normal distribution was tested by Kolmogorov-Smirnov. The results were analyzed by Shapiro-Wilk test followed by independent T-test to evaluate the significance of the difference between two groups and correlated T-test to evaluate the significance of the difference between pre-test and post-test. These statistical tests were performed using Statistical Package for Social Science (SPSS 22.0 for windows). A significant change was accepted at P-value$\leq$0.05.

Results

As shown in Table 1, inexperimental group chronic aerobic activity caused a significant reduction in the weight and a significant increase in maximum oxygen consumption. However there were no significant change in other indexes in both groups.

Table 2 demonstrates the plasma levels of OXA, leptin, glucose, insulin, and insulin resistance in control and experimental groups before and after chronic aerobic exercise. Chronic aerobic exercise causeda significant increase in OXA, and a significant decrease in glucose, insulin, and insulin resistance, and leptin.

Discussion

Findings showed that eight weeks of physical activity resulted in a significant increase in plasma levels of OXA, significant decrease in plasma levels of glucose, insulin, and insulin resistance, and no significant effect on plasma levels of leptin.

Studies reported that aerobic physical activity through two simultaneous events, that is decreasing in weight and increasing in GLUT4 expression in the skeletal muscles, can increase insulin sensitivity in subjects with insulin resistance (20). The molecular mechanisms that increase glucose uptake and reduce the insulin resistance with physical activity, involves key signaling proteins regulating glucose uptake in skeletal muscle. Recent evidences suggested that exercise-induced reduction in insulin resistance is associated with elevated effective proteins in insulin signaling such as AMP-activated protein kinase (AMPK). AMPK is a intracellular metabolic sensor that regulates glucose hemostasis by acting upon insulin sensitive tissues (21,22). In insulin resistance, insulin signaling pathways may be disturbed due to multiple processes including changes in the levels of proteins, enzymes, transcription factors and signaling molecules involved in it,and Insulin did not function properly on its target tissue. In such cases, usually due to dysfunction of the insulin receptors, glucose transporter GLUT4 are not stimulated in order to transfer them to the cell membrane and insulin resistance occur. This process can lead to type 2 diabetes, if it is not treated in early stages (23-25). Exercise can increase insulin sensitivity by impacting on insulin substrate receptors (IRS-1, IRS-2) and/or activation of phosphatidylinositol 3-kinase. Increasing in

Table 1. Anthropometric, physiological and physical composition of the participants before and after chronic aerobic exercise.

Variables	Control (mean ± SD)		Experimental (mean ± SD)	
	before	after	before	after
Age (year)	46.60 ± 5.42	46.60 ± 5.42	44.20 ± 3.70	44.20 ± 3.70
Height (cm)	174.98 ± 6.39	174.98 ± 6.39	179.00 ± 5.03	179.00 ± 5.03
Weight (kg)	79.30 ± 6.35	79.74 ± 4.73	82.53 ± 5.47	78.32 ± 5.45*
Body fat (%)	32.82 ± 3.82	33.02 ± 2.32	30.27 ± 3.70	29.01 ± 1.34
BMI (kg/m^2)	26.02 ± 2.62	26.43 ± 1.80	24.81 ± 2.76	23.67 ± 1.63
Waist-hip ratio (m)	0.96 ± 0.42	0.96 ± 0.61	0.94 ± 0.50	0.93 ± 0.80
VO_{2max}(ml/kg/min)	35.09 ± 4.07	35.86 ± 4.15	37.09 ± 3.03	41.05 ± 4.18*

* Significant difference in $P \leq 0.05$.

Table 2. Plasma levels of OXA, leptin, glucose, insulin, and insulin resistance before and after chronic aerobic activity in control and experimental groups.Data are expressed as mean ± SD.

Variables	Control group (mean ± SD)		Experimental group (mean ± SD)	
	before	after	before	after
OXA (ng/ml)	50.87 ± 3.28	51.28 ± 1.31	52.96 ± 1.15	55.13 ± 1.16*
leptin (ng/ml)	9.41 ± 1.18	9.46 ± 1.18	8.02 ± 1.18	6.21 ± 1.16*
glucose (mg/dl)	163.10 ± 16.76	157.80 ± 15.11	157.70 ± 16.68	144.30 ± 13.01*
insulin (IU/ml)	12.52 ± 2.23	13.95 ± 2.51	11.04 ± 1.92	8.52 ± 1.25*
Insulin resistance	5.64 ± 0.66	6.10 ± 1.28*	4.26 ± 0.06	3.10 ± 0.50*

* Significant difference in $P \leq 0.05$.

the sensitivity of insulin receptors initiates a complex cascade of phosphorylation and dephosphorylation reactions, leading to widely metabolic and mitogenic effects of insulin. For example, activation of phosphatidylinositol 3-kinase induces the translocating of glucose receptors (GLUT4 etc.) to the cell surface, resulting in glucose uptake in muscle cells (26,27). Our findings showed thatchronic aerobic activity increases the plasma levels of OXA. Studies suggested that OXA prevented the insulin resistance in liver by affecting on central nervous system (28). Also, some studies have shown that OXA increases the activity of the AMPK and UCPs, increasing in glucose utilization, fatty acid oxidation, mitochondrial biogenesis, and insulin sensitivity, resulting in weight loss and reducing insulin resistance (21,29-31).

In addition, OXA increased glucose uptake by elevated translocating of glucose transporter GLUT4 from cytoplasm into the plasma membrane (9). Other evidences suggested that plenty of OXA in mice protects the body against the development of insulin resistance caused by aging. Furthermore, orexin receptors signaling (OX2R) resists to diet-induced obesity and decreases insulin sensitivity by improving leptin sensitivity (10). Exercise-induced increasing in orexin can also improve the function of brown adipose tissue, increases metabolic thermogenesis, and prevents weight gain and metabolic disorders such as insulin resistance (32). In addition to direct impact of physical activity on plasma levels of orexin, the resulting weight loss can also be effective in increasing of orexin, thus obese subjects have high levels of orexin and low levels of leptin as compared with lean people (33).

Our findings showed a decrease in plasma levels of leptin after aerobic exercise in males with type 2 diabetes. Studies reported that aerobic exercise reduces the plasma levels of leptin through decreased body fat levels and negative energy balance (34). In agreement with this study, we found a reduction in the levels of body fat together with plasma levels of leptin. According to Gomez et al. decreased levels of glucose can reduce leptin secretion in adipose tissue (35). Exercise-induced reduction in leptin may be due to decreased plasma levels of glucose and negative energy balance (36), and can increase catecholamines, and decrease insulin (37). Here we found a decrease in both insulin and leptin. Researches observed that endurance training reduces ob gene mRNA in mice (38). Insulincan affectthe expression ofob geneand therebycontrol theplasma levels ofleptin (39). So insulin and leptin have reciprocal effects. In addition insulin and leptin are sensitive to changes in blood glucose, as found in adipoinsular axis (35,40). In the recent study, these simultaneous changes were observedin leptin, insulin and glucoseas a result of chronic aerobic activity. Moreover, changes in plasma levels of OXA were observed along with leptin changes in adipoinsular axis. Researches suggested that orexin receptor signaling can prevent obesity and increases leptin sensitivity (11). So it is possible that reduction in leptin secretion as a result of leptin sensitivity is due to increase in plasma levels of OXA.

Blood glucose reduction in the brain as a result of insulin injections increases mRNA of OXA precursors in the lateral hypothalamus. Stimulation of OXA secretion and its role in increased desire to take food is because of reduction in glucose levels. On the other hand, increased levels of leptin, which is a sign of increasing energy reserves of adipose tissue, inhibits the OXA secretion. Leptin can decrease insulin secretion in the adipoinsular axis using autonomous nervous system by inhibiting parasympathetic system or activating sympathetic system. In addition insulin effects on hypothalamic expression of OXA or its secretion is depend on glucose concentration (41,42). Taken together, it seems that changes in blood glucose as a result of physical activity is initial point of changes in orexin cycle and adipoinsular axis, because orexin, leptin, and insulin are hormones that regulate the body's energy balance, and react with nutritional status of the body.

Our research including the limited researches that evaluate the effect of chronic aerobic exercise on changes in OXA and adipoinsular axis. It is recommended to do similar research with longer time and greater number of subjects.

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