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Effect of endurance training on plasma levels of AGRP and HOMA-IR in diabetic rats

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Abstract. The hypothalamus is a strong central controller of appetite that secretes different neuropeptides including AGRP. Plasma levels of AGRP are effective in controlling obesity and hunger. Therefore, the current study was performed with the aim of investigating the effect of endurance training on plasma levels of AGRP and HOMA-IR in diabetic rats. The Current study was experimental by posttest and control group. Eighteen male Wistar rats (200-220 grams with 8-10 weeks) were randomly divided into the control group and diabetic training. Eight weeks endurance training program included in the group of animal diabetic training for 5 days per week (15-40) minutes at 50 to 65 percent of vo2max. To determine the serum concentrations of AGRP was used by ELISA. A comparison of two groups showed significantly increased plasma concentrations of AGRP (p=0.006) and insulin resistance index, decreased significantly (p=0.002) compared to the control group after eight weeks, endurance training. According to the results, increased plasma concentrations of AGRP can be attributed to the negative balance caused by training. This agent destroys the body's energy balance and hypothalamus for balancing increases the secretion of AGRP. This neuropeptide is likely will cause higher fat metabolism.

Keywords. Agouti-related protein, endurance training, insulin resistance, diabetes.

Introduction

Insulin is associated with energy storage materials. Defects in intracellular events after insulin binding agent in patients with type 2 diabetes are insulin resistance and response to insulin will be impaired (Gorgisen et al., 2015). Diabetes is one of the most common chronic metabolic disorders. The twenty-third International Congress on world diabetes federation held in 2016 in Vancouver, Canada. In this congress statistics of over 400 million people with diabetes in the world reported that unfortunately, according to the International Diabetes Federation, 48% of the inhabitants of the Middle East, including Iran, have diabetes without the diagnosis. In other words, out of every two people, one person is unaware of his illness (World Diabetes Congress, 2015). Nowadays the incidence of this disease has been increasingly more in the world because of reducing the amount of activity and increasing obesity.

HPA (hypothalamic-pituitary-adrenal gland cortical) is a collection of neuroendocrine that regulates vital functions and thus ensures survival (Luzi, 2012). Hypothalamus and its neural circuits play an important role in the regulation of feeding behavior and body weight gain. The main areas of the hypothalamus, that are involved in food intake and energy expenditure, are arcuate is nucleus (center of satiety) and ventricular adjacent cells (hunger center). Arcuate nucleus is the place of important areas in response to hormonal messages such as insulin and leptin.

There are two types of main neurons in the arcuate nucleus. They are sensitive to nutritional status and one of them is anti-appetite neuropeptides (POMC, CART, and CRH) and the other is appetite stimulant neuropeptide (NPY, AGRP, MCH, Orexin, and

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Galanin) that are essential to regulate food intake and energy expenditure (Levin & Dunn-Meynell, 2004; Luzi, 2012). So it seems that energy regulations in the body are integrated by the hypothalamus (Luzi, 2012). The most important peptide known to appetite is Agouti-Related Protein or AGRP that specifically expressed in humans and rats in the arcuate nucleus of the hypothalamus (Hosseini-Khakhak et al., 2009). AGRP is a powerful peptide that stimulates appetite and increases food intake. In humans, AGRP polymorphism is always has been associated with resistance to whites and blacks obesity and diabetes type II resistance in black Africans (Ilnytska & Argyropoulos, 2008).

AGRP inhibits the melanocortin receptors that causes food intake and body weight gain (Sternson & Atasoy, 2014). With regard to the role of physical activity in increased level of activity and weight loss, most people who control their diabetes, exercise regularly.

Actually, physical activity is one of the three bases of diabetes treatment in addition to diet and medications (Stenlöf et al., 2012). During physical activity, due to the release of specific proteins identified (GLUT1, GLUT2, GLUT3, GLUT4, GLUT5) that are released when muscle contractions, glucose transport increased into muscle fibers (Santer & Klepper, 2016).

Studies have shown that physical activity is one of the factors in the analysis of cellular energy sources such as glucose and glycogen that can be effective peptides including AGRP changes in the regulation and create balance.

The imbalance between food intake stimulators and inhibitor peptides as factors involved in the mechanism could increase the percentage of body fat, obesity and overcome to the process of appetizing on anorexia.

History reviews show contradictory findings associated with markers of this study. Also due to AGRP role in energy balance and the importance of energy balance in the prevention and treatment of diabetes management necessity, it seems be necessary to perform the study by the effect of moderate-intensity endurance training and also can AGRP have a positive impact on the plasma concentration of people with diabetes and serum levels of these peptides or not?

The present study was performed with the aim of determining the effect of endurance training on plasma concentration Agouti-Related Protein and insulin resistance index in diabetic male rats.

Materials & Methods

Subjects

This study was experimental with posttest control group. 18 male Wistar rats (weight 10±200 g) and the age of eight weeks were prepared from the center of propagation and breeding of laboratory animals Baghiatallah in Iran. These animals were kept in the laboratory, under controlled conditions of temperature (22°C±2), humidity (between 25 to 30 percent) and light-dark cycle 12:12. All the animals had access to especially rat food and water, freely. To ensure proper monitoring of environmental conditions circadian humidity, temperature and ventilation (Modification of environment pollution and reduction of odor and the risk of respiratory disease in animals) were used for the air conditioning system (In line with the chill laboratory animals used for scientific purposes and laboratory support). After two weeks familiarity with the new environment, the rats were randomly divided based on body weight (the matching method) to the endurance training group (n=9) and control (n=9) group.

Diabetes induction

In order to induce diabetes in rats, the combination of streptozotocin (STZ) and nicotinamide intraperitoneally at a rate of 95 mg per kg nicotinamide were dissolved in saline solution and after 15 min of 55 mg kg STZ in citrate buffer solution was 1.0 mM and by ph of 5.4 injected intraperitoneally.

Five days after injection, a drop of blood was placed on the glucometer kit with a small wound in the tail of animal by the Lancet. The criterion for being diabetic was blood glucose levels higher than 250 mg per dL.

Measurement of maximum oxygen consumption

Measurement of maximum oxygen consumption was used according to the research conducted by Hoydal et al. (2007) indirectly but by high credit. Accordingly, after ten minutes of low- intensity warm-up, the test began with rats running on a treadmill at a speed of 15 meters per minute for 2 minutes and speed increased every two minutes to 0.03 meters per second to reach to the failure. Thus Maximum oxygen consumption was calculated.

Training protocol

The rats in endurance training group performed an increasing training for eight weeks and 5 days a week (Table 1).

Training protocol's speed, increased of 10 meters per minute (first week) to 25 meters per minute (six weeks). Initial speed was 15 minutes and increased to 45 minutes and the slope was zero. In addition, 5 minutes of total time considered for warm up and 5 minutes for cool down. It should be noted that all environmental conditions for the control group were similar to the training group, except main training protocol in the testing day.

Sample collection

At the end of eight weeks and after 12 hours of fasting, the rats were weighed. They were anesthetized with a combination of ketamine and xylazine and after the splitting of the abdomen and chest, blood samples were taken directly from the heart. To prevent the hemolysis of blood samples, poured in tubes containing EDTA and gently mixed. In order to separate the blood plasma, was centrifuged For 15 min at -4°C at a speed of 3000 rpm and for next measurement, at -80°C were kept.

Measurement of biochemical variables

Plasma concentrations of Agouti-Related Protein (AGRP) was measured by ELISA ZellBio (GmbH, Germany) kit, GmbH by a factor of Sensitivity of 1.1 pg/ml and CV% equal to 6.4 for male rats according to the manufacturer's instructions. Plasma levels of insulin by using the ultrasensitive rat insulin ELISA kit (Crystal Chem, USA) and the sensitivity of 0.5 ml micro units and glucose levels were determined by using photometric methods by using the human standard kit (Pars Azmoon Inc, Iran) with a sensitivity of 1 mg per deciliter. Insulin resistance index HOMA-IR were measured using the following homeostasis equation (10):

Insulin resistance = [plasma insulin (micro unit / dl) × plasma glucose (mmol / L)] / 22.5

Statistical analysis

The collected data were analyzed by SPSS version 20. After determining normality by the Shapiro-Wilk test and verifying data, in order to compare variables between two groups, independent t-test was used at the significant level $p<0.05$.

Results

In Table 2, the average of blood factors examined in this study has been put and also the result of independent t-tests, are shown separately.

According to the results, after eight weeks of endurance training concentrations of plasma AGRP in diabetic rats of 201.22 ± 27.99 and in the control group, 169.56 ± 11.70 nanograms per liter were shown.

Figure 1 shows, the comparison of the groups after eight weeks of endurance training. The plasma AGRP increased significantly compared with the control group.

After eight weeks of endurance training in diabetic rats, insulin concentrations was 0.206±0.02 microunit/dl and plasma glucose concentration was 276.88 ± 27.59 mmol per liter and in the control group respectively was 0.235 ± 0.02 and 324.55 ± 16.04.

Table 1
Endurance training protocol on treadmill.

Weeks	1	2	3	4	5	6	7	8
Duration (min)	15	15	20	20	30	30	40	40
Speed (m/min)	10	10	15	15	20	20	25	25
VO_2max (percent)	50	50	55	55	60	60	65	65

Table 2
Mean and standard deviation of variables in studied groups.

	Glucose (mmol/L)	Insulin (mU/L)	HOMA-IR	AGRP (ng/L)
Endurance training (n=9)	276.88 ± 27.59	0.206 ±0.02	2.55 ±0.495	201.22 ± 27.99
Control (n=9)	324.55 ± 16.04	0.235 ± 0.02	3.40 ±0.468	169.56 ±11.70
Significance	p=0.001§	p=0.033	p=0.002§	p=0.006§

§ Significant difference with control group with independent t-test.

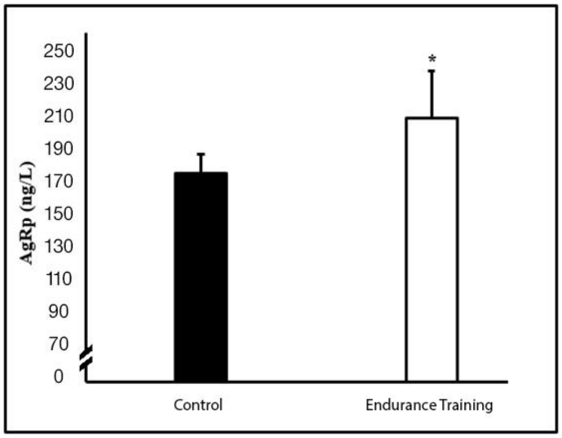

Figure 1. Amount of plasma AGRP**;** * Significant difference with independent t-test.

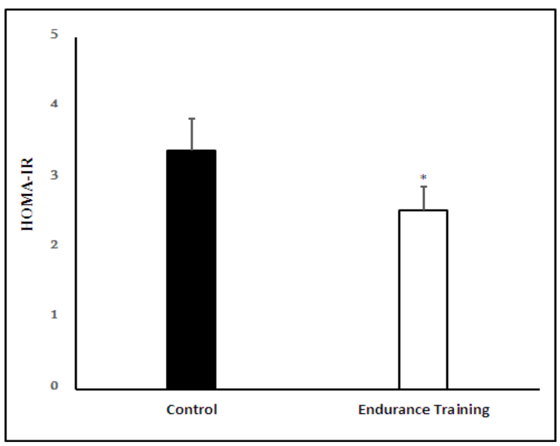

Figure 2. HOMA-IR index; *Significant difference with independent t-test.

Finally, insulin resistance index with homeostasis equation 2.55±0.495 in the training group and 3.40±0.468 in the control group were obtained. These results in Figure 2 in compared with control group, shows the significant decrease in insulin resistance after eight weeks of endurance training.

Discussion

The results showed that after eight weeks of endurance training, levels of blood glucose, insulin, and insulin resistance in the training group has decreased compared to the control group and the amount of AGRP has increased. It can be suggested that endurance training increases levels of AGRP in diabetic rats and this issue has a negative balance on their energy intake. Because AGRP neuropeptide is a strong stimulator of appetite and if it not controlled, can lead to obesity. Since diabetes is characterized by hyperglycemia due to the lack of absolute or relative insulin, As a result it can be said in some chronic diseases such as diabetes that weight gain is one of the causes, so it can lead to change the process of some neuropeptides of hypothalamus and can be effective on weight control so that weight changes is very important in this disease.

Levin & Dunn-Meynell (2004) examined the effects of calorie restriction and physical activity on the wheel and neuropeptide expression in the hypothalamus of rats and the results showed a significant increase in the expression of AGRP in the training group in compared with sedentary control group with no calorie restriction. Hosseini-Kakhak et al. (2009) examined the effect of endurance training on AGRP gene expression in skeletal muscle of rats and then he told that endurance training can lead to upregulation of AGRP gene in skeletal muscle.

Rashidlamir et al. (2010) after eight weeks circuit training said that eight weeks training increased significantly in gene expression of lymphocyte AGRP and concluded that increasing of AGRP gene expression can express this peptide in the hypothalamus and also in response to the negative balance of energy induced by circuit training for increasing the appetite seems to compensate the energy sources of subjects that are similar to the results of this research. On this issue, it can be said since the fuel in endurance training is glycogen, may reduction of muscle glycogen stores could show the negative energy balance and transmit to the brain as the signal till the brain by secreting of AGRP, stimulate loss of deposits of glycogen and food intake behavior, so can cause this increase in level of AGRP.

Abdi et al. (2014) in the research, on the effect of using glucose on response of AGRP after a single session of endurance training, they observed no significant increase in AGRP immediately after training but in 90 minutes after the training, the levels of plasma AGRP in the group of glucose consumer compared to water consumption was decreased significantly. They concluded that oral glucose by increasing insulin levels can decrease the increased levels of AGRP after endurance training that contrasts with results in this research. However, this study only evaluated the effect of training on changes of AGRP and the interference of matter intake, haven't been done, which is probably due to the antithetic results.

However, Levine et al. (2004) demonstrated that, although 6 weeks running on a treadmill causes a significant decrease in rat's body weight, but the training hadn't a significant effect on the expression of AGRP which wasn't consistent with results of the current study. Regarding changes of AGRP after the training, in some studies, it has been reported that this peptide increases in negative energy situations. Shrestha et al. (2006) and Chen et al. (2004) in their studies, found that NPY and AGRP incidences metabolic and caloric changes by a bunch of secreted common neuron and very similar behavior. According to the physiological roles of these peptides, it can be said that these peptides increases in order to prevent the process of catabolism induced by training and increasing the process of anabolic after the training. This may help to restore carbohydrate stores and glycogen replenishment (Shrestha et al., 2006; Chen et al., 2004).

Generally, the results have shown that using oral glucose can decrease the increasing of AGRP induced by training (Luzi, 2012; Abdi & Abbasi, 2012). Maybe increasing AGRP of lymphocytes is because of increased insulin and leptin induced by using glucose. However, Xiaosong et al. (2008) showed that glucose maybe through the effect of leptin on hypothalamic neurons; control the secretion of them. In some studies, it has been shown that, by increasing the levels of glucose, the level of leptin increased which has been associated with the amount of AGRP and in the lower levels of glucose, the levels of leptin decreases which in turn have led to an increase in the amount of AGRP. Because of this correlation, the measurement of blood glucose and insulin with this peptide appears to be necessary. In this study, it was observed that after eight weeks the levels of blood glucose and insulin, has been decreased and because of this reason, insulin resistance index which measured of homeostasis, also reduced. Insulin is the main controller of blood glucose levels which the secretion of this, largely depends on blood glucose. However, the response of beta cells to glucose depends on body fat (Woods et a., 2004). Insulin receptors are in specific areas in the brain that are involved in the control of energy homeostasis including, within the hypothalamus. Inside the hypothalamus, the arcuate nucleus has the highest density of insulin receptors. Insulin resistance is the most important factor for diabetes and has a strong association with obesity and cardiovascular disease. On the other hand, physical activity increases the expression of GLUT4. Thus increasing of GLUT4 can decrease glucose and blood insulin in diabetes. Probably in this study, increasing in GLUT4 due to the adaptation of endurance training can be the reason of increased AGRP.

Conclusion

In this study, changes of AGRP in response to eight weeks endurance training were examined. Further researches in this field, were studied this peptide with training by high intensity or resistance training. Very few studies examined with moderate endurance training and it hasn't been studied in the diabetic with this protocol. Generally, it seems that probably improving in insulin resistance with endurance training is an effective factor in increased levels of plasma AGRP in diabetic rats. Perhaps this study was one of the studies in effect of endurance training in diabetic rats. Therefore more studies are needed to determine the mechanism of effect of endurance training on AGRP associated with insulin resistance after training.

References

Abdi A, Abbasi A. The response of Agouti-Related Protein (AGRP) after a single session of aebobic training in untrained men. Journal of Sports Medicine and Physical Fitness, 2014; 1(2): 37-46.

Chen HY, Trumbauer ME, Chen AS, Weingarth DT, Adams JR, Frazier EG, Shen Z, Marsh DJ, Feighner SD, Guan XM, Ye Z, Nargund RP, Smith RG, Van der Ploeg LH, Howard AD, MacNeil DJ, Qian S. Orexigenic action of peripheral ghrelin is mediated by neuropeptide Y and agouti-related protein. Endocrinology, 2004; 145(6): 2607-2612.

Gorgisen G, Balci MK, Celik FC, Ozes ON. Differential actıvation and expression of insulin receptor substrate 1 (IRS1) in mononuclear cells of type 2 diabetes patients after insulin stimulation. Cellular and molecular biology (Noisy-le-Grand, France), 2015; 62(2): 25-30.

Hosseini-Khakhak SA, Ghanbari Niaki A, Rahbarizadeh F, Mohagheghi MA, Khabazian BM, Fathi R, Hedayati M. The effect of treadmill running on plasma and muscle agouti-related protein (AGRP) concentration in male rats. Iranian Journal of Endocrinology and Metabolism, 2009; 11(4): 455-461.

Ilnytska O, Argyropoulos G. The role of the Agouti-Related Protein in energy balance regulation. Cellular and molecular life sciences, 2008; 65(17): 2721.

Levin BE, Dunn-Meynell AA. Chronic exercise lowers the defended body weight gain and adiposity in diet-induced obese rats. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 2004; 286(4): R771-R778.

Lin CY, Higginbotham DA, Judd RL, White BD. Central leptin increases insulin sensitivity in streptozotocin-induced diabetic rats. American Journal of Physiology-Endocrinology and Metabolism, 2002; 282(5): E1084-E1091.

Luzi L. Cellular Physiology and Metabolism of Physical Exercise, 2012.

Ma X, Zubcevic L, Ashcroft FM. Glucose regulates the effects of leptin on hypothalamic POMC neurons. Proceedings of the National Academy of Sciences, 2008; 105(28): 9811-9816.

Rashidlamir A, Ghanbari-niaki A. Effect of 8-week circuit training on lymphocyte AGRP gene expression in well-trained wrestlers. Daneshvar, 2010; 18(89): 67-72.

Santer R, Klepper J. Disorders of glucose transport, in Inborn metabolic diseases. 2016, Springer. p. 175-183.

Shrestha YB, Wickwire K, Giraudo SQ. Role of AgRp on Ghrelin-induced feeding in the hypothalamic paraventricular nucleus. Regulatory Peptides, 2006; 133(1): 68-73.

Stenlöf K, Cefalu WT, Kim KA, Alba M, Usiskin K, Tong C, Canovatchel W, Meininger G. Efficacy and safety of canagliflozin monotherapy in subjects with type 2 diabetes mellitus inadequately controlled with diet and exercise. Diabetes, Obesity and Metabolism, 2013; 15(4): 372-382.

Sternson SM, Atasoy D. Agouti-related protein neuron circuits that regulate appetite. Neuroendocrinology, 2014; 100(2-3): 95-102.

Woods SC, Benoit SC, Clegg DJ, Seeley RJ. Regulation of energy homeostasis by peripheral signals. Best Practice & Research Clinical Endocrinology & Metabolism, 2004; 18(4): 497-515.

World Diabetes Congress. http://www.vancouver conventioncentre.com/events/world-diabetes-congress-2015.