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Research Article

THE EFFECT OF EIGHT WEEKS ENDURANCE TRAINING ON APELIN RECEPTOR GENE EXPRESSION IN ADIPOSE TISSUE OF OLD MALE RATS

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ABSTRACT
The aim of this study was to investigate the effect of eight weeks endurance training on Apelin receptor gene expression in adipose tissue of old male rats.In order to conduct the study, 12 male rats were randomly divided into control and experimental groups for 20 weeks. The rats in the experimental group practiced on the treadmill for 8 weeks, 5 days a week with an intensity of 20 meters per minute (equivalent to 75 to 80 % of maximum oxygen uptake) for 60 minutes. After 8 weeks practicing, and 72 hours after the last practice session, adipose tissue samples were collected. Then, REALTIME PCR method was applied to identify the definite variables. Data analysis was conducted based on independence t-test in a significant level ($p<0.05$). The findings of this study revealed that Apelin receptor gene expression levels in the experimental group in compare to the control group indicated a significant increase after eight weeks endurance training ($p<0.00$). Increasing Apelin receptor mRNA in rats may possibly regulate the adipose tissue and increase unpaired protein expression to determine its role in energy metabolism.

***Keywords**: Endurance Training, Apelin Receptor Gene Expression, Adipose Tissue, Old Male Rats*

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases which occurs by increasing blood sugar level due to the deficiencies in insulin secretion, insulin resistance, or a combination of both (Coskun*et al.,* 2004). Obesity is the most important health problem in both developed and developing countries and can increase the risk of various diseases including heart attack, arthritis, type 2 diabetes, stroke, high blood pressure and other diseases(Crampes*et al.,* 2003; Horowitz and Klein, 2000). Adipose tissue is a major endocrine organ which produces various factors (Adipocytes) affecting the insulin sensitivity and energy balance (Rosen and Spiegelman, 2006). Excessive Adipose tissue in obesity causes some changes in the Adipocytes levels which play a main role in the adjustment of obesity-related disorders such as type 2 diabetes or cardiovascular disease (Mlinar *et al.,* 2007).

In the past decade, many studies have proved the existence of a large number of secreted factors derived from adipocytes affecting the control of energy homeostasis and glucose metabolism (Deng and Scherer, 2010). There are several Adipocytes such as Interleukin- 6, Resistin, Necrosis factor alpha, Vaspin, Adiponectin, Apelin and Amntin. Apelin is one of the adipocytes expressed in central neurons and peripheral tissues with its receptor (APJ)(Carpéné *et al.,* 2007; De Mota *et al.,* 2004). It was discovered by professor Fujino in 1998. This hormone is a peptide that acts together with the receptor APJ.

Apelin is composed of 36 amino acids, which in turn derived from 77 amino acids (pre-pro Apelin) (Tatemoto *et al.,* 1998). APJ is the receptor of 377 amino acids which transfers them from membrane - bound and is attached to G protein and its gene is translated on the long arm of chromosome 11 (Japp and Newby, 2008). Several factors, including physical activity, were identified to effect on Adipocytes secretion. Depending on the intensity and duration of the physical activity, they can affect Adipocytes secretion in different ways (Hida *et al.,* 2005). However, few studies have been conducted on the effect of physical activityon Apelin. On the other hand, in one study, Drake *et al.,* examined the effects of Apelin on glucose uptake in normal fat insulin -resistant rats and found Apelin may be the goal of encouraging on the management of insulin resistance. According to available data, the intravenous injection of Apelin in the rats reduced blood glucose levels and increased glucose utilization in skeletal muscles.

Over the past years, several studies have shown a clear link between energy metabolism and Apelin environmental practices (Dray *et al.,* 2008; Frier *et al.,* 2009; Higuchi *et al.,* 2007). Under normal conditions, a positive correlation between plasma Apelin and insulin levels were observed during the transition from hunger to satiety (Boucher *et al.,* 2005; Heinonen *et al.,* 2005; Soriguer*et al.,* 2009). Under pathophysiological conditions, it seemed the environmental Apelinergic system was set out. However, some researchers found that Apelin plasma levels in patients and rats in terms of obesity, diabetes (Heinonen *et al.,* 2005; Soriguer *et al.,* 2009) and insulin resistance (Dray*et al.,* 2008) were increased. Other studies have shown that the level of Apelin in patients with type 2 diabetes were reduced (Erdem *et al.,* 2008). Recently, it was indicated that intravenous injection of Apelin reduces blood glucose and increases glucose consumption in the whole body of rats with normal diet through the stimulation of glucose consumption, especially in skeletal muscle via AMPK and endothelial nitric oxide synthase dependent pathway (eNOS).

Insulin sensitivity in the rats with high-fat diet, the Apelin glucose tolerance and insulin sensitivity were also improved (Dray *et al.,* 2008) which indicated the potential compensatory role of high levels of Apelin observed in the obese subjects. According to the importance of this hormone as a possible factor in the prevention or warning for diseases, metabolic processes and the functions of the different organ systems, limited number of studies and conflicting results of the previous studies done in this field, the present study sought to investigate the effect of eight weeks endurance training on Apelin receptor gene expression in adipose tissue of old male rats.

MATERIALS AND METHOD

Subjects

In this study, 12 Wistar male rats aged 20 months were purchased from Razi institute as the study samples. The rats were kept in groups of two and in the environment of4/1 ± 22 ° C average temperature, humidity of 4 ±% 55, and light - dark cycle of 12:12 hourin special polycarbonate cages. All animals had free access to water and special food for rats prepared by Pars animals feed company. In all phases of the study, the water needed for animals were freely available to them. All the rats were kept and then killed with respect to the charters on the ethical committee for Animal of Shahid Beheshti University. The subjects were randomly divided into control and experimental groups after three days living in the laboratory environment.

The Practicing Program for the Participants

The rats in the experimental group practiced for 8 weeks, 5 days a week. The total course of practice was divided into the three phases: introducing to the new environment, overloading, and maintaining or stabilizing the intensity of the work. In the introductory phase (first week), the rats walked on a treadmill specialized for rodents every day for 10-15 minutes at a speed of 10 meters per minute. In the overloading phase (second to fourth week), the rats initially walked for 15 minutes at a speed of 12 meters per minute and gradually over a period of 3 weeks, the intensity and duration of the activity were increased to the final rate determined for each group. In the maintaining or stabilizing phase (fifth to eighth week), the rats ran on a treadmill for 60 minutes during 4 weeks with the determined intensity of 28 meters per minute, equivalent to 75-80 % of maximum oxygen uptake and in the all stages the treadmillincline was zero degree. In addition, from the total duration of activity, 5 minutes to warm up and 5 minutes to cool down the rats at a speed of 7 meters per minute were considered.

Blood Sampling and Laboratory Analysis

The rats 72 hours after the last session, while being satiated (4 hours before killing had no access to the food, but could drink water), were anesthetized and killed by Intraperitoneal injection of an anesthetic mixture of Ketamine (mg/kg 30-50-) and Xylazine (mg/kg 3-5).Immediately after removing adipose tissue, theywere weighed with a digital scale andwere immediately frozen in the liquid nitrogen (at -196 ° C) were taken to the laboratory and keptat -80 ° C until the desired laboratory protocols. Tissues were implemented through homogenization and applying REALTIME PCR method for detecting changes in the variables. To avoid the effect of circadian rhythm, sampling started at 8 and finished at 11:30.

Method of Statistical Analysis

Independence t-test was implemented for statistical analysis and comparing the groups. Significance level of the tests was considered $p<0.05$.

RESULTS AND DISCUSSION

Results

The findings of this study revealed that Apelin receptor gene expression levels in the experimental group in compare to the control group indicated a significant increase after eight weeks endurance training ($p<0.00$).

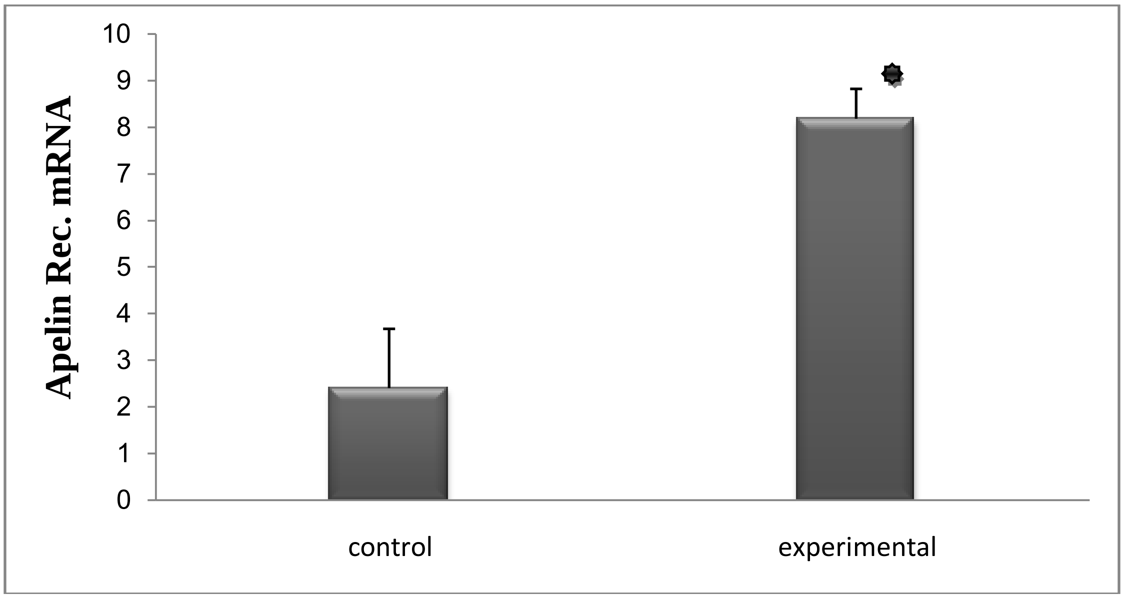

Figure 1:Apelin receptor gene expression changes in the experimental and control groups

Discussion and Conclusions

The findings of this study revealed that Apelin receptor gene expression levels in the experimental group in compare to the control group indicated a significant increase after eight weeks endurance training ($p<0.00$). Few studies have been conducted on the effect of physical activity on Apelin. Adipose tissue is a major endocrine organ that produces various factors (Adipocytes) affecting insulin sensitivity and energy balance (Rosen and Spiegelman, 2006).

Excessive Adipose tissue in obesity caused changes in the levels of Adipocytes which has A major role in modulating obesity-related disorders such as type 2 diabetes or cardiovascular disease (Mlinar *et al.*, 2007).Apelin is an endogenous ligand from the receptor paired with G protein called APJ (Tatemoto *et al.*, 1998). mRNAAPJ Widely expressed in human tissues and is related to the functional effects on the central nervous system and peripheral tissues (Carpéné *et al.*, 2007). Several studies also indicated the role of Apelin in energy metabolism. Central injection of Apelin has been shown to reduce food intake in rats, but conflicting and opposing effects were also reported (Carpéné *et al.*, 2007; Valle *et al.*, 2008). In these studies it was confirmed that serum levels of Apelin are related to nutritional status and simultaneously Apelin plasma insulin levels in rats and humans (Boucher*et al.*, 2005; Castan-Laurell *et al.*, 2008). Moreover, Apelin plasma levels were increased in obese subjects (Heinonen *et al.*, 2005) and subjects with type 2 diabetes such as obese rats suffering Hyperinsulinemia (Boucher *et al.*, 2005). Apelin stimulates glucose-mediated insulin secretion from rats islets (Sörhede *et al.*, 2005), indicating that there is association with glucose homeostasis.

Recently Apelin treatment for a period of 14 days in rats showed that it regulates Adipose tissue (Higuchi *et al.*, 2007) and increases unpaired protein expression to determine the role of Apelin in increasing the energy metabolism. Cavallo *et al.,* (2012)expressed in a study that changes in glucose homeostasis is associated with increasing serum Apelin levels in patients with type 2 diabetes. The findings confirmed that Apelin levels are higher in people with type 2 diabetes compared with their counterparts in the control group and those with Type 1 diabetes.Apelin concentrations are directly related to the fasting blood glucose, body mass index, age, and diabetes (Cavallo *et al.*, 2012).Dray *et al.,* (2010) evaluated the regulation of Apelin and its receptor in the adipose tissue and skeletal muscle in individuals and rats with type 2 diabetes. Apelin and its receptor gene expression in adipose tissue of obese mice compared with the control group were increased. Apelin and its receptor gene expression in skeletal muscle were similar. These data indicates that Apelin expression and its receptor in rats and human are regulated through tissue -dependent manner and in accordance with the intensity of insulin resistance (Dray *et al.,* 2010).

In the present study, after 8 weeks of endurance training and subsequent adaptations, Adipose tissue is more likely to engage in physical activity which increases mRNA of Apelin receptors in Adipose tissue.

Mohebi *et al.,* (2013), investigated the effects of 8 weeks moderate-intensity aerobic exercise on plasma Apelin levels and insulin resistance in women with type 2 diabetes. The results revealed that the weight of the participants in the experimental group was significantly decreased compared to their weight before the treatment and alsoApelin levels of plasma, insulin, and insulin resistance index were significantly decreased. It was claimed that the changes in the Apelin levels of plasma and insulin resistance index, were probably due to weight loss and inflammatory nature of aerobic exercise (Mohebi *et al.,* 2013). Another factor that may contribute to increase mRNA of receptor Apelin is the weight loss of the rats during 8 weeks endurance training. Finally and according to the findings of the study, 8 weeks endurance training increases mRNA of Apelin receptor in Adipose tissue of old male rats. It is likely that the increase in mRNA of Apelin receptor in rats could regulate adipose tissue (Higuchi *et al.,* 2007), and increase unpaired protein expression to determine its role in energy metabolism.

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