

## The effect of eight weeks high intensity interval training (HIIT) and continuous training on apelin levels of cardiac tissue in healthy male rats

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### Abstract

*Introduction:* Pathway apelin/APJ is one of the positive powerful inotropic substances which is an important regulator of cardiovascular operation and a significant factor in cardiovascular tissue. The aim of the present study is to investigate the effect of eight weeks high intensity interval

training (HIIT) and continuous Training on apelin levels of cardiac tissue in healthy male rats.

*Material & Methods:* In this research, 30 Wistar 2-month male rats were chosen with average weight  $180 \pm 20$  gr and randomly divided into 3 groups: continuous training (n=10), HIIT (n=10) or control group (n=10). Continuous training and HIIT groups have been engaged in exercising according training program in five days a week for 8 weeks. For the comparison among the groups, one-way analysis of variance (ANOVA) was performed.

*Results:* No significant differences were observed in apelin levels between continuous training, HIIT and control group (P=0.29).

*Conclusion:* According the results of this study, both exercises increased the apelin levels in heart; however this increase was not significant. It seems that the response of this hormone is different in organs so that it has more limitation in heart tissue than other organs. Therefore, it has been assumed that making noticeable changes in apelin levels of heart required much more time.

**Keywords:** Apelin, Continuous training, High Intensity Interval Training, Cardiac tissue

## 1. Introduction

Adipokines are so important physiological factors in heart system that can be affected by physical activity and exercise. Sport exercises (HIIT and continuous training) may increase the sensitivity of the heart to disorder in this Physiological factor (1). Based on the findings in athletes, 90% of heart attacks and sudden death takes place during the intensive activity or immediately after that. Researchers bring up the disturbance of Peptide balance as a reason for the increase of heart failure. There are different reasons in balancing cardiovascular physiological process such as peptide apelin that is assumed it has potential role in cardiovascular system (2). Apelin is appetite hormones

with amino acid 77 which is produced by many types of cells; it contains fat cells, artery stromal cells, and heart cells. During its process several different activity materials such as peptide 36 of amino acid (apelin36), peptide 17 of amino acid (apelin17), peptide 13 of amino acid (apelin13) is produced and it seems that it is the most active apelin form. These materials describe various apelins and express signals via coupled *receptor* G, APJ, in cardiovascular cells and even in hypothalamus neurons. Apelins take part in multiple physiological process such as central appetite control, much urine, blood pressure control and regulation of cardiac contraction. Indeed, appleins are the expanders which related to extremely strong endothelium. In addition to that, it is in the human heart. Apelin is one of the most powerful positive endogenous inotropic factors that have already been found. Levels increase in obesity and diabetes where insulin and even TNF- $\alpha$  stimulates the apelin production (3). Besides, apelin is induced by hypoxia, as it takes place in ischemic cardiomyopathy and the pathological development of adipose tissue. Apelin shows anti-inflammatory properties and may deregulate the angiogenesis. It has been illustrated that apelin stimulates exit, proliferation, formation of the andotelian cells duct and upgrades the angiogenesis inside the body (4). In fact, it has shown that apelin is essential for the symmetrical angiogenesis caused by hypoxia (5). In addition, the results shown that siRNA intermediate suppression in apelin, angiogenesis responses shall decrease in rat model with fat grafting(6). This shows that apelin may play the role of physiopathology in artery development in adipose tissue especially in low oxygen conditions (7). APJ apelin *receptor* is a lot in human and rat myocardial and also human coronary arteries, aorta and smooth vein. Apelin is a factor in heart tissue and artery which is well-known in autocrine and paracrine and its one of the strong positive inotropic that has been already found. Apelin effect cause strong stimulation and contractile feature of the heart muscle; therefore, apelin has effect on blood flow and blood pressure in this way. The results have indicated that apelin density has been increased in the left ventricle in people with chronic heart disease (8). APJ is an important regulator of artery and heart operation (9-10) and plays a significant role in accessing and developing artery-heart diseases such as arteriosclerosis, coronary

artery disease, heart failure, high blood pressure, pulmonary hypertension, reflow myocardial ischemia and atrial fibrillation (11). The purpose of setting apelin way APJ is the protection against diseases that is one of the major reasons of death in the world (12). However, it has been reported, there is a direct and meaningful relationship between apelin and physical activity level. The researches have shown the apelin increased level in order to create an adoptable response against disease conditions in diseases such as diabetes type 2, hepatic and cardiovascular. Wright et al. (2009) found apelin mRNA in the heart and plasma levels of apelin increase four times in response to activity in compare with control group in rats and this increase remained high for 24 hours after the exercise; while plasma levels apelin from the skeletal muscle didn't changed dramatically (13). In another study, the search of plasma apelin matter and heart tissue through high pressure level in mice which have high blood pressure after 9 weeks, swimming exercises indicated that the activities may improve the high level pressure by adjusting apelin levels after finishing the activities during this term (14).

As we found few studies on the impact of HIIT and aerobic training on Apelin levels of heart tissue and with contradiction of the results, so this study was done to examine the effect of eight weeks HIIT and continuous training on apelin levels of cardiac tissue in healthy male rats.

## 2. Material & Methods

### *Animals*

This is an experimental study that is done via control and examination group procedure. 30 two month old Wistar male rats, weighing  $180 \pm 20$  g, were selected and randomly divided into 3 groups: continuous training (10), HIIT group (10) and control (10). Rats were kept in the Animal House of Faculty of Physical Education and Sport Sciences of Tehran University with a  $22 \pm 2$  degree temperature, humidity of 50-60% and light-darkness cycle of 12-12 with food and water made available *ad libitum*. All animals in the experiment were used and treated according to Iran's Convention policy for the protection of vertebrate animals for experimental and scientific purposes; the protocol was adopted by the

ethics committee of Endocrine Sciences Research of Shahid Beheshti Institute.

### *Exercise protocol*

In the first 2-week of the preliminary experiments, the rats were adapted to treadmill. The adaptation consisted of 10 min of exercise at a speed of 12 m.min<sup>-1</sup> on a 0° incline. Then, they started exercising according to the training protocol of aerobic group, 5 sessions per week for 8 weeks. The overall running time for rats was about 42 minutes (6-minute warm-up, 50-60% VO<sub>2max</sub>), 30 minutes of aerobic exercise (70-75% VO<sub>2max</sub>) and 6-minute cool-down (50-60% VO<sub>2max</sub>). The HIIT group performed exercise program 5 times per week with 90-100 % of VO<sub>2max</sub> for 8 weeks. Running duration on treadmill for 30 minutes (50-60 % of VO<sub>2max</sub>) and 6 minutes to warm up (50-60 % of VO<sub>2max</sub>), 3 sets for 4 minutes in high rate, (90-95 % of VO<sub>2max</sub>) and 2 minutes in slow rate for cool down in 6 minutes (50-60 % of VO<sub>2max</sub>). The treadmill's slope didn't change for 8 weeks during the exercise. During the study, the control group did not do any exercise at all.

### *Biochemical measurements*

In order to eliminate the acute effects of exercise and uncontrollable variables such as subjects' stress while performing the exercise program, rats were ethically anesthetized 24 hours after the last exercise session by intraperitoneal injection of a mixture of ketamine (30 to 50 milligrams per kilogram of body weight, intraperitoneal) and Xylazine (3 to 5 mg per kg of bodyweight, intraperitoneal). The cardiac muscle of the rat was removed from its chest, rinsed in physiological saline and weighed on a digital scale with 0.0001 gram sensitivity. Then, it was immediately frozen using liquid nitrogen for subsequent measurements and kept in the lab freezer at -70 °C in the Cellular and Molecular Endocrine Research Center of the Institute for Endocrinology and Metabolism of Shahid Beheshti University. Apelin was measured using ELISA kits for rats apelin made in Cusabio Biothech Company, China, with 0.078 ng/ml sensitivity via ELISA method and according to the manufacturer's instructions on Chinese Elisa Reader of HUISONG Company.

*Statistical analysis*

In this study, descriptive statistics were used so as to calculate the mean and standard deviation. For the comparison among the groups, one-way analysis of variance (ANOVA) was performed. Data analyses were performed using SPSS software for windows (version 19, SPSS, Inc., Chicago, IL) and statistically significant differences were established at  $P < 0.05$ .

**3. Results**

The results on apelin levels in the continuous training, HIIT and control groups are presented in the Table 1 and Figure 1. The results indicated that there were no significant differences in apelin levels between continuous training, HIIT and control group ( $P = 0.29$ ).

Table 1. One-way ANOVA analysis for comparing of apelin levels between three groups

Group	Average	Standard deviation	F	Sig
Control	0.64	0.13	1.26	0.29
Continuous training	0.76	0.23		
HIIT	0.68	0.20		

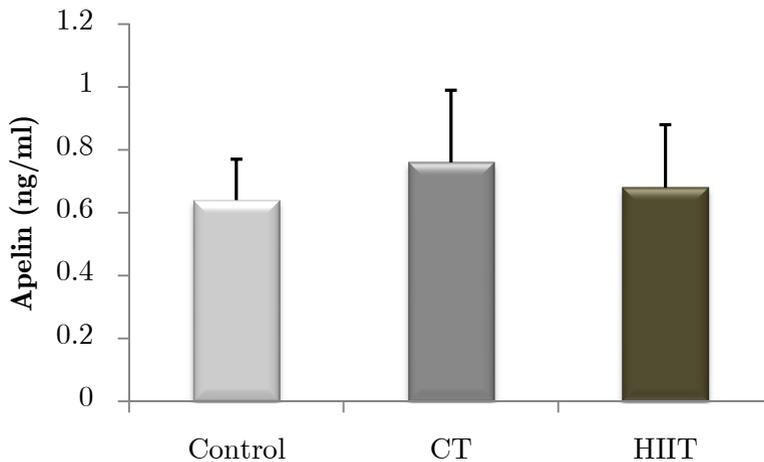


Figure 1. Apelin levels in control group, continuous training group and HIIT group

#### 4. Discussion

The results of this research shown that the apelin hormone levels would be increased after the continuous training and HIIT, however this increase was not significant in compare with the control group.

The apelin may have a protection role in the myocardium injury. In laboratory studies and *in vivo* had been shown that apelin and APJ gene will be regulated in the peripheral tissues and heart on the response of sport activity (15). There are different factors which have effect on the Adipokines exudation specially apelins that it can be referred to sport activity. Sport activity can be effective in different ways and doing effective exercise (intensity, duration, type, number of sessions in week) leads to reduce the cardiovascular disease. Kechyn et al. (2015) reported that apelin concentration was decreased after endurance exercise with 80% of  $VO_{2max}$  in healthy population. (16). These discrepant results may be attributed to differences in exercise protocol and study population. The results showed that the response of apelin is different in various tissues and it seems that the response of this hormone has higher threshold in the heart tissue than the other organs. Recently, many reports have been presented about apelin and heart diseases which show the reduction of apelin levels in this disease. mRNA of apelin gene has shown noticeable reduction in myocyte which exposed mechanical pull. It has been specified that regulation of apelin plasma levels is a therapy and predictable important factor in cardiovascular patients (17). We can refer to different operations of apelin in order to explain this contradiction to the response of apelin hormone to sport activity. On the other hand, apelin is a strong stimulant to widen artery and it needs to be increased in people with cardiovascular diseases. Apelin plays a role in artery receiver and the control of blood pressure. Activating this receiver cause create a new artery. The effect of apelin on blood pressure cause activates the receptors in endothelial cells levels (2). It seems still that doing sport activity causes the reduction of hormones reactions and the increase of apelin sensitivity. The research results showed that high intensity exercise lead to increase of apelin levels, thus it seems that intensity of exercise is a one of main factors for decrease the level of this hormone. In addition, Wright et al. (2009) found apelin mRNA in the heart and plasma levels of apelin increase four times in response to

activity in compare with control group in rats and this increase remained high for 24 hours after the exercise; while plasma levels apelin from the skeletal muscle didn't changed dramatically (13). As the level of applein has increased in these two studies, physical activity has an increasing effect on this hormone. There is an apelin receptor in the heart and the impact of apelin cause strong stimulation and contraction quality of heart muscle; thus, the apelin affects bloodstream and blood pressure. HIIT exercise and continuous training might enhance the apelin levels and it's receptor in the heart muscle. Apelin is an effective protein on cardiovascular function such as vasodilation related to endothelium, vascular stiffness can have a strong role in people with cardiovascular disease. It has been seen that the apelin viscosity has been increased in left ventricle with chronic disease (18,19). Therefore, generally putting the apelin way against these diseases is the aim which is the major reasons of death in the world (12).

## 5. Conclusion

Generally, apelin is a hormone that affected heart structure and function. Our results indicated that continuous training and HIIT exercise had not significant effects on this protein. Apelin release from the different organs such as myocardium, adipose tissue and skeletal muscles and this is very difficult to determine the participation of these organs in plasma levels of apelin after the exercise. It seems that exercise intensity is a main factor for determine the plasma levels of apelin. Although the results of the present study indicated that there were no significant differences in plasma levels of apelin between continuous training and HIIT exercise and control group, but future studies are needed to examine the effects of these exercise on apelin levels of cardiac tissue.

**Conflict of interests:** There was no conflict of interest among authors.

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