THE RESPONSE OF PLASMA ADROPIN LEVELS AND INSULIN RESISTANCE INDEX TO HIGH INTENSITY INTERVAL TRAINING IN SEDENTARY MEN

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ABSTRACT

Adropin is one of the peptide hormones that plays a role in energy homeostasis, metabolic adaptation, adjustment of insulin sensitivity and obesity. Therefore, in this study, the response of plasma adropin levels and insulin resistance index to High Intensity Interval Training in Sedentary men was investigated. In this paper, 22 inactive menaged 21-27 years were randomly classified into two groups of experimental (n=11) and control(n=11). The eight-week training program inthe experimental group included high intensity interval training (HIIT)under amaximum intensity of 90% heart rate via three sessions per week. Then,blood sampling was performed to measure the serum adropin, insulin, and fasting glucose before and after the intervention. In this paper, the paired-*t* and independent-*t* statistical tests were employed atP<0.05.The comparison between thegroups indicated that the values of adropin and insulin resistance index did not change significantly in the post-test of the experimental groupcompared to the control group (P>0.05). Moreover,the intra-group changes inadropin, insulin resistance index and BMI revealeda significant difference inthe meanbefore and after the experimental group (P<0.05). Furthermore,regarding the relationship of adropin with insulin resistance and their HIIT effectiveness, adropin may play an essential role in weight control as well as prevention of type 2-diabetes in inactive people.

Keywords: Adropin, Glucose, Insulin, Insulin resistance index

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A RESPOSTA DOS NÍVEIS PLASMÁTICOS DE ADROPINA E ÍNDICE DE RESISTÊNCIA INSULÍNICA AO TREINAMENTO INTERVALADO DE ALTA INTENSIDADE EM HOMENS SEDENTÁRIOS

RESUMO

A adropina é um dos hormôniospeptídicos que desempenha um papelnahomeostaseenergética, adaptaçãometabólica, ajuste da sensibilidade à insulina e obesidade. Portanto, nesteestudo, foiinvestigada a resposta dos níveisplasmáticos de adropina e do índice de resistência à insulinaaoTreinamentoIntervalado de Alta Intensidadeemhomenssedentários. Neste trabalho, 22 homensinativos com idade entre 21-27 anosforamclassificadosaleatoriamenteemdoisgrupos de experimental (n=11) e controle (n=11). O programa de treinamento de oitosemanas no grupo experimental incluiutreinamentointervalado de altaintensidade (HIIT) sob umaintensidademáxima de 90% da frequênciacardíacaemtrêssessõesporsemana. Emseguida, foirealizada coleta de sangue para

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dosagem de adropinasérica, insulina e glicemia de jejum antes e após a intervenção. Neste trabalho, os testes estatísticos t pareado e t independenteforamempregadosem P<0,05. A comparação entre osgruposindicou que osvalores da adropina e do índice de resistência à insulinanão se alteraramsignificativamente no pós-teste do grupo experimental emrelaçãoaogrupocontrole (P>0,05). Alémdisso, as alteraçõesintragrupo de adropina, índice de resistência à insulina e IMC revelaramumadiferençasignificativanamédia antes e depois do grupo experimental (P<0,05). Alémdisso, emrelação à relação da adropina com a resistência à insulina e suaeficácia no HIIT, a adropinapodedesempenhar um papelessencial no controle de peso, bemcomonaprevenção do diabetes tipo 2 empessoasinativas.

Palavras-Chave: Adropina, Glicose, Insulina, Índice de resistência à insulin

LA RESPUESTA DE LOS NIVELES PLASMÁTICOS DE ADROPINA Y EL ÍNDICE DE RESISTENCIA A LA INSULINA AL ENTRENAMIENTO INTERVÁLICO DE ALTA INTENSIDAD EN HOMBRES SEDENTARIOS

RESUMEN

La adropina es una de las hormonaspeptídicas que desempeña un papelen la homeostasis energética, la adaptaciónmetabólica, elajuste de la sensibilidad a la insulina y la obesidad. Por lo tanto, enesteestudio, se investigó la respuesta de losnivelesplasmáticos de adropina y elíndice de resistencia a la insulina al entrenamientointerválico de altaintensidaden hombres sedentarios. Enesteartículo, 22 hombres inactivos de entre 21 y 27 años se clasificaronaleatoriamenteen dos grupos de experimental (n=11) y de control (n=11). El programa de entrenamiento de ochosemanasenelgrupo experimental incluyóentrenamientoenintervalos de altaintensidad (HIIT) con unaintensidadmáxima del 90 % de la frecuenciacardíacaentressesionesporsemana. Luego, se realizó un muestreo de sangre para medir la adropinasérica, la insulina y la glucosaenayunas antes y después de la intervención. Enestedocumento, las pruebasestadísticas t pareadas e independientes se emplearonen P<0.05. La comparación entre losgruposindicó que losvalores de adropina e índice de resistencia a la insulina no cambiaronsignificativamenteenel post-test del grupo experimental encomparación con elgrupo control (P>0.05). Además, loscambiosintragrupoenadropina, índice de resistencia a la insulina e IMC revelaronunadiferenciasignificativaen la media antes y después del grupo experimental (P<0,05). Además, encuanto a la relación de la adropina con la resistencia a la insulina y sueficaciaenel HIIT, la adropinapuededesempeñar un papelesencialenel control del peso, asícomoen la prevención de la diabetes tipo 2 en personas inactivas.

Palabras Clave: Adropina, Glucosa, Insulina, Índice de resistencia a la insulin

INTRODUCTION

Obesity is known as a metabolic complication which, is now increasing noticeably in developing and developed countries. According toWorld Health Organization (WHO),obesity isdefined asan abnormal or excessive accumulation of fat in the body, which may harm health (OMIDI GHANBARI, SOORI, HEMMATFAR, 2020).Besides,itmainly causes some chronic inflammation and the spread of several diseases such as increased blood lipids, blood pressure, atherosclerosis, increased risk of insulin resistance, type 2-diabetes, and cardiovascular diseases (ROSHDIBONAB, EBRAHIM, GHAZALIAN, et al, 2019). It is worth

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noting thatobesity has differentperipheral and central causes.

In addition to hereditary factors, one of the factors affecting the rate of weight gain islifestyle change, nutritional status,lack of physical activity, anddisruption inthe body's energy balance. More importantly,there is no desire in obese and inactive people to dophysical activity (OMIDI GHANBARI, SOORI, HEMMATFAR, 2019). As such,the increasing trend of obesity has prompted researchers to conduct several studies in terms of factors influencing obesity as well asdistinguishing hormonal signals related to metabolic diseases and body homeostasis. Interestingly, thebody weight regulation in sedentary people has attracted the attention of manyresearchers to distinguish interesting hormonal signals related to metabolic diseases and body homeostasis (INOUE, FUJIE, HASEGAWA, et al, 2020).

In this regard, investigatingobesityand the related metabolic diseases hasdrawn the attention of many researchers to demonstrate the factors affecting these peptidesthrough discovering new peptides regulating energy homeostasis (OLIVEIRA, MONTEIRO, JACOME, et al, 2017).Of note,adropin is one of the peptide hormones, which is a newly identified protein hormone including 76 amino acids. It is encoded using a gene related to energy homeostasis,in which its high expression levels have been reported in the central nervous system as well as peripheral tissues such as the cardiac, liver, skeletal muscle, and endothelium (RAMEZANKHANI, SOORI, RAVASI, et al, 2019).This hormone can be expressed by different body tissues such as the skeletal muscle in animal and human samples (ZHANG, JIANG, YANG, et al, 2017). Therefore, discovering adropin has opened up a whole new field of research on obesity.Adropin levels can be affected by the changes in metabolic conditions, which can bereduced significantly in obesity, diabetes mellitus, cardiovascular diseases and hypertension (FUJIE, HASEGAWA, SATO, et al, 2015).Accordingly,adropin plays a critical role in energy homeostasis, metabolic adaptation, and adjustment of insulin sensitivity and obesity. Meanwhile,in addition to these important metabolic effects, it affects non-metabolic properties such as the regulation of endothelial function andprotects the vessels against atherosclerosis (MAEDA, ZEMPO-MIYAKI, SASAI, et al, 2015).

Different studies have indicated that adropincan result in weight loss, improved glucose tolerance, and liver lipid metabolism (GAO, MCMILIAN, ZHU, et al, 2015).The impact of adropin on the carbohydrate metabolism may berelated to the activation of pyruvate dehydrogenase (PDH). This mechanismincreases the use of sugar as fuel in skeletal muscles as well as glucose oxidation and insulin signaling activity (KORKMAZ, SAYILANOZGUN, 2019).In contrast,reducing the concentration of adropin increasesobesity without changing the dietary habits. In the first study conducted on this peptide hormone, the researchers reported a reduction in the circulating concentration of adropinas well as its expression in the liver tissue of obese rats.In these rates, fat tissue increased whilefood intake and energy consumption were both normal. Moreover, in hyperinsulinemic conditions, therats suffered from lipid disorders and suppression of endogenous glucose production, which was associated with insulin resistance. They reported that adropinprevented hyperinsulinemia and the fatty liver associated with obesity by regulating lipid and glucose metabolism (KUMAR, TREVASKIS, LAM, et al, 2008).According to the available results, adropin levels can vary depending on the level of physical activity such

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as doing sports (OMIDI GHANBARI, SOORI, HEMMATFAR, 2020).

Studies on adropinindicate that adropinaffectsmetabolic homeostasis wherelow levels of serum adropin are associated with dyslipidemia and insulin resistance. To confirm this hypothesis, an increase in adiposity and fasting triglycerides, insulin resistance, and impaired glucose tolerance were observed in ratssuch thatadropin was removed (KUMAR, ZHANG, GAO, et al, 2012).It seems that low levels of adropin may raise insulin resistance and other characteristics of metabolic syndrome associated with obesity.In another study,chronic calorie limitation ledto metabolic adaptation and reprogramming of fat metabolism in rate. This decreased metabolic adaptation of lipogensenhanced bothketogenesis and lipolysis. Moreover, the regulation of adropin was noticeably disrupted, which could be the reason forthe reducedlipogenesis (KUHLA, HAHN, BUTSCHKAU, et al, 2014).

Alizadeh et al.explored the impact of a session of aerobic activity viaintensity proportional to the maximum of fat oxidation on both theadropin levels and insulin resistance in overweight women. However,they did not observe anyconsiderable changes in the adropinlevel. Afterwards,they described the possible reason for the lack of change as the effect of the fasting state in increasing adropin or the duration and intensity of the activity (ALIZADEH, GOLESTANI, MORADI, et al, 2018). Hosseini et al.reported an increase in adropin anda decrease in insulin resistance in elderly men following a period of exercise in water (HOSSEINI, ABEDI, FATOLAHI, 2019).

Choui and Yim. investigated a significant relationship between obesity and its complications with reducedadropin levels in obese Korean men and women (CHOI, YIM, 2018).Accordingly, adropin can provide hope for improving the treatment of obesity-related metabolic disorders. Veryfew studies have investigatedserum adropin levels and their relationship with insulin resistance and body composition as well asmetabolic syndrome indicators in inactive people. Hence, the role of adropin in increasing overweight and obesity in inactive people is not well known andrequires more studies.

Unfortunately, there exists a paucity of researchon the effect of sports activity,particularly high intensity interval training (HIIT), onadropin levelsin inactive people. So far, there has been no consensus on the impact of the intensity of sports activity on peptides regulating energy homeostasis including adropin. Thus, this paper aims to investigate the response of plasma adropin levels, insulin resistance index and lipid profile to HIIT in inactive men.

MATERIALS AND METHODS

The current semi-experimental research has apre-testpost-test designincluding a control group. The statistical population includedinactive men aged21 to 27 years whowere chosen voluntarily based on the criteria for entering the research.For this purpose, 22 volunteers were randomly divided into two experimental and control groups. All subjects participated in the orientation session wherethe research procedures andexercise protocols were explained to them in detail. Afterwards,they signed a written consent to participate in the research after knowing the aim of the research as well as how to perform the workout.

In the two steps of pre-test and post-test, height, weight (using calipers and scales), and body mass index (divided by weightto the square of height in meters) were measured.After that,all subjects were asked not

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to change their diet until the end of the research protocol so that they couldcontinue their normal diet as before. The inclusion criteria werethe absence of known physical diseases such as cardiovascular, thyroid, and respiratory diseases, diabetes, muscular-skeletal conditions, and high blood fat, professional exercise, and being on a diet. In contrast, theexclusion criteria werethe absence of more than one session in the sports training program, the occurrence of an accident, injury, suffering from other disruptive diseases, smoking, andany interfering factor influencing the effective participation of the subjects in the training sessions.

Measurement of biochemical variables

To measure the resting levels of biochemical variables, blood sampling was performed in the morning and after 12 hours of fasting in the pre-test stage as well as 48 hours after the last training session in the post-test stage.Next, 5 cc of blood was taken from the subjects' arm veins by a specialist, following the hygiene principles. Blood samples were poured into tubes containing anticoagulant Ethylene Diamine Tetra Acetic Acid (EDTA). After that, the blood samples were centrifuged at 3000 rpm for 15m. Following centrifugation and plasma separation, they were frozen at -80°C andused to measure the research variables.

Biochemical analysis and measurement of the serum levels of adropin were performed by the sandwich ELISA method and the research kit of Sunlog company with asensitivity of0.05 pg/ml. Fasting blood sugar was measured by Hitachi 902 machine made in Japan and Glucose Pars Azmon kit made in Iran.Moreover, insulin was measured using ELISA method byMonobind Inc special kit made in America with a sensitivity of 0.75 microunits/ml and intragroup variation coefficient of 6.3%.The insulin resistance index was calculated as follows:

HOMA-IR = Fasting insulin (µU/mL) ×Fasting glucose (mmol/L)/22/5

The training protocol

After a week of preparation and familiarity with the protocol implementation method, the participants performed their training programs, including Shuttle Run Test for a distance of 20 m, shown by 3 cones, for 8 weeks in training hall at a temperature of 26 °C during fall according to the following procedure. After warming up, including 10 min of jogging and 5 min of active stretching exercise, the participants ran from the starting point (cone 1) towards the cone 2 (path 1) with a maximum speed, and then returned and ran in the inverse direction towards the cone 3 (path B) with a maximum speed of 20 m. Finally, they re-returned, and ran towards the starting point (cone 1) with a maximum speed (path C) to complete the 40-m distance. The participants continued the trend with a maximum speed and completed a 30-second period of training protocol, and repeated the training protocol after 30 seconds of rest. The training was progressed by increasing frequency of 30-second repetition from 4 times in the first and second weeks to five times in the third to fourth weeks, six times in fifth and sixth weeks, and eight times in the seventh and eighth weeks. The training intensity was measured for all participants at all protocol stages, over a Heart Rate Maximum (HRmax) of 90% using a formula of HRmax= 220-age, and it was controlled by a heart rate monitor, made in Finland. The training program was derived from a 40-m Shuttle Run Test with a maximum speed as it was a valid test for evaluating the anaerobic performance (15). At the end of each training session, the participants walked and did stretching training and exercise for cooling down during 10 minutes.

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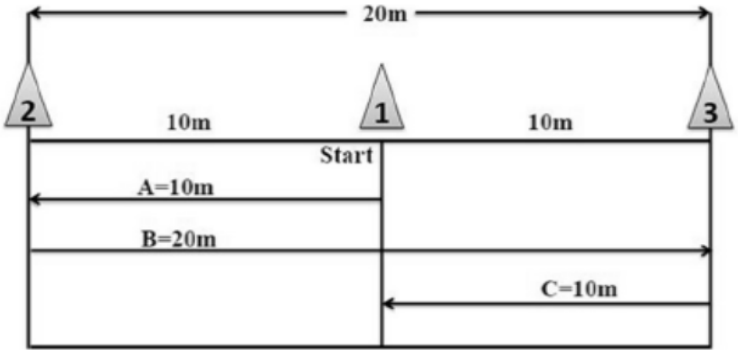

Figure 1. Schematic diagram of HIIT protocol

Statistical method

SPSS version 23 software was employed for data analysis. In addition, Kolmogorov-Smirnov test was designed to assess the data homogeneityof different variables. Because no significant difference was observed in the pre-test between the groups in terms of several factors, inferential statistics and paired *t*-testwere utilized to compare the pre-test and post-test in each group;the independent *t*-test was employed for the comparisonbetween the groups at the significance level of $P < 0.05$.

RESULTS

Table 1 reports the anthropometric features of the subjects of the proposed groups. It can be observed that body weight (P=0.001) and body mass index (P=0.001) of the post-test inthe experimental groupdecreased significantly ascompared tothe pre-test. Moreover,the results of intergroup analysis revealedno significant difference between the post-test values of the experimental group and the control group.

Table 1. Investigation of inter- and intra-group changes in general characteristics in two groups.

Variables	Group	Pre-test	Post-test	In-group		Intergroup	
		Mean and SD	Mean and SD	t	P	t	P
Weight (kg)	experimental	88±10.5	84±9.93	6.05		**0.223**	
				*0.001		**0.826**	
	Control	85.5±7.2	84.8±7	1.3			
				0.224			
BMI (kg/m^2)	experimental	27.25±2.25	26.03±2.36	6.3	*	**1.41**	
				0.001		**0.172**	
	Control	27.52±2.64	27.56±2.97	0.239			
				0.816			

*In-group Statistical significance

The results of intra-group analysis indicated that eight weeks of intense intermittent trainingresulted in a significant increase in the amount of adropinin the post-test in the experimental group (P=0.022). Besides,the inter-group results showed that there wasno significant difference between the experimental group andthe control group in terms of adropin in the post-test (P=0.196) (as depicted in Fig 2).

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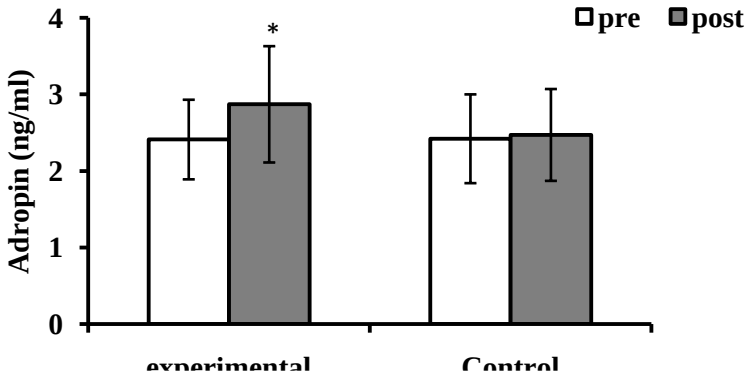

Figure 2. Mean (±Standard error) of Adropin before and after training in groups. * In-group Statistical significance

Furthermore, eight weeks of intense interval training significantly reducedthe amount of glucose (P=0.001), insulin (P=0.012) and insulin resistance index (P=0.011) in theexperimental group in the post-test compared to the pre-test. Nevertheless, no significant difference was observed between the post-test of the experimental group and the control group regardingglucose, insulin and insulin resistance index (Table 2).

Table 2. Investigation of inter- and intra-group changes in variables in two groups

Variables	Group	Pre-test	Post-test	In-group		Intergroup	
		Mean and SD	Mean and SD	t	P	t	P
Adropin (ng/ml)	experimental	2.41±0.52	2.87±0.76	2.71	*0.022	**1.33**	**0.196**
	Control	2.42±0.58	2.47±0.6	1.78	0.104		
Glucose (mg/dl)	experimental	102.2±13.35	95.91±13.61	4.87	*0.001	**1.13**	**0.207**
	Control	106.37±19.89	105.64±20.65	1	0.34		
Insulin (IU/ml)	experimental	16.72±7.25	11.55±6.53	3.06	*0.012	**1.74**	**0.096**
	Control	16.18±5.15	15.91±5.11	0.89	0.39		
Insulin resistance (HOMA)	experimental	4.4±2.38	2.78±1.73	3.12	*0.011	**2.05**	**0.054**
	Control	4.2±1.28	4.13±1.32	0.827	0.43		

*In-group Statistical significance

DISCUSSION AND CONCLUSION

In this paper, the effect of intense interval training on adropin levels as well as insulin resistance index in inactive men was investigated.To the best of our knowledge, no research has been conducted onthe effect of intense interval training on serum adropin levels. In our study,eightweeks of intense intermittent trainingresulted in a significant increase in adropin levels as well as a significant decrease in the insulin resistance index and BMI in the experimental group.Noteworthy,although

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thedifferences between groups were not significant, the amount of adropin increased (about 14%) in the experimental group. The insulin resistance index (about 32%) was observed to decrease in the experimental group.

The increase in the serum adropin levels observed with intense intermittent activity was associated with a reduction concerning both body composition index and insulin resistance. In this regard,few studies have been conducted to investigate the effect of physical activity on serum adropin levels.The results of this paper are in accordance with the findings of (FUJIE, HASEGAWA, KURIHARA, et al, 2018)in terms of increased serum adropin levels following HIIT.

The results ofthestudies conducted on adropinindicate that Dyslipidemia, glucose intolerance and insulin resistance can be related to low levels of serum adropin (KUHLA, HAHN, BUTSCHKAU, et al, 2014). In this regard,it seems thatincreasedadropin raisesthe oxidation of glucose via activating the Pyruvate dehydrogenase complex (PDC). Through this action, adropin reduces the oxidation of muscle fatty acids by inhibiting carnitine palmitoyl transferase 1B (CPT1B) whichis the key enzyme for transferring fatty acids to muscle mitochondria for β-oxidation.

It should be noted that adropin treatment by inhibiting free fatty acids can have therapeutic roles by improving metabolism and glucose intolerance in obesity. Throughreducing theregulation ofPyruvate Dehydrogenase Kinase 4 (PDK4), adropinincreases the activity of pyruvate dehydrogenase (GAO, MCMILIAN, ZHU, et al, 2015). On the other hand, adropin is a regulator of endothelial nitric oxide synthase and NO releaser. In this regard, Fuji et al. concluded that adropinprobably participates in decreasingexercise-induced resistance (FUJIE, HASEGAWA, SATO, et al, 2015). Asfat tissuescontain several capillaries and autonomic nerves, all their metabolic actions can be controlled by thyroid, sex, and nerve hormone factors. Accordingly, dilating vascular by NO can improve blood supply to tissues, including fat tissue,subsequently increasing its metabolism as well (ALIZADEH, GOLESTANI, MORADI, et al, 2018).

Suri et al. surveyed the effect of 16 weeks of aerobic activity and caloric restriction on adropin levels, anthropometric indices, insulin and glucose, and HOMA-IR in sedentary obese women. They showed a significant increase in adropin levels in both aerobic activity and caloric restriction groups. In addition,a significant relationship was observed between adropin changes with BMI changes and insulin resistance index (SOORI, RAMEZANKHANI, RAVASI, et al, 2017).Nonetheless, theadropin gene expression mechanism after the physical exercises was not well understood (CELIK, BALIN, KOBAT, et al, 2013),necessitating future researchon theadropin expression.Additionally,some clinical studies revealed a reduction in adropin in cardiovascular patients and type 2-diabetes (TOPUZ, CELIK, ASLANTAS, et al, 2013). This paper indicated that in addition to a major increase in adropinin the experimental group, insulin resistance can besignificantly improved.

In this regard, it was reported that adropinwas a regulator of lipid and carbohydrate metabolism, and its secretion could be furtherregulated by dietary sugar and fat consumption (ZHANG, ZHAO L, XU, et al, 2014).Based on the results of some studies,adropin couldlead to weight loss and improve glucose tolerance and liver lipid metabolism. The effect of adropin on carbohydrate metabolism probably depends on the activation of pyruvate dehydrogenase (PDH). By increasing the consumption of sugar

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as fuel in skeletal muscles, pyruvate dehydrogenase causes glucose oxidation and increases insulin signaling activity (BUTLER, TAM, STANHOPE, et al, 2012).Likewise,exercise increases glucose uptake in skeletal muscle by increasing the density of transporter protein (GLUT4) on the sarcolemma whichis independent of insulin. With theactivation of this mechanism, insulin sensitivity increases as well.

The results of Assarzadeh et al. revealed thatcombined training in inactive menresulted in a significant decrease in insulin concentration and insulin resistance index (LAMBADIARI, TRIANTAFYLLOU, DIMITRIADIS, 2015). Besides, the improvement inthe insulin resistance index may be partially due to the potential effect of adropin on muscle vasodilation. Moreover, theincreased blood flow can facilitate access to glucose andenhance glucose metabolism (WU, FANG, CHEN, et al, 2014).Inactivity, overweight, and obesity can increase the production of pro-inflammatory factors involved in the pathogen of insulin resistance by creating inflammatory conditions. Thus, thechange in theproduction of inflammatory factors by adipose tissue plays an essential role in insulin resistance and obesity-related metabolic problems.Therefore, regular and continuous exercise and physical activity can improve glucose transport and metabolism by generating special biochemical changes in the muscles, including increased capillary density, augmented oxidative enzymes, increased GLUT4mRNA content, and improved insulin signaling. These changes reduce the need for insulin. Hence, a reduction in the pathogenesis of insulin resistance may be achieved using aerobic exercise-induced weight loss. Nevertheless, regarding the insulin-like effect of regular and long-term physical activity and that the increase in insulin resistance occurs over a relatively long period, it can be expected that regular and long-term exercise plays a significant role inenhancing the insulin sensitivity, particularlyaerobic exercise (WONG, WANG, LEE, et al, 2014).

In the present study, high intensity interval training increased adropineand decreasedinsulin resistance index in the experimental group in inactive people. It can be concluded that intense periodic training, by increasing adropineand reducing insulin resistance in inactive men,can culminate in the prevention of type2 diabetes and cardiovascular diseases in old age. In addition, probablydue to the increase in fatty acid metabolism as a result of high intensity interval training, an improvement can also be observed in the lipid profile.

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