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A Comparison of the Effects Continuous and Interval Exercises on Fibrillin-1 and Asprosin in Obese Male Rats

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ABSTRACT

Introduction: Obesity is still a health problem for humanity. Although the favorable role of exercise on weight loss has been reported. But the effect of the type of exercise is still unclear. The present study compared the effects of continuous exercise (CE) and interval exercise (IE) on fibrillin-1 and asprosin in obese male rats.

Methods: Forty- eight male rats were divided into six groups including 1) obese IE, 2) obese CE, 3) healthy IE, 4) healthy CE, 5) obese control and 6) healthy control. Groups 1- 4 performed exercises for 8 weeks and 72 hours. Insulin resistance index, fasting glucose, insulin, fibrillin-1 and asprosin were measured after the last training session. Data analysis was performed by Two-way analysis of variance and Kruskal-Wallis tests with SPSS software (P≤0.05).

Results: There were significant differences in insulin resistance (P=0.001), fibrillin-1 gene expression (P=0.001), fasting glucose (P=0.001), asprosin serum levels (P=0.001), and insulin (P=0.002) levels between obese IE, obese CE, healthy IE, healthy CE, obese control and healthy control groups.

Conclusions: Although obesity increased fibrillin-1 and asprosin, but IE and CE decreased fibrillin-1 and asprosin. Thus, IE and CE can be used for controlling fibrillin-1 and asprosin levels. IE and CE can be considered as effective methods to reduce weight in obesity.

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Introduction

Many factors are involved in human obesity.In the same vein, more than 40 diseases are attributed to obesity and the most important of which are cardiovascular disease, type 2 diabetes, stroke, hypertension, hyperlipidemia, respiratory diseases, gastrointestinal complications, and liver disease (1,2).Many adipokines have been identified so far, which directly and indirectly affect the control of obesity and type 2 diabetes(3)Therefore, the investigation of the fat cells and the hormones secreted from them with a direct effect on increasing blood glucose is very important for obese people(4). Most physical education and medical sciences specialists agree on the method of controlling diets along with physical activity as the most basic and scientific method of weight loss, however, there is still no general agreement on the rate of the role of exercise and diet in weight loss (5). The present study used sports intervention to consider the positive changes in gene expression level and the secretion of hormones affecting obesity(5).Obesity causes changes in the levels of some hormones secreted by adipose tissue, which can severely activate the production of glucose in the liver in the long-term. The increased levels of glucose in the long term reduce the sensitivity of muscle cells to insulin and lead to insulin resistance (6). Insulin resistance and its complications are very effective in developing obesity(7).Insulin is an axial regulatory hormone, which is responsible for the energy balance and glucose homeostasis.Since insulin is no longer able to get glucose into the cells in obese people,blood glucose levels are constantly rising and both glucose and insulin in the blood are rising at the same time(8). The recently identified adipokine, called 'asprosin' hormone is synthesized through

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FBN1 gene expression and enters the blood circulation. Asprosin can increase glucose release in liver via move towards liver. Controlling the asprosin secretion among people with diabetes is very important, since the effect of asprosin on glucose release in liver is reduced by decreasing its synthesis and secretion (9,10). Exercise along with diet are considered as very important factors in controlling obesity and weight loss. Different exercises can improve glucose control via increasing glucose uptake in muscles (11). Some studies indicated that blood pressure is lowered after exercise, especially strenuous exercise and the metabolic stress caused by sport activities can improve the oxygen uptake following trainings and carbohydrates utilization during training. The type of training protocol is still under consideration (12). Considering a few studies conducted on the asprosin secretion and FBN-1 gene responsible for its secretion among healthy and obese people, the need for conducting study is direly felt in this regard.Further, given the positive effect of exercise on the improvement of the obesity, review the impact of sport activities on the asprosin is very critical. According to investigations, in the less researches reviewed the effects of continuous exercise (CE) and interval exercise (IE) on fibrillin-1 and asprosin. Thus present study performed to compare the effects of continuous exercise (CE) and interval exercise (IE) on fibrillin-1 and asprosin in obese male rats.

Materials and Methods

Animals Caring

This experimental study, 48 two-month Wistar male rats (mean weight of 180.23±7.59 g) were prepared from Mashhad University of Medical Sciences (MUMS) and transferred to Ferdowsi University of Mashhad. The situation of lab was under standard conditions (temperature: 20-24˚C, humidity: 40-50%, and 12 hours cycle of light/darkness). All ethical principles were according to Research Ethics Committee of Medical Kerman University with code IR.KMU.REC.1399.688.

Induction of Obesity

After transferring to the laboratory, the high-fat diet used for animals and kept until they reached the desired weight.In the present study, some rats became obese for two objectives(2).

Exercise Training Protocol

During two weeks, the rats were familiarized with IE and CE protocols for 10 sessions (in separated training protocols). At the beginning of training sessions all animals were placed on the treadmill in calmness situation with uniform and very low speed. In later sessions, when the rats were well on schedule, the treadmill speed was gradually increased equal to running protocol. Further, the training time was increased during two weeks in order that the rats could reach the actual training time in the main part of the training at the end of two weeks.After two weeks, without any problems with the protocols and familiarity of the rats, the main training started and ended for eight weeks(13). IE performed with intensity near to VO_{2max} with zero incline to improve oxidative capacity, oxygen absorption and aerobic power in skeletal muscles. For performing IE, the animals ran 2- 4 minutes (with 1- 2 minutes active recovery) for 5 sessions per week. In the first week, the training was started with 35% Vo2max and a speed of 14 m/min at a low intensity and 85% VO_{2max}. Similarly, the training period was increased by one meter per minute every two weeks and it reached 18 m/min and 38 m/min at the end of the eighth week(13).

Sampling

3 days after last IE and CE, all animals anesthetized by xylazine (90 mg/kg) and ketamine (90 mg / kg). Blood sampling gathered directly from heart and immersed in sodium citrate as well as centrifuged for 10 minutes. After tissue sampling, all tissues kept in liquid nitrogen and transferred to lab.

Measuring Variables

1. Asperosin test was performed using HANGZHOU EASTBIOPHAR.withCat.No : CK-E91570,ELISA kit. (Sensitivity : 0.23ng/ml)

2. First, RNA was extracted using the instructions of Trizol Kit (Bioneer made in South Korea) in white Adipose tissue and then cDNA was synthesized using Takara Jaben Kit. Also, the primers of studied genes for reverse transcription are presented in Table 1. In order to quantify the ratio of the desired gene to the reference gene, the formula 2-ΔΔCT was used.

3. Enzymatic colorimetric method was used for measurement of glucose concentration by Pars Azmoun company kit (Tehran, Iran) with measurement sensitivity of 5 mg/dL.

Table 1. Sequence of primers used in the research

Genes	Primer Sequences	Sizes (bp)
β Actine	*Forward: 5'TGGCCCACATATTCCTTGGT 3'* *Reverse: 5'-GTAGCTGCGGACATTTCAGG -3'*	147
FBN1	*Forward: 5'- AGCCTTCCTTCCTGGGCATGG -3'* *Reverse: 5'- AGCACTGTGTTGGCGTACAGGTC-3'*	183

Table 2. The test results of two-way analysis of variance

Source of changes	Sum of squares	DF	Mean square	F value	Significance level
Obesity	2854.400	1	1096.341	94.700	P=0.001
Exercise	1711.876	2	855.938	73.935	P=0.001
The interaction of obesity and age	46.183	2	23.091	1.995	P=0.149
Error factor	486.233	42	11.577		
Total	58299.500	48			

*Significance level: P <0.05

4. ELISA method was used for measurement of serum insulin by Demeditec Diagnostic insulin ELIZA kit (Germany).

5. HOMA- IR formula was used for measurement of insulin resistance according to below formula:

$$\text{HOMA} - \text{R} = \frac{\text{Fasting Insulin } \left(\mu\frac{\text{U}}{\text{ml}}\right) x \; Fasting\; Glucose \left(\frac{mmol}{l}\right)}{22.5} (14).$$

Data Analysis Procedure

Two-way analysis of variance (for asprosin) and Kruskal- Wallis (insulin resistance, FBN1 gene expression, fasting glucose and insulin, levels) tests were used for statistical analysis of data by SPSS software (P≤0.05).

Table 3. The mean asprosin among obese and non-obese rats

Group	Mean	Standard error	95% confidence interval	
			Lower bound	Upper bound
Obese	38.61	0.695	37.21	40.01
Non-obese	29.05	0.695	27.65	30.46

Table 4. The paired comparison of asprosin hormone level in untrained, high intensity interval training and continuous aerobic training groups by post hoc test

Exercise i	Exercise j	Meandifference (i-j)	Standard error	Significance level	Lower bound	Upper bound
Untrained	**-**	9.28	1.20	P<0.001	6.28	12.28
CE	**Untrained**	-9.28	1.20	P<0.001	-12.28	-6.28
	IE	5.14	1.20	P<0.001	2.14	8.14
IE	**Untrained**	-14.43	1.20	P<0.001	-17.43	-11.43
	CE	-5.14	1.20	P<0.001	-8.14	-2.14

Results

As shown in Table 3, the mean asprosin was lower in non-obese rats compare to obese rats. Further, obesity increases asprosin in male rats.

Based on the Table 4, asprosin hormone levels were not the same in CE and untrained rats (P=0.001). Further Considering the difference between the mean asprosin of CE and untrained rats; CE significantly reduced the level of asprosin hormone in rats as well as IE significantly reduced the level of asprosin hormone in rats. Also the results showed that IE reduced asprosin levels more than CE. Mean weights of rats in pre and posttest are presented in Figure 1.

The results of the Kruskal- Wallis test showed that there are significant differences in FBN1 levels between groups. The results of Mann–Whitney U-test showed that obesity control increased FBN1 (P=0.001) nevertheless CE control, IE control, CE Obese and IE Obese significantly reduced FBN1 (P=0.001) in obese rats. Indeed exercise did not significantly changed FBN1 in healthy rats. IE reduced asprosin secretion in obese rats compared to CE. This has been shown in Table 5.

The results of Kruskal- Wallis test showed that in obese control rats, the insulin levels were significantly higher than non-obese rats (P=0.002) as well as CE and IE (CE control, IE control, CE Obese and IE Obese) significantly increased insulin in healthy rats and decreased in obese rats. IE reduced insulin in obese rats compared to CE.

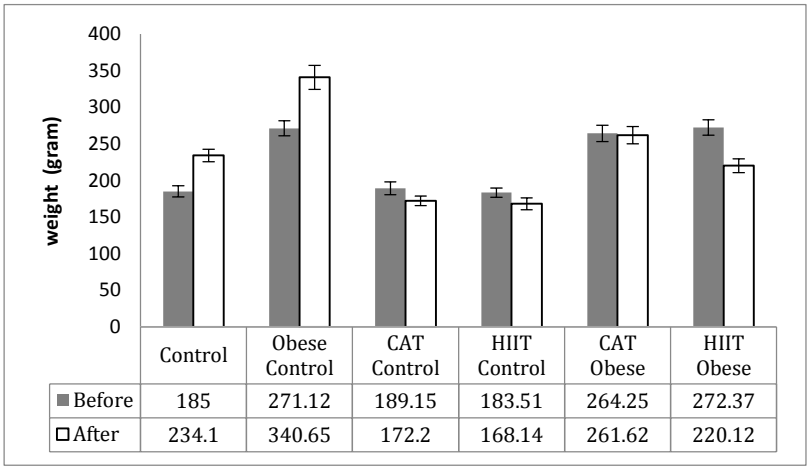

Figure1. The mean weight of the studied rats

Table 5. A comparison of the mean and standard deviation of FBN1 gene expression, insulin, fasting glucose levels, and insulin resistance index in the study groups by Kruskal- Wallis test

Variable	Study Groups						Kruskal-Wallis test results
	Control	Obeseity control	CE	Obesity and CE	IE	Obesity and IE	
FBN1	Mean±SD 0.00±1.00	Mean±SD 0.50±3.19	Mean±SD 0.26±1.16	Mean±SD 0.41±2.24	Mean±SD 0.97±0.97	Mean±SD 0.41±1.73	X2=38.01، df=5، P=0.001
Insulin	0.27±1.96	0.77±3.14	0.19±2.03	0.92±2.27	0.20±2.10	1.92±2.29	X2=19.55، df=5، P=0.002
Fasting Glucose Level	10.05±198.62	28.22±313.5	10.14±179.37	38.12±282.75	9.77±179.62	43.34±267.12	X2=42.27، df=5، P=0.001
Insulin Resistance Index	2.55±17.32	9.63±43.87	1.80±16.24	6.28±28.53	2.05±15.95	6.10±27.05	X2=36، df=5، P=0.001

In healthy rats, the fasting glucose levels were lower than obese rats (P =0.001) as well as IE and CE (CE control, IE control, CE Obese and IE Obese) significantly decreased fasting glucose in obese and healthy rats. IE reduced glucose in obese and healthy rats compared to CE.

The results of Kruskal-Wallis test showed that insulin resistance levels in healthy rats were significantly lower than obese rats (P =0.001) as well as IE and CE (CE control, IE control, CE Obese and IE Obese) significantly decreased insulin resistance in obese and healthy rats. IE insulin resistance in obese and healthy rats compared to CE.

Discussion

In present study insulin resistance, fasting glucose, insulin, FBN1 and asprosin levels were different in all research groups. Progress in metabolic diseases can be due to imbalance in factors secreted from adipose tissues. According to results of present study in obese training, healthy training, obese control and healthy control groups, the insulin resistance, fasting glucose, insulin, FBN1 and asprosin levels were significantly different. In recent years, the central elements regulating energy homeostasis, such as food intake behavior and energy expenditure, have been considered to better understand the pathophysiological mechanisms of obesity, as the main cause of metabolic disorders (15).The studies conducted on the mechanisms associated with the obesity and the occurrence of cardiovascular disease identified increased fat mass as the most important factor(16). In recent studies, adipose tissue is known as an active

endocrine and paracrine organ by synthesizing and secreting a set of adipocytokines and bioactive mediators, which controls body weight balance and explains the association of overweight and obesity with insulin resistance, diabetes, and cardiovascular disease by influencing the lipid, metabolic, and inflammatory profile (14). The recently discovered hormone, called asprosin, is secreted from fat cells during fasting. Via cAMP signaling pathway asprosin can affect liver to release glucose into the bloodstream (17). As a result, the hormone insulin is secreted.It is worth noting that asprosin is secreted during starvation and in response to an excessive decrease in blood glucose, aiming to regulate glucose homeostasis(17).Therefore, the this research initially reviewed whether obesity in rats could cause changes in asprosin levels. Based on the results, obesity in rats significantly increased asprosin secretion levels. Asprosin levels in healthy rats were significantly lower than obese rats. Thus reducing the asprosin for obese people, is very important to prevent the effect of this hormone on the release of liver glucose into the bloodstream. Given that increased plasma glucose levels, followed by increased insulin secretion, has a negative effect on the secretion of hormones influencing lipolysis, a strategy for weight loss in obese people is to lower their glucose levels during fasting. Also fasting glucose and insulin levels in healthy rats were lower obese rats, so that it may be related to lower levels of asprosin in healthy rats compared to obese rats (14). It has been reported that increase in insulin secretion and blood glucose levels in obese people can be due to the secretion of more than normal level of asprosin. Indeed high levels of asprosin hormone in obese people can affect liver to increase the release of glucose into the bloodstream. Due to dysfunction in GLUT4 which is a special glucose transporter, glucose cannot enter into the muscle cells. Therefore for decrease the secretion and synthesis of asprosin, adjustments in nutrition and exercise interventions can be important.

Results showed that CE significantly decreased blood glucose and asprosin levels in obese and healthy rats. Accordingly, obese people can prevent the increased asprosin via performing CE and managing their glucose levels in bloodstream. FBN1 gene (in humans) can encode the Fibrillin-1 glycoprotein. Researchers recently pointed that Fibrillin-1 can plays a important role in adipogenesis. The researchers reported that in inbred mouse species the FBN1 levels are associated with the changes in adipocyte levels. Further, a decrease or increase in fibrillin-1 levels in fat cells, caused by gene mutations or lifestyle, is associated with the changes in fat cell levels and FBN1 gene expression in obese mice more than normal-weight or healthy mice with adipocyte size. The relationship between asprosin and obesity largely depends on its upstream gene, namely, FBN1(18).

In present study obesity in rats raised FBN1 levels. Thus, the synthesis and secretion of asprosin increased in response to the enhancement of gene expression. Finally the main aim of controlling glucose and asprosin levels is to reduction of FBN1. In present research IE and CE significantly decreased FBN1 levels as well as FBN1, increase in obese rats. In order to explain the impact of obesity and trainings on fasting glucose homeostasis, it should be noted that obesity is a disease-causing change in fat cells.Therefore, there may be changes in the level of liver cells, leading to the release of more glucose into the bloodstream (19). The obese people may experience increased insulin secretion level and insulin resistance during fasting if their blood glucose levels rise. Based on the results, the insulin resistance, insulin, glucose, asprosin and FBN1 levels in healthy rats were significantly lower than obese rats. In addition, exercise reduced asprosin levels and FBN1 gene expression in adipocytes. Therefore, long-term exercise helps the glucose homeostasis in obese people by reducing the activity of glycogenolysis and hepatic gluconeogenesis pathways. Decreased plasma glucose means the decreased insulin secretion and reduced insulin resistance.Additionally, exercise increase glucose uptake by muscle cells in obese individuals by increasing AMPK and GLUT4 activity(20).Therefore, exercise through changes in the levels of fat and muscle cells leads to a reduction in insulin resistance and glucose in the long term. Results showed that IE lowered insulin resistance, glucose, asprosin secretion, and FBN1 gene expression compared to CE,which can be attributed to the impact of exercise on GLUT4 activity. The long-term aerobic exercise contributes to the activation of GLUT4s on the surface of muscle cells by increasing intracellular calcium levels and calmodulin

calcium(21).However, HIIT increases AMPK levels sharply, due to the high conversion rate of ATP to AMP.AMPK has a greater effect on the activation of GLUT4s on the surface of muscle cells(22).Therefore, the greater effect of HIIT protocol compared to CAT on lowering blood glucose levels and insulin resistance for more activation of AMPK is due to the high intensity of exercise in HIIT. As FBN1 and asprosin recently discovered, less studies found regarding to effect of IE and CE. Although in present study IE had high positive effects on FBN1 and asprosin in obese rats compared to CE.

Conclusion

Although obesity increased fibrillin-1 and asprosin, but IE and CE decreased fibrillin-1 and asprosin. Thus IE and CE can be used for controlling fibrillin-1 and asprosin levels as well as an intervention method contributing to the reduction of weight and obesity.

Statements

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Conflict of Interest

There is no conflict of interest.

Animal Rights

All ethical principles were according to Research Ethics Committee of Medical Kerman University.

References

1. Lönn A, Kallings L V, Andersson G, Paulsson S, Wallin P, Eriksson JS, et al. Lifestyle-related habits and factors before and after cardiovascular diagnosis: a case control study among 2,548 Swedish individuals. Int J Behav Nutr Phys Act. 2023;20(1):1–12.
2. Davari F, Alimanesh Z, Alimanesh Z, Salehi O, Hosseini SA. Effect of training and crocin supplementation on mitochondrial biogenesis and redox-sensitive transcription factors in liver tissue of type 2 diabetic rats. Arch Physiol Biochem. 2022;128(5):1215–20.
3. Chakraborti CK. Role of adiponectin and some other factors linking type 2 diabetes mellitus and obesity. World J Diabetes. 2015;6(15):1296.
4. Roy PK, Islam J, Lalhlenmawia H. Prospects of potential adipokines as therapeutic agents in obesity-linked atherogenic dyslipidemia and insulin resistance. Egypt Hear J. 2023;75(1):1–28.
5. Gokee-LaRose J, Gorin AA, Raynor HA, Laska MN, Jeffery RW, Levy RL, et al. Are standard behavioral weight loss programs effective for young adults? Int J Obes. 2009;33(12):1374–80.
6. Meex RCR, Schrauwen-Hinderling VB, Moonen-Kornips E, Schaart G, Mensink M, Phielix E, et al. Restoration of muscle mitochondrial function and metabolic flexibility in type 2 diabetes by exercise training is paralleled by increased myocellular fat storage and improved insulin sensitivity. Diabetes. 2010;59(3):572–9.
7. Tatoń J, Czech A, Piątkiewicz P. Insulin as the main regulator of cellular glucose utilization-aetiological aspects of insulin resistance. Endokrynol Pol. 2010;61(4):388–94.
8. Hosseini SA, Hamzavi K, Safarzadeh H, Salehi O. Interactive effect of swimming training and fenugreek (Trigonella foenum graecum L.) extract on glycemic indices and lipid profile in diabetic rats. Arch Physiol Biochem. 2023; 129(2):349-53
9. Romere C, Duerrschmid C, Bournat J, Constable P, Jain M, Xia F, et al. Asprosin, a fasting-induced glucogenic protein hormone. Cell. 2016;165(3):566–79.
10. Yuan M, Li W, Zhu Y, Yu B, Wu J. Asprosin: a novel player in metabolic diseases. Front Endocrinol (Lausanne). 2020;11:64.
11. Colberg SR, Sigal RJ, Fernhall B, Regensteiner JG, Blissmer BJ, Rubin RR, et al. Exercise and type 2 diabetes: the American College of Sports Medicine and the American Diabetes Association: joint position statement. Diabetes Care. 2010;33(12):e147–67.
12. Asano RY, Sales MM, Browne RAV, Moraes JFVN, Júnior HJC, Moraes MR, et al. Acute effects of physical exercise in type 2 diabetes: a review. World J Diabetes. 2014;5(5):659.
13. Pirani H, Bakhtiari A, Amiri B, Salehi OR. Beneficial Mitochondrial Biogenesis in Gastrocnemius Muscle Promoted by High-Intensity Interval Training in Elderly Female Rats. Cell J. 2023;25(1):11.
14. Czech MP. Insulin action and resistance in obesity and type 2 diabetes. Nat Med. 2017;23(7):804–14.
15. Watts K, Jones TW, Davis EA, Green D. Exercise training in obese children and adolescents. Sport Med. 2005;35(5):375–92.
16. Alizadeh R, Salehi O, Rezaeinezhad N, Hosseini SA. The effect of high intensity interval training with genistein supplementation on mitochondrial function in the heart tissue of elderly rats. Exp Gerontol. 2023;171:112039.
17. Mazur-Bialy AI. Asprosin—A Fasting-Induced, Glucogenic, and Orexigenic Adipokine as a New Promising Player. Will It Be a New Factor in the Treatment of Obesity, Diabetes, or Infertility? A Review of the Literature. Nutrients. 2021;13(2):620.
18. Davis MR, Arner E, Duffy CRE, De Sousa PA, Dahlman I, Arner P, et al. Expression of FBN1 during adipogenesis: Relevance to the lipodystrophy phenotype in Marfan syndrome and related conditions. Mol Genet Metab. 2016;119(1–2):174–85.
19. Triplitt C, DeFronzo RA. Exenatide: first-in-class

incretin mimetic for the treatment of type 2 diabetes mellitus. Expert Rev Endocrinol Metab. 2006;1(3):329–41.
20. Elnagar A, El-Dawy K, El-Belbasi HI, Rehan IF, Embark H, Al-Amgad Z, et al. Ameliorative Effect of Oxytocin on FBN1 and PEPCK Gene Expression, and Behavioral Patterns in Rats' Obesity-Induced Diabetes. Front Public Heal. 2022;10.
21. Rose AJ, Frøsig C, Kiens B, Wojtaszewski JFP, Richter EA. Effect of endurance exercise training on Ca2+–calmodulin-dependent protein kinase II expression and signalling in skeletal muscle of humans. J Physiol. 2007;583(2):785–95.
22. O'neill HM. AMPK and exercise: glucose uptake and insulin sensitivity. Diabetes Metab J. 2013;37(1):1–21.