Effect of Resistance Training with Two Different Volumes on Serum Myonectin Levels in Rats Fed with Sucrose Solution

[1]Majid Adigozalpour, [1]Alireza Safarzade*

[1]Department of Exercise Physiology, Faculty of Sport Science, University of Mazandaran, Babolsar, Iran.

ABSTRACT

Background. Moyenctin, is a new identified myokine and belongs to the CTRPs family which plays role in lipid and glucose metabolism. There is limited information available regarding the effects of exercise training on serum myonectin levels. **Objectives.** he aim of this study was to investigate the effects of sucrose solution intake and resistance training (RT) with two different volumes on serum myonectin levels and HOMA-IR in rats. **Methods.** Forty-eight male Wistar rats (10-12 weeks old) were randomly divided into six groups; Control (C), Low volume RT (L), High Volume RT (H), Sucrose Control (SC), Sucrose Low volume RT (SL) and Sucrose High volume RT (SH). The RT groups were subjected to a resistance training program with the use of a ladder (3 days/week, for 8 weeks). Body weight and serum levels of myonectin, insulin, glucose, and HOMA-IR were measured. **Results.** The results two way ANOVA showed that interaction between RT and sucrose solution significantly increased serum levels of insulin (P=0.007) and HOMAL-IR (P=0.002). Consumption of sucrose solution, significantly reduced serum myonectin (P=0.0001) and increased serum glucose, insulin and HOMA-IR (P=0.0001). Also RT significantly reduced serum myonectin, insulin and HOMA-IR (P=0.0001). No correlation was found between serum myonectin and insulin resistance in groups fed with normal foods (P=0.456), but in the groups fed with sucrose solution there was positive and significant correlation (P=0.0001). **Conclusion.** This data indicated that resistance training regardless of volume of training can reduce serum myonectin levels as well as insulin resistance in rats fed with sucrose solution or normal diet groups.

KEY WORDS: *Myonectin, Insulin Resistance, Sucrose Solution, Resistance Training.*

INTRODUCTION

Insulin resistance in skeletal muscles is one of the most important defective factors in maintaining normal glucose levels in the blood. Insulin resistance is associated with several diseases like hypertension, atherosclerosis, impaired glucose tolerance, and Type 2 diabetes (1). The main link between these diseases is increasing insulin levels as a result of insulin resistance (1). It has been shown that a diet with a higher ratio of glucose increases insulin resistance (2). For example, researchers reported that plasma levels of insulin and glucose showed a significant increase after receiving a diet with more sucrose (2). Also, an increase in the proportion of sucrose in the diet was associated with an increase in the abdominal obesity index. These are considered the parameters of classic insulin resistance (2). Exercise training plays a

*. Corresponding Author:
Alireza Safarzade
E-mail: a.safarzade@umz.ac.ir

significant role as a major factor in the prevention, control and even improvement of insulin resistance and diabetes (3). Several factors may improve glucose homeostasis and reduce insulin resistance caused by acute and chronic physical activities (1). Among these factors, changes in the levels of some proteins or peptides can be mentioned. Skeletal muscles actually secrete various bioactive polypeptides that are collectively called myokines (myocytokine) and may be involved in autocrine, paracrine or endocrine in the regulation of metabolism and inflammatory processes (4). Myokines can also play an important role in reducing insulin resistance (5-7).

Myonectin is known as an important myokine that is secreted in response to exercise as well as consumption of glucose and fatty acids (sugar and fat). It has been proposed to play an important role in the increased consumption of glucose and lipids in the liver and adipose tissue, and may be able to prevent the development of insulin resistance (8, 9). Myonectin secretion is stimulated by two primary factors: exercise and nutrition. Changes in circulation myonectin levels are very slow in the blood. It has been shown that it increases two hours after receiving diet with high glucose or lipid. Also, an increase in the secretion of myonectin has been seen after two weeks of training in laboratory rats (9), though it has not been specified that increased levels of myonectin due to the two weeks of exercise have occurred alone or following the consumption of fat and glucose in combination with exercise. The effects of myonectin on glucose and lipid metabolism have created promising therapeutic opportunities. However, the molecular mechanisms of expression, secretion and action of myonectin have not been determined yet.

Regular physical activity has a protective role in sedentary individuals over several pathological conditions such as insulin resistance, obesity, atherosclerosis, type 2 diabetes, neurological disorders and cancers, such as breast cancer (7). Since skeletal muscles are the main origin of cytokine secretion (myokines), it seems muscle contractions are a major factor in stimulating the secretion of myokines (10-12). Resistance training plays a

prominent role in the expression of key signaling proteins in the regulation of glucose consumption and metabolism in muscles (13, 14). The studies that focused on the effect of resistance training have shown a positive correlation between resistance training and diabetes control (8, 15). There is also an association between insulin sensitivity and resistance exercises. It has been shown that resistance exercise increases insulin sensitivity (13-16).

Little information is available about the effects of resistance training with different volumes associated with insulin resistance and secretion of myokines (17, 18). Researchers still have not reached any consensus on this matter. For example, it has been shown that high-volume and moderate-intensity exercise lead to a further reduction of insulin resistance (19). On the other hand, a study showed that exercise at low to moderate intensity is more effective than exercise with moderate to high intensity (20), while another study reported that high intensity exercise is more effective in improving glycemic control (21). Also, different types of myokines have different changes caused by different volumes of resistance training; low volume—in some myokines—and high volume—in others—increase myokines (22). Therefore, taking into consideration the abovementioned factors, the aim of this study was to investigate the effect of sucrose solution consumption and resistance training with two different volumes on serum myonectin levels and insulin resistance index.

MATERIALS AND METHODS

All animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication 86-23 revised 1985).

Animals. 48 adult male Wistar rats (10 to 12 weeks, with an average weight of 157.3 ± 13.8 g) were prepared from the Pasteur Institute of Iran, and were divided into six equal groups based on the homogenization of weight (eight in each group and four in each cage). These animals—after a week's familiarization with the laboratory environment—were divided into two equal groups at the first (three groups per

category) for feeding with and without solution of sucrose 30%. After four weeks, each category was divided into a control group and two exercise groups. In them, finally, groups of this study included (1) control (C), (2) training with low volume (L), 3- training with high volume (H), (4) control + feeding with sucrose (SC), (5) training with low volume + feeding with sucrose (SL), (6) training with high volume + feeding with sucrose (SH).

The rats subjected in this study were kept in cages with dimensions of $20 \times 27 \times 47$ cm. The ambient temperature was $22 \pm 2°C$, and light cycle was 12:12 h. Rats were fed with feed normal pellets and water ad libitum. Also, 30% sucrose solution was added for groups fed with sucrose (23).

Resistance Training. Resistance training included climbing the ladder for one meter (26 steps). The ladder was inclined to the floor at an angle of 85 degrees. The resistance training started four weeks after the beginning of feeding with sucrose solution, and lasted eight weeks. In order to reduce the stress, electric shock, air pressure, cold water, rewards, or food deprivation were not used at all; rather, only touching and grooming of the rat tail were

carried out. Resistance training was held three days a week, every other day; the first training session was done with a load equal to 50% of the weight of the rats, for all the training groups in two times and five repeats every time. Within two weeks, the number of training sessions for groups of high volume gradually increased and reached four times and five repeats at every time, while in the group with low volume, the number of times and repetitions were constant.

Also, in accordance with the principle of overload, the loads from the second week were respectively 75, 100, 125, 150, 175, and 200 percent of the animals' weight. In order to determine the appropriate exercise weight, the rats were weighed every week, and in the last few weeks (two weeks), the work load was constant. There was a one-minute rest between each repetition and also two minutes' rest between each time (26). Before and after each training session, rats climbed the stairs two to three times without weights for warm-up and cool-down. The amount of work done (training volume) was calculated on the basis of the amount of the mass moved by the animal multiplied by the acceleration of gravity and distance displacement (Figure 1).
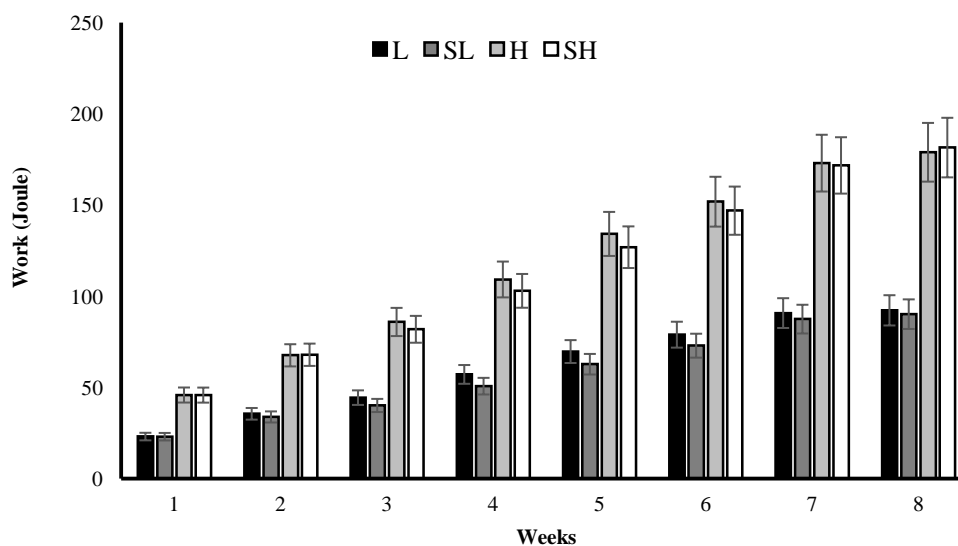

Figure 1. *Workouts for each week.* Low volume RT (**L**), High volume RT (**H**), Low volume RT+ sucrose (**SL**), High volume RT+sucrose (**SH**)

Measurement. In order to eliminate the acute effects of exercise, samples were taken 72 hours after the last training session and after an

overnight fast (eight to 12 hours). Rats were anesthetized by intraperitoneal injection of ketamine (70 mg/kg) and Xylazine (3-5 mg/kg).

Blood samples were taken from the superior vena cava and collected in the tubes. Samples collected were centrifuged at 3,000 rpm for 15 min at 4°C; its serum was isolated, and then transferred to the freezer with a temperature of -20°C for the next stages of research. Myonectin and insulin serum concentrations were measured by ELISA method and using the kits for rats (ZellBio Germany for Myonectin and Mercodia Sweden for insulin). To evaluate insulin resistance, the HOMA-IR index and the following calculation formula were used.

HOMA-IR = glucose concentration (mmol /liter) × insulin concentration (m units / liter) ÷ 22.5

Data Analysis. In order to describe the data, descriptive statistical indices, including mean and standard deviation, were used. In the analysis (deduction) section, after making sure of the normal distribution of data using Kolmogorov–Smirnov test, two-way analysis of variance and LSD post hoc tests were used. To investigate the relationship between levels, myonectin serum and insulin sensitivity Pearson's correlation coefficient was used. All analyses were performed using *SPSS Version 16* and threshold for statistical significance was $p<0.05$.

RESULTS

All rats in the four experimental groups could do eight-week resistance training. These animals were capable of carrying up to 200% of their weights. The weights of the rats are given in Table 1. At the beginning of the study, there was no significant difference between the weights of animals in different groups.

Table.1 Changes in rat's weight (gr) before and after 8 week RT (mean ± SE)

	C	L	H	SC	SL	SH
Baseline weight (gr)	160 ± 12	157 ± 92	156 ± 8	159 ± 12	156 ± 8	156 ± 8
Final weight (gr)	350 ± 32	362 ± 28	349 ± 28	367 ± 54	373 ± 37	369 ± 29
Weight gain (gr)	190 ± 27	206 ± 22	193 ± 41	208 ± 50	217 ± 40	213 ± 26

Control (**C**), Low volume RT (**L**), High volume RT (**H**), Control+sucrose (**SC**), Low volume RT+ sucrose (**SL**), High volume RT+sucrose (**SH**)

The results of the two-way analysis of variance for myonectin serum levels indicated no significant interaction of sucrose solution and training ($p=0.205$ and $F=1.657$). However, the significance effect of sucrose solution contributed to a reduction in the amount of myonectin in the groups fed with sucrose, compared with the groups fed with normal food ($p=0.0001$ and $F=7.860$). The significance effect of resistance training ($p=0.0001$ and $F=467.794$) was also observed for eight weeks. LSD post hoc test results showed that there is a significant difference between the myonectin levels of control groups compared with the low-volume and high-volume training groups ($p=0.0001$). But no significant difference was observed between low- and high-volume training groups in the levels of myonectin ($p=0.707$). Myonectin levels significantly decreased as a result of eight weeks of resistance training (fig.2).

The results of two-way ANOVA for glucose serum levels showed no significant interaction of sucrose solution and training ($p=0.144$ and $F=2.079$). However, the serum level of glucose significantly increased in response to sucrose solution ($p=0.0001$ and $F=22.269$).

The results of the two-way ANOVA for insulin serum levels indicated a significant interaction of sucrose solution and training ($p=0.007$ and $F=5.987$). LSD post hoc test results showed that there is a significant difference between the insulin levels of control groups compared with the low volume and high volume training groups ($p=0.0001$). However, no significant difference was observed between low- and high-volume training groups in the levels of insulin ($p=0.911$).

The results of two-way ANOVA for insulin resistance index showed the significant interaction of sucrose solution and training ($p=0.002$ and

F=7.513). HOMA-IR was significantly improved in the groups fed with the sucrose solution. LSD post hoc test results showed that there is a significant difference between the insulin levels of control groups compared with the low-volume and high-volume training groups (p=0.0001). But no significant difference was observed between low- and high-volume training groups in the levels of insulin (p=0.911).

According to Pearson correlation test results, it is clear that there is no significant correlation between myonectin levels and insulin resistance index in the groups fed with normal food (p=0.456). The results also showed that there is a significant positive correlation between the myonectin levels and insulin resistance index in the groups fed with sucrose solution p=0.0001).

Table.2 Serum levels of variables after 8 weeks RT (mean ± SE)

	C	L	H	SC	SL	SH
Insulin	7.38 ±2.78	5.69±2.66*	5.08±2.60*	20.91±5.64#	8.78±4.17*#	9.71±2.97*#
Glucose	5.03±0.40	5.58±0.79	5.48±0.66	6.98±1.20#	6.93±0.68 #	6.390.71#
HOMA-IR	1.64±0.61	1.37±0.56*	1.30±0.68*	6.51±2.11#	2.69±1.27*#	2.77±0.74*#

*p<0.05 main effect of resistance training; #p<0.05 main effect of sucrose. Control (**C**), Low volume RT (**L**), High volume RT (**H**), Control+sucrose (**SC**), Low volume RT+ sucrose (**SL**), High volume RT+sucrose (**SH**)
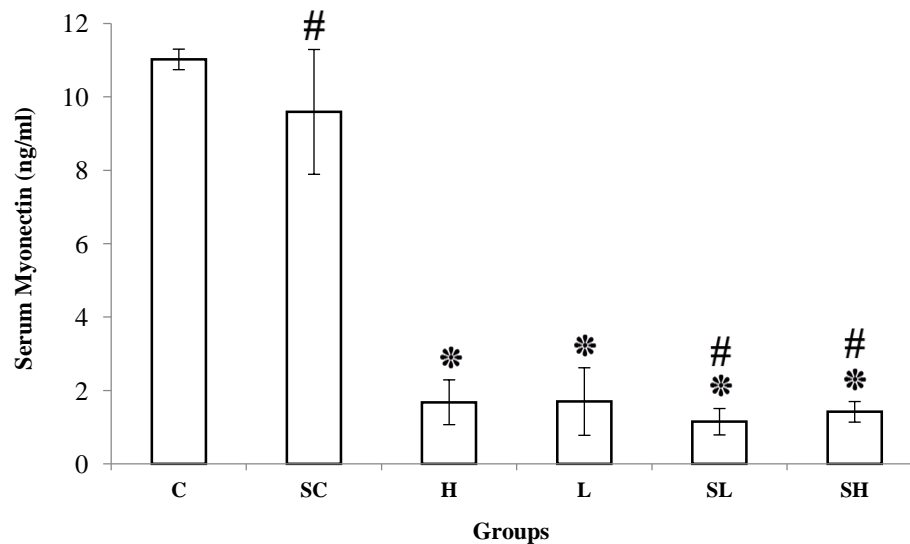

Figure 2. *Serum Myonectin levels (ng/ml) after 8 weeks of RT.* mean ± E | ***:** p<0.05 RT versus controls; **#:** p<0.05 sucrose fed versus normal fed; Control (**C**), Low volum RT (**L**), High volum RT (**H**), Control+sucrose (**SC**), Low volume RT+ sucrose (**SL**), High volume RT+sucrose (**SH**)

DISCUSSION

The results of the present study showed that in the groups fed with sucrose solution, serum myonectin levels were reduced compared to the groups fed with normal diet. Myonectin is highly dependent on food to the extent that it is referred to as hormonal food (9). Myonectin also reduces

in obesity state. In the study conducted on rats got fat with a normal diet compared with rats with a low-fat diet, it was observed that circulating levels of myonectin are significantly less in obese rats (9). In the initial study of Seldin et al., reduced levels of myonectin in rats fed with a high-fat diet were considered due to

reduced mRNA levels in skeletal muscle (9). In the present study, myonectin levels in rats fed with sucrose were lower than in rats fed with a normal diet as well. However, a recent study by Peterson et al. showed an increase in myonectin circulation in obese Zucker rats (24). Since Zucker rats are genetically deficient in leptin receptor and show continually increased hyper-leptinmy (increased levels of leptin), such effects may be due to chronic stimulation of the muscle cells by leptin. It was recently shown that leptin acts on muscle cells to stimulate the myonectin gene (25). However, leptin levels were not measured in this study. On the other hand, it has been observed that myonectin serum levels remain unchanged in rats with calorie restrictions (26).

This study showed that eight weeks of resistance training—regardless of the volume of training—significantly reduces myonectin levels in all training groups fed with sucrose and normal, compared to the control group. In 2012, Seldin et al. investigated the expression and systematic circulation of myonectin following two weeks of treadmill-free exercise on male rats model of C57BL/6; the results of this study indicated the increased expression of myonectin in muscle and also its increased serum levels (9), which contradict the results of the present study. In another study—in line with the results of this study—rats model Zucker, both lean and obese, exercised aerobic training for nine weeks and five days a week on the animal treadmill. At the end, the expression of the myonectin gene—regardless of the state of obesity, both in lean rats and obese rats—was significantly reduced (6, 24).

One of the interesting points, which can be a reason for these contradictions, is the training period in these two studies. In the study of Seldin et al. (27), the duration of training was two weeks, which increased the myonectin expression. But in the study of Peterson et al. (25), the effect of long duration (nine weeks) on myonectin was assessed for the first time, which reduced the expression of myonectin. In the present study, myonectin serum levels decreased after eight weeks of resistance training in all rats, including normal and those fed with sucrose. Peterson et al. showed the reduction of myonectin mRNA in Zucker rats after exercise

training (6). The weakness of the study of Peterson et al. was the lack of measurement of myonectin in serum samples: the authors assumed that circulating myonectin levels can be associated with its intra-muscular levels. Myonectin plasma levels and insulin resistance index were investigated in another study using concurrent training on elderly women. In this study, 40 elderly women, in different groups, conducted concurrent training (endurance-power or intermittent) for eight weeks and three sessions per week. The results of this study showed no significant difference in myonectin and plasma insulin levels between training and control groups (28). This means that exercise training had no effect on the plasma myonectin. However, the authors of this study reported that insulin resistance index decreased significantly in training groups.

Various myonectin changes by exercise training in previous studies may be because of differences in experimental animal models in particular, or may be relevant due to differences in the action of leptin and exercise type (strength, endurance, combination and optional running). Overall, the available results show that the expression of myonectin may be regulated by leptin, the secretion of which may also be stimulated by nutritional status; as shown, it decreases after exercise. Decreased myonectin expression in the study of Peterson et al. may reflect reduced leptin after training, though the authors have not measured it. In total, there is still no consensus in connection with myonectin changes with diet and exercise, and more research is required in this area.

The results of the present study also suggested that insulin resistance in the groups fed with sucrose solution was greater compared to the groups fed with normal food. Also, the insulin resistance index of the control groups was higher compared with the training groups, regardless of whether training volume and resistance training reduced insulin resistance. The results also showed that there is a significant positive correlation between the serum levels of myonectin and insulin resistance index in the groups fed with sucrose solution. The impaired secretion and action of myokines—like myonectin—may be involved in the development of insulin resistance. On the other

hand, several studies have shown that insulin resistance in skeletal muscle may alter the expression and secretion of Myokines. This may subsequently affect the metabolism of fat and glucose in adipose tissue, leading to a vicious cycle between impaired myokine production and insulin resistance (8). Although muscles directly increase GLUT4 transport and glycogen synthesis in response to insulin, myokines affect the metabolism of glucose and lipids in the whole body, and the energy balance as well. Also, as has been demonstrated, they act on the adipose tissue, liver, pancreas and intestines (8). Therefore, simultaneous increase of myonectin and insulin resistance through feeding with sucrose sucrose as well as a concurrent reduction of myonectin and insulin resistance in this study seems reasonable.

There is less information available from studies on the effects of resistance training with different amounts on insulin resistance and myokine secretion (17, 18); researchers still have not reached any consensus on this matter. For example, it has been shown that various types of myokines show different changes to resistance training with different volumes; low volume in some myokines and high volume in others may cause changes or no changes in them (22). On the other hand, it has been shown that high-volume and moderate-intensity exercise leads to further reduction of insulin resistance (19), which is not consistent with the present study. This contradiction in results may be because of the nutritional status of this study and exercise duration. On the other hand, a study showed that exercise at low to moderate intensity is more effective, relative to the exercise with moderate to high intensity (20), while another study reported that high intensity exercise is more effective to improve glycemic control (21). In this respect, Gorge et al. (2011) also observed no changes in HOMA-IR index after 12 weeks of combined aerobic and resistance exercises (29). This difference in results may reflect the different methods used to assess sensitivity to insulin. For example, researchers have suggested that glycemic–hyper-insulin clamping is more sensitive in the function assessment of insulin (29).

In the study of Stefano et al., it was observed that a significant inverse relationship between physical activity levels and insulin resistance exists, in line with previous reports, emphasizing the beneficial effects of exercise on insulin sensitivity in different populations (30). It has been shown that physical activity increases the insulin sensitivity through changes in body fat mass as well as through mechanisms independent of fat mass loss, such as: Increasing the transfer of GLUT4 and the subsequent use of glucose in skeletal muscle, improving the capacity of skeletal muscles for fat oxidation, increasing fat transfer of muscle cells, and reducing the amount of fat metabolites (30).

CONCLUSION

The results of the present study showed that eight weeks of resistance training—regardless of training volume—leads to reduced levels of myonectin and insulin resistance in all training groups. Hence, it seems that even low volume resistance training can lead to almost similar improvements in insulin resistance and serum myonectin change. So, further research is required in this area.

APPLICABLE REMARKS

- Resistance training regardless of volume of training can improved serum insulin levels and insulin resistance index in both normal and insulin resistance conditions, and these improvements accompanied by decline of serum myonectin levels.

- It seems that improvement of metabolic condition would occur even by low volume of resistance training.

REFERENCES

1. Henriksen EJ. Invited review: Effects of acute exercise and exercise training on insulin resistance. Journal of Applied Physiology. 2002;93(2):788-96.

2. Folmer V, Soares JC, Rocha J. Oxidative stress in mice is dependent on the free glucose content of the diet. The international journal of biochemistry & cell biology. 2002;34(10):1279-85.

3. Sigal RJ, Kenny GP, Wasserman DH, Castaneda-Sceppa C, White RD. Physical activity/exercise and Type 2 diabetes A consensus statement from the American Diabetes Association. Diabetes care. 2006;29(6):1433-8.

4. Petersen KF, Shulman GI. Pathogenesis of skeletal muscle insulin resistance in type 2 diabetes mellitus. The American journal of cardiology. 2002;90(5):11-8.

5. Pedersen BK. Muscle as a secretory organ. Comprehensive Physiology. 2013.

6. Peterson JM, Mart R, Bond CE. Effect of obesity and exercise on the expression of the novel myokines, Myonectin and Fibronectin type III domain containing 5. PeerJ. 2014;2:e605.

7. Peterson JM, Seldin MM, Wei Z, Aja S, Wong GW. CTRP3 attenuates diet-induced hepatic steatosis by regulating triglyceride metabolism. American Journal of Physiology-Gastrointestinal and Liver Physiology. 2013;305(3):G214-G24.

8. Gamas L, Matafome P, Seica R. Irisin and Myonectin Regulation in the Insulin Resistant Muscle: Implications to Adipose Tissue: Muscle Crosstalk. J Diabetes Res. 2015;2015:359159.

9. Seldin MM, Peterson JM, Byerly MS, Wei Z, Wong GW. Myonectin (CTRP15), a novel myokine that links skeletal muscle to systemic lipid homeostasis. J Biol Chem. 2012;287(15):11968-80.

10. Akerstrom T, Steensberg A, Keller P, Keller C, Penkowa M, Pedersen BK. Retraction: Exercise induces interleukin-8 expression in human skeletal muscle. The Journal of Physiology. 2011;589(13):3407-.

11. Febbraio MA, Pedersen BK. Muscle-derived interleukin-6: mechanisms for activation and possible biological roles. The FASEB Journal. 2002;16(11):1335-47.

12. Febbraio MA, Pedersen BK. Contraction-induced myokine production and release: is skeletal muscle an endocrine organ? Exercise and sport sciences reviews. 2005;33(3):114-9.

13. Ibañez J, Izquierdo M, Argüelles I, Forga L, Larrión JL, García-Unciti M, et al. Twice-weekly progressive resistance training decreases abdominal fat and improves insulin sensitivity in older men with type 2 diabetes. Diabetes care. 2005;28(3):662-7.

14. Poehlman ET, Dvorak RV, DeNino WF, Brochu M, Ades PA. Effects of Resistance Training and Endurance Training on Insulin Sensitivity in Nonobese, Young Women: A Controlled Randomized Trial 1. The Journal of Clinical Endocrinology & Metabolism. 2000;85(7):2463-8.

15. Stouthard J, Romijn JA, Van der Poll T, Endert E, Klein S, Bakker P, et al. Endocrinologic and metabolic effects of interleukin-6 in humans. American Journal of Physiology-Endocrinology And Metabolism. 1995;268(5):E813-E9.

16. Shaibi GQ, Cruz ML, Ball GD, Weigensberg MJ, Salem GJ, Crespo NC, et al. Effects of resistance training on insulin sensitivity in overweight Latino adolescent males. Medicine and science in sports and exercise. 2006;38(7):1208.

17. Pedersen B, Steensberg A, Fischer C, Keller C, Keller P, Plomgaard P, et al. Searching for the exercise factor: is IL-6 a candidate? Journal of Muscle Research & Cell Motility. 2003;24(2-3):113-9.

18. Pedersen BK, Steensberg A, Fischer C, Keller C, Ostrowski K, Schjerling P. Exercise and cytokines with particular focus on muscle derived IL-6. Exercise immunology review. 2001;7:18-31.

19. Talebi-Garakani EAS, Alireza. The effect of resistance training intensity and volume on serum vaspin concentration and insulin resistance index in adult male rats. Daneshvar. 2012;19(100):75-82.

20. Hansen D, Dendale P, Jonkers R, Beelen M, Manders R, Corluy L, et al. Continuous low-to moderate-intensity exercise training is as effective as moderate-to high-intensity exercise training at lowering blood HbA1c in obese type 2 diabetes patients. Diabetologia. 2009;52(9):1789-97.

21. Dunstan DW, Daly RM, Owen N, Jolley D, De Courten M, Shaw J, et al. High-intensity resistance training improves glycemic control in older patients with type 2 diabetes. Diabetes care. 2002;25(10):1729-36.

22. Izquierdo M, Ibañez J, Calbet JA, Navarro-Amezqueta I, González-Izal M, Idoate F, et al. Cytokine and hormone responses to resistance training. European journal of applied physiology. 2009;107(4):397-409.

23. Ribeiro R, Lautt W, Legare D, Macedo M. Insulin resistance induced by sucrose feeding in rats is due to an impairment of the hepatic parasympathetic nerves. Diabetologia. 2005;48(5):976-83.

24. Peterson JM, Mart R, Bond CE. Effect of obesity and exercise on the expression of the novel myokines, Myonectin and Irisin. PeerJ PrePrints. 2014;2:e410v1.

25. Rodríguez A, Becerril S, Méndez-Giménez L, Ramírez B, Sáinz N, Catalán V, et al. Leptin administration activates irisin-induced myogenesis via nitric oxide-dependent mechanisms, but reduces its effect on subcutaneous fat browning in mice. International Journal of Obesity. 2015;39(3):397-407.

26. Sharma N, Castorena CM, Cartee GD. Greater insulin sensitivity in calorie restricted rats occurs with unaltered circulating levels of several important myokines and cytokines. Nutrition & metabolism. 2012;9(1):1.

27. Mathur N, Pedersen BK. Exercise as a mean to control low-grade systemic inflammation. Mediators of inflammation. 2009;2008.
28. Kazemi A, Mizani R. The comparison of different order of concurrent training on plasma myonectin levels, insulin resistance index and anthropometric characteristics of elderly women. EBNESINA- Journal of Medical. 2015;16(4):39-46.
29. Jorge MLMP, de Oliveira VN, Resende NM, Paraiso LF, Calixto A, Diniz ALD, et al. The effects of aerobic, resistance, and combined exercise on metabolic control, inflammatory markers, adipocytokines, and muscle insulin signaling in patients with type 2 diabetes mellitus. Metabolism. 2011;60(9):1244-52.
30. Stefanov T, Temelkova-Kurktschiev T, Koehler C, Henkel E, Schaper F, Hanefeld M. Association of physical activity with insulin resistance, subclinical inflammation, coagulation, and fibrinolytic biomarkers among population at high risk for type 2 diabetes. Folia medica. 2012;54(2):32-9.