

ORIGINAL ARTICLE



The Effect of Aerobic Continuous Training on Myonectin, Insulin Resistance and Liver Enzymes in Rats with Nonalcoholic Fatty Liver Disease

¹Zahra Koohestani Sini, ¹Mohammad Esmail Afzalpour, ¹Mohsen Mohammadnia Ahmadi, ²Mohammad Ali Sardar*, ³Sattar Gorgani-Firuzjaee

¹Department of Sport Sciences, University of Birjand, Birjand, Iran. ²Department of General Courses, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. ³Department of Medical Laboratory Sciences, School of Allied Health Medicine, AJA University of Medical Sciences, Tehran, Iran.

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ABSTRACT

Background. Non-alcoholic fatty liver disease, is the most common reason of liver disorders that can be controlled by dieting and regular physical activity. **Objectives.** The aim of this study was to investigate the effect of aerobic continuous training on serum levels of myonectin, insulin resistance and liver enzymes in rats with non-alcoholic fatty liver disease. **Methods.** Thirty-four male Wistar rats (6 - 8 weeks old) were randomly assigned into two groups: normal diet (N = 9) and high-fat diet (N = 25). Through 12-week high-fat diet, induction of fatty liver was performed. In order to confirm non-alcoholic fatty liver induction, seven rats fed with high-fat diet were tested. The 18 remaining rats were randomly assigned to two groups: high-fat diet plus sedentary activity (n = 9) and high-fat diet plus aerobic continuous training (n = 9). The aerobic group has performed running on a treadmill at the intensity of 50 - 60% VO₂max, for eight weeks and five sessions per week. The results were analyzed by one-way ANOVA and Tukey's post hoc tests at the statistical significance level of p<0.05. **Results.** The significant body weight increase induced by high-fat diet was controlled by aerobic continuous training (p<0.05). The serum levels of myonectin, insulin, glucose, alanine aminotransferase and aspartate aminotransferase enzymes and insulin resistance improved significantly after eight weeks of aerobic continuous training (p<0.05). **Conclusion.** It seems that aerobic continuous training can improve insulin resistance and liver enzymes by reducing serum myonectin levels in rats with non-alcoholic fatty liver disease.

KEYWORDS: *Aerobic Continuous Training, Myonectin, Insulin Resistance, Liver Enzymes, Non-Alcoholic Fatty Liver Disease.*

INTRODUCTION

Obesity is a global health problem that could lead to cardiovascular disease, diabetes, cancer and Nonalcoholic fatty liver disease (NAFLD) (1). NAFLD is the most common cause of liver disorders and it is closely related to obesity and insulin resistance (2). Approximately 50 percent of people with NAFLD have been afflicted with it due to an unhealthy lifestyle (1). So far, no

definite pharmacotherapy has been introduced for the disease (3). Nevertheless, among the most important recommendations for NAFLD, one can mention body weight loss through modifying the diet and doing regular physical activities (4). Unfortunately, most people with NAFLD do not engage in physical activities and due to their working conditions, the excuse of dearth of time

*. Corresponding Author:

Mohammad Ali Sardar, Ph.D

E-mail: sardarma@mums.ac.ir

or some physical restrictions are not adequately active (5). Studies on high fat diet fed rats showed that exercise reduce visceral fat, body weight, food intake and fat mass (6).

Some studies suggest that intense exercise may be more effective in weight loss, glucose regulation and insulin resistance (7). Meanwhile, high-intensity interval training (HIIT) and continues exercise training along with a HFD decreased fat mass and slowed down weight gain in high-fat diet-fed rats (8). However, recent research suggests that physical activity regardless of its duration and intensity is effective in improving NAFLD (3). It has been reported that regular physical activity causes diminished fat of the liver and increased energy expenditure; it also improves lipid oxidation, reduces abdominal fat tissue, and increases transport of fatty acids to the liver (9).

In a systematic review on exercise training and NAFLD, exercise was proposed as the first therapeutic solution for NAFLD (10). The authors reported useful therapeutic effects of performing aerobic exercise with 4.8 metabolic equivalents (METs) for 40 min/session, three times/week for 12 weeks on NAFLD (10).

Insulin resistance and reduction of plasma glucose uptake are among the primary disorders observed in NAFLD (11). Elevation of free fatty acids inside the liver, which is directly associated with insulin resistance, puts a considerable metabolic burden on hepatocytes. Then, in response to intensified lipotoxicity of hepatocytes and endoplasmic reticulum stress, cellular damage occurs, which itself leads to progression of the disease and nonalcoholic steatohepatitis (NASH) (2). Regarding insulin resistance and exercise, a recent study reported that optional running in rats led to an increase in the release of hepatic insulin sensitizing substance (HISS) and reduced insulin resistance (12).

Myokines has been introduced as one of the factors involved in modification of the body metabolism and inflammation. Myonectin (C1q Necrosis Tumor a-Related Protein: CTRP15) which is a newly recognized myokine that is associated with fat metabolism, and through overexpressing the genes involved in transportation of fatty acids (such as FATP1, FAT/CD36, Fabp1 and Fabp4) causes fatty acid uptake by mature lipid cells and hepatocytes, thereby reducing the plasma free fatty acids. Previous research have suggested the role of

environmental factors, such as physical activity and nutrition in myonectin expression (13, 14).

It has been shown that myonectin levels have an inverse relationship with obesity (14). Obesity decreases the levels of myonectin and exercises increase the expression of the myonectin gene (14). Myonectin is also expected to play a significant role in improving insulin resistance due to its importance in glucose and lipid metabolism (15, 16). Also, it has been indicated that aerobic exercise training led to diminished insulin resistance and elevated myonectin levels in overweight and obese women (17). Nevertheless, based on the limitations of myonectin-based sport studies, this issue requires further investigations in the future.

In addition, NAFLD is marked by upregulated liver enzymes, such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (18). In this regard, it has been showed that in patients with NAFLD, ALT and AST enzymes are both sensitive to the effects of aerobic exercises, and physical activity reduces the amount of these enzymes (19). Although exercise and weight loss could be recommended to improve NAFLD, there is still controversy on the impact of exercise on this disease (5). Accordingly, concerning the high prevalence of NAFLD, obesity, sedentary lifestyle, high health care costs and absence of any definite pharmacotherapy for the disease, the present study investigated the interventional effect of the nonpharmacological method involving aerobic continuous training on the serum levels of myonectin, insulin resistance and liver enzymes in rats with NAFLD.

MATERIALS AND METHODS

The present research was approved by the ethics committee of Mashhad University of Medical Sciences, Mashhad, Iran (IR.MUMS.MEDICAL.REC.1397.063).

Animals and Diet. Thirty-four male Wistar rats (6 - 8 weeks old) with a body weight of 170-200 g as the research sample were kept in animal laboratory of Faculty of Medicine, Mashhad University of Medical Sciences. The rats were housed in a controlled condition with 12:12 light/dark cycle at $22 \pm 3^\circ\text{C}$ and humidity of around 45%. After a one-week familiarization with the housing conditions, the rats were randomly assigned into two primary groups of normal diet (ND) (n = 9) and high-fat diet (HFD) (n = 25). Through a 12-week high-fat diet,

induction of NAFLD was performed. To confirm fatty liver induction, seven rats were fed with the high-fat diet under anesthesia. Then, blood sample was collected and biopsy was taken from the liver to conduct the relevant tests [Figure 1](#).

Then, the 18 remaining rats with NAFLD that fed with high-fat diet were randomly assigned to two groups: high-fat diet plus sedentary activity (HFD + SED) (n = 9) and high-fat diet plus aerobic continuous training (HFD + ACT) (n = 9).

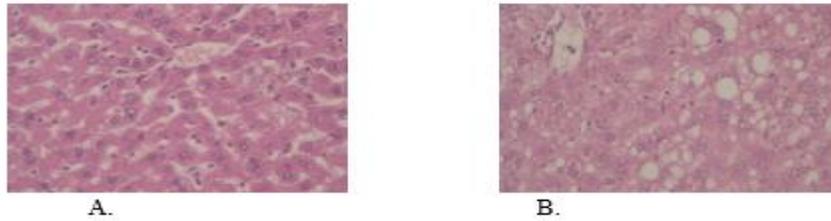


Figure 1. Histopathology Results for Confirm NAFLD Induction. A: Normal Diet, B: High Fat Diet

In order to induce NAFLD, high-fat diet was used for 12 weeks (high-fat diet: 20% carbohydrate, 20% protein and 60% fat) and the composition of normal diet (standard rodent chow) was: 70% carbohydrate, 20% protein and 10% fat [\(20\)](#).

Exercise Training Protocol. The exercise training protocol was conducted in a motorized treadmill, specially designed for rats (T.S. 8000, Sanat Tower, Iran). To familiarize the rats with the exercise protocol, the training group practiced in the first week for five days lasted for 10 - 30 min per day with the speed of 10-15 m/min. Then, the VO₂max was measured and based on the exercise training protocol, the speed of running on the treadmill was specified. VO₂max test was performed with the speed of 10 m/min and then

the speed was progressively increased 5 m/min every 3 min until the rat reached a criterion of exhaustion. Studies have shown a strong correspondence between the speed of treadmill and rats' VO₂max (r = 0.94 - 0.98) [\(21\)](#).

The aerobic continuous training was eight weeks, in a schedule of weekly five days and lasted for 50 min per each session. It was implemented as running at 50-60% of VO₂max on a treadmill with a zero inclination. Warm up was performed for five minutes with the speed of 10 m/min. The rats of HFD+SED and ND+SED groups were exposed to the same environment such as the exercising group and were placed on the treadmill without running for as long as the exercising group [\(22\)](#). Study design is presented in [Figure 2](#).

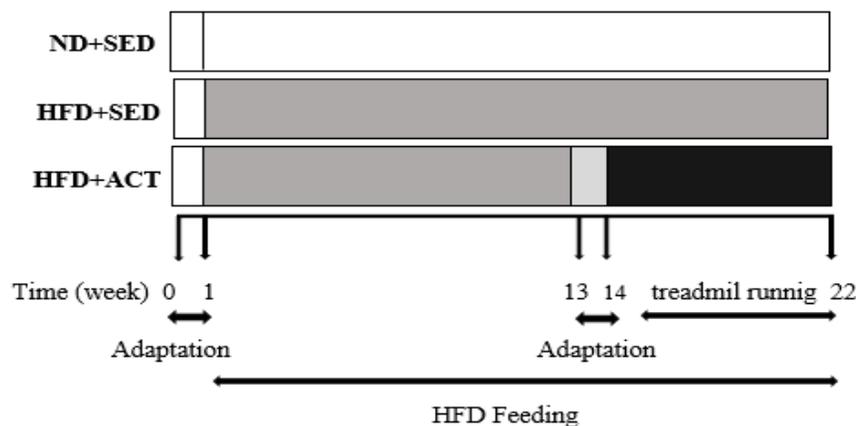


Figure 2. Study Design. ND+ SED: Normal Diet Plus Sedentary Activity; HFD+SED: High-Fat Diet Plus Sedentary Activity; HFD+ACT: High-Fat Diet Plus Aerobic Continuous Training.

Blood Sample. After eight weeks of aerobic continuous training, the rats were fasted overnight and sacrificed. Blood samples were obtained by cardiac puncture and plasma was separated by

centrifugation (3000 × γ, 10 min). All procedures were performed in accordance with the United States Public Health Service Guide for the Care and Use of Laboratory Animals.

Biochemical Assays. The serum glucose concentration was measured through enzymatic, calorimetric method for single point spot measurement using photometric method by Pars Azmoon Co. kit (Iran), while the insulin serum levels was measured by the ELISA kit (Mercodia Co, Sweden, sensitivity: 0.15 µg/l) and myonectin was measured by ELISA kit (ZellBio Co, Germany, sensitivity: 0.02 ng/ml). ALT and AST was measured by quantitative detection kit (Pars Azmoon Co, Iran, sensitivities: 4 U.L and 2 U.L, respectively). To evaluate the insulin resistance, the following formula was used: Blood glucose (mg/dl) × fasting plasma insulin (IU mg/l in the fasting state) divided by 405 (23).

Statistical Analyses. Collected data were analyzed using SPSS software (version 19.0). All data are reported as the means ± SD. Initially Shapiro-Wilk test was performed to test normality. One-way ANOVA and Tukey's Post Hoc test was used to determine within group differences. In all of the comparisons, the significance level was defined at $p \leq 0.05$.

RESULTS

The descriptive statistics of each variable for the studied groups as well as the results of one-way ANOVA are presented in Table 1.

Changes in the Body Weight. The results in Table 1 show that the body weight of the rats who were fed with high-fat diet (HFD + SED and HFD + ACT) along the research period significantly increased compared to the ND+SED group ($p < 0.05$). Also, eight weeks of aerobic continuous training led to a significant reduction in the final body weight in the rats with NAFLD ($p = 0.001$).

Changes in the Serum Levels of Glucose, Insulin and Insulin Resistance. The study showed a significant difference in the serum levels of insulin and glucose between groups ($p < 0.05$) (Table 1). In addition, the changes in the serum levels of glucose and insulin in the rats fed with high-fat diet (HFD+SED and HFD+ACT) increased along the research period. Indeed, eight weeks of aerobic continuous training caused a significant reduction in insulin resistance in the rats with NAFLD ($p = 0.001$).

Table 1. The Comparison of Body Weight, Serum Levels of Glucose, Insulin, ALT, AST, Myonectin, and Insulin Resistance among the Groups

| Variable | Normal Diet | | High Fat Diet | | p |
|-----------------------------|----------------|-----------------------------|-------------------------------|-------------------------------|-------|
| | ND+SED | HFD+SED | HFD+ACT | HFD+ACT | |
| Body weight (g) (1st week) | 189.00 ± 12.06 | 192.7 ± 14.91 | 193.14 ± 6.80 | 193.14 ± 6.80 | 0.410 |
| Body weight (g) (13th week) | 290.71 ± 17.86 | 358.28 ± 30.31 ^a | 351.21 ± 7.84 ^a | 351.21 ± 7.84 ^a | 0.001 |
| Body weight (g) (22th week) | 335.57 ± 14.92 | 452.40 ± 28.71 ^a | 398.42 ± 5.82 ^{a, b} | 398.42 ± 5.82 ^{a, b} | 0.001 |
| Glucose (mg/dl) | 114 ± 19.48 | 219.01 ± 29.21 ^a | 155 ± 21.40 ^b | 155 ± 21.40 ^b | 0.000 |
| Insulin (µg/l) | 0.70 ± 0.42 | 4.01 ± 1.42 ^a | 1.21 ± 0.73 ^b | 1.21 ± 0.73 ^b | 0.000 |
| HOMA-IR | 0.20 ± 0.13 | 2.30 ± 1.09 ^a | 0.48 ± 0.29 ^b | 0.48 ± 0.29 ^b | 0.001 |
| ALT (u/l) | 130.60 ± 19.13 | 174.14 ± 19.95 ^a | 132.42 ± 15.59 ^b | 132.42 ± 15.59 ^b | 0.001 |
| AST (u/l) | 115.40 ± 13.10 | 163.14 ± 15.25 ^a | 125.00 ± 16.91 ^b | 125.00 ± 16.91 ^b | 0.002 |
| Myonectin (ng/ml) | 1.32 ± 0.04 | 1.53 ± 0.11 ^a | 1.37 ± 0.10 ^b | 1.37 ± 0.10 ^b | 0.001 |

^a $p < 0.05$ compared with ND+SED; ^b $p < 0.05$ compared with HFD+SED

Data in the table are presented as Mean ± SD.

The Changes in AST and ALT Serum Levels. There is a significant difference in ALT and AST between groups ($p < 0.05$) (Table 1). Also, high-fat diet resulted in elevation of the liver enzymes. Eight weeks of aerobic continuous training led to a significant reduction in the serum levels of ALT ($p = 0.001$) and AST ($p = 0.002$) in the rats with NAFLD.

Changes in the Serum Levels of Myonectin. There is a significant difference between groups in the serum levels of myonectin ($p < 0.05$) (Table 1) whose levels significantly increased in the HFD+SED group compared to the ND + SED group. The results from Tukey's post-hoc test showed that eight weeks of aerobic continuous

training could control the elevation of myonectin levels in the HFD+ACT group ($p = 0.001$).

DISCUSSION

This study suggests that the group fed with high-fat diet showed an ascending trend in body weight changes, while exercise intervention controlled this body weight gain. Also, we observed a significant increase in the insulin resistance index in the rats with NAFLD compared to the healthy group. However, the increased level of serum glucose in the rats with NAFLD significantly decreased after aerobic continuous training. As well, aerobic continuous training led to improved insulin resistance in the rats with NAFLD. The liver enzymes also

significantly decreased following aerobic continuous training. In addition, the serum myonectin levels increased in the group fed with high-fat diet, which significantly decreased after the aerobic continuous training.

In a recent study, it was observed that both aerobic and resistance exercise led to diminished fat percentage, insulin resistance, and serum levels of ALT and AST in men with NAFLD (24). Also, in consistent with our findings, it has been shown that both aerobic and resistance exercises (over a period of four months) in people with NAFLD and type II diabetes, reduced body fat and visceral fat and improved insulin resistance (25). In contrast, another study demonstrated no significant change in the body weight of men and women with NAFLD following 22 weeks of aerobic or resistance exercise (26). Studies have indicated that exercise is associated with a reduction in the amount of liver fat and a reduction in the prevalence of NAFLD (27).

Exercise modifies the cellular organization of the liver associated with lipid metabolism and gluconeogenesis. One of the causes of diminished lipid content of the liver and improved steatosis phenotypes may be diminished fat anabolism alongside increased fat catabolism. Regular physical activity may improve the mitochondrial function or content (increase of carnitine palmitoyltransferase I (CPT-1) activity, citrate synthase activity, thyroid hormone receptor and cytochrome c oxidase). Also, increased use of lipid substrates in the rest state or during exercise to provide the required energy demands is one of the possible mechanisms for diminished hepatic lipid content, visceral fat, and body weight loss (27).

In present study, an improvement in insulin resistance and glucose levels were observed in the aerobic exercise training group compared to the control group on high-fat diet. In another study, similar result such as decreased insulin resistance was reported in people with NAFLD and type II diabetes after four months of aerobic or resistance training (25), while other researchers declared that three months of moderate aerobic exercise training did not lead to a significant change in insulin resistance levels in obese middle-aged women with type II diabetes (28). Insulin resistance and diminished plasma glucose uptake are among the first disorders observed in NAFLD (11). Accumulation of the muscular intracellular lipids in skeletal muscles interferes with insulin signaling and impairs glucose uptake. This leads to the

development of insulin resistance and a compensatory hyperinsulinemia, which ultimately leads to an increase in the delivery of fatty acids to the liver (2). Regarding the effect of exercise on glucose homeostasis, it has been reported that prolonged regular exercise improves insulin sensitivity. In other words, exercise increases the responsiveness to insulin through increasing the amount of glucose transporters inside muscle cells (glucose transporter4: GLUT4) and increasing the muscle mass (29).

In our study, ALT and AST levels were significantly improved following aerobic continuous training. Our results were confirmed by other study indicating that both aerobic and resistance exercises training for eight weeks resulted in a decrease in the enzymes of ALT and AST in men with NAFLD (24). On the contrary, in another study, 12 weeks of aerobic exercise in obese men did not change ALT levels (30). Although the mechanisms involved in the reduction of hepatic enzymes have not been clearly established, it seems that body weight loss and especially reduction of body fat percentage are among the influential factors in improving the liver enzyme index. As observed in the present study, aerobic continuous training led to body weight loss in rats with NAFLD, and possibly body weight loss has been effective in improving the liver enzymes.

One of the most important findings of the present research was increased serum myonectin levels in the group fed with high-fat diet, which decreased significantly following aerobic continuous training. In this regard, it has been reported that a nine week endurance exercise reduced the expression of myonectin gene in Zucker rats (31). Whereas, in another study, eight weeks of aerobic exercise resulted in a decrease in insulin resistance and an increase in the myonectin levels in overweight and obese women (17). Increased levels of myonectin rises phosphorylation of AMPK, causing elevated concentration of glucose transmitters across the cell membrane and enhanced glucose uptake (32). Further, myonectin has a similar function as insulin, and with delay, it stimulates uptake of fatty acids and glucose (14).

Insulin levels increase instantly after feeding, while circulating myonectin increase two hours after glucose or lipid consumption, in other words, myonectin carries out the removal of fatty acids and glucose by delay (14). Apparently,

myonectin inhibits the development of insulin resistance by regulating glucose and lipid levels (16). It seems that in cases where insulin resistance occurs such as type II diabetes and NAFLD, both insulin resistance, and myonectin levels are likely to increase, parallelly. In this regard, researchers suggest that myonectin stimulates the mTOR pathway, leading to hepatic autophagy and may also have an anti-protective role which is considered as a major contributor to metabolic disorder (13). In present study, increase in myonectin levels caused by NAFLD was modified following aerobic continuous training. Understanding the function of myonectin requires further studies in order to present involved mechanism following exercise.

CONCLUSION

The aerobic continuous training (eight weeks, five days / week for 50 min / session) could improve insulin resistance in rats with NAFLD through reducing serum myonectin levels. Besides, the body weight, serum levels of glucose, insulin, insulin resistance and levels of liver enzymes in the rats with NAFLD may improve following aerobic continuous training.

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However, further research needs to be conducted so that the mechanisms involved in the effectiveness of aerobic continuous training on myonectin and insulin resistance in non-alcoholic fatty liver disease are fully comprehended.

APPLICABLE REMARKS

- This study supports that aerobic continuous training can improve non-alcoholic fatty liver disease by reducing weight gain, insulin resistance and myonectin in high-fat diet-fed rats.

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CONFLICT OF INTEREST

The authors declare that they have no conflict interest.

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