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Effect of Short-Term Aerobic Exercise Training on Adropin Levels in Obese Rats

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ABSTRACT

Hieda M, Takakura H, Komine H. Effect of Short-Term Aerobic Exercise Training on Adropin Levels in Obese Rats. **JEPonline** 2019;22(7):64-71. This study examined the effects of short-term aerobic exercise on blood adropin levels and adropin expression in the liver and heart of obese Zucker rats. Male obese Zucker rats were randomized into control (CO) and exercise training (EX) groups. The EX group trained on a treadmill for 30 min·d^{-1}, 5 d·wk^{-1} for 6 wks. Serum adropin levels and adropin expression in the organs were measured by enzyme-linked immunosorbent assay and Western blot technique, respectively. Upon completion of the program, body weight of the EX group was lower than that of the CO group (P<0.05). However, plasma adropin levels were not significantly different. Adropin expression in the heart was higher in the EX group than in the CO group (P<0.05). Thus, short-term aerobic exercise training affected adropin expression in the heart of obese Zucker rats in the EX group. These findings suggest a new link between aerobic exercise and adropin levels.

Key Words: Adropin, Exercise Training, Zucker Rats

INTRODUCTION

Obesity puts one at risk for several health problems such as heart disease, diabetes, and metabolic syndrome. Aerobic exercise training is among one of the methods strongly suggested to mitigate metabolic risk factors and prevent cardiovascular diseases. The American Society of Exercise Physiologists (3) and the American College of Sports Medicine recommend regular aerobic exercise for overweight and obese adults to improve health (5).

Adropin is a newly discovered peptide hormone that was found to play an important role in energy homeostasis and insulin sensitivity (10). Low adropin levels may be associated with a higher risk of obesity and insulin resistance (8). Although it is mostly expressed in the liver and brain (10), Aydin et al. (2) showed that adropin is expressed in other tissues as well. Garcia-Hermoso et al. (9) reported that the effects of aerobic exercise improved insulin sensitivity and glucose profile.

Fujie et al. (7) reported that serum adropin levels increased after 8 wks of aerobic exercise training in middle-aged and older adults. Similarly, Zhang et al. (16) showed elevated serum adropin levels after 12 wks of aerobic exercise training in obese individuals. However, the effect of short-term aerobic exercise training (i.e., >8 wks) on adropin levels is not clear. Thus, we hypothesized that short-term aerobic exercise training can upregulate adropin protein expression in the heart and liver and also increase plasma adropin levels. This was attempted to examine the period of aerobic exercise training required to affect adropin levels and the extent to which such training affects them.

Hence, the purpose of this study was to investigate the effects of short-term aerobic exercise training on blood adropin levels using an obese animal model (obese Zucker rats). In addition, this study examined the changes in adropin protein expression in the rats' liver and heart.

METHODS

Animals

We performed this study using male obese (*fa/fa*) Zucker rats aged 8 wks. The animals were housed in an animal care facility for 7 days prior to the experiment. The experimental protocols for this study were approved by the Institutional Animal Care and Use Committee of the National Institute of Advanced Industrial Science and Technology. The rats were housed in pairs in cages at 22°C (12:12 hr light:dark cycle) with free access to food and water. Food intake and body weight were monitored weekly. The rats were randomly grouped into control (CO) and exercise training (EX) groups.

Procedures

Exercise Training Protocol

The rats belonging to the EX group trained on a treadmill for 30 $min \cdot d^{-1}$, 5 $d \cdot wk^{-1}$ for 6 wks. The initial speed of the treadmill was set at 14 $m \cdot min^{-1}$. Then, it was increased 2 $m \cdot min^{-1}$ after each minute until a maximum speed of 24 $m \cdot min^{-1}$ was reached (Sebai et al. 2011).

Collection and Storage of the Sample
At the end of the exercise training program, the rats were fasted overnight. Blood as well as heart and liver samples were obtained to analyze the biochemical parameters and, ultimately, the rats were sacrificed. The heart and liver samples were frozen until analysis.

Blood Chemistry
Plasma adropin was measured using enzyme-linked immunosorbent assay (ELISA) (Phoenix Pharmaceuticals, CA, USA).

Western Blot Analysis
Samples of equivalent protein content were separated by electrophoresis on 10% sodium dodecyl sulfate–polyacrylamide gel and transferred to polyvinylidene difluoride (PVDF) membranes. Membranes were then incubated in a blocking buffer followed by incubation with anti-adropin (7527, ProSci, CA, USA) or anti-alpha-tubulin (ab176560, Abcam, Cambridge, UK) antibodies at a temperature of 4°C overnight. After exposure to secondary antibody for an hour, membranes treated with chemiluminescent (ECL) prime reagents (GE Healthcare, NJ, USA) and chemiluminescent signals were visualized using a ChemiDoc™ MP system (Bio-Rad, Hercules, CA, USA). Signal intensities were quantified using Image J imaging software (NIH, MD, USA).

Statistical Analyses

The results are expressed as means ± standard error (SE). The SPSS version 24 was used for statistical analysis. Differences between the groups were determined using Student's *t*-test. Differences were considered significant at an alpha level of P<0.05.

RESULTS

Body weight of the subjects had increased with age in both groups. After the 6-wk intervention, body weight was lower in the EX group than in the CO group (P<0.05, Table 1). However, the average food intake was not different between the groups throughout the experimental period.

Table 1. Body Weight and Food Intake in the CO and EX Groups.

	CO	EX
Body Weight (g)	611.9 ± 9.3	566.6 ± 7.2*
Average Food Intake ($g \cdot d^{-1}$)	37.2 ± 0.7	36.3 ± 0.7

Values are expressed as mean ± SE. Each group contained 7 rats. *Significant difference vs. CO (P<0.05)

Plasma insulin and glucose levels were not significantly different in both the groups. There was also no difference in plasma adropin levels (Table 2).

Table 2. Blood Chemistries of Samples Collected from the CO and EX Groups.

	CO	EX
Insulin (ng·mL^{-1})	4.28 ± 0.86	7.67 ± 1.83
Glucose (mg·dL^{-1})	297.7 ± 25.2	237.5 ± 43.0
Adropin (ng·mL^{-1})	18.0 ± 1.6	20.6 ± 0.8

Values are expressed as mean ± SE. **CO** = Control Group (n = 6); EX = Exercise Training Group (n = 6)

Expression of the adropin protein was higher in the heart samples of the EX group than in the heart samples of the CO group (P<0.05, Figure 1).

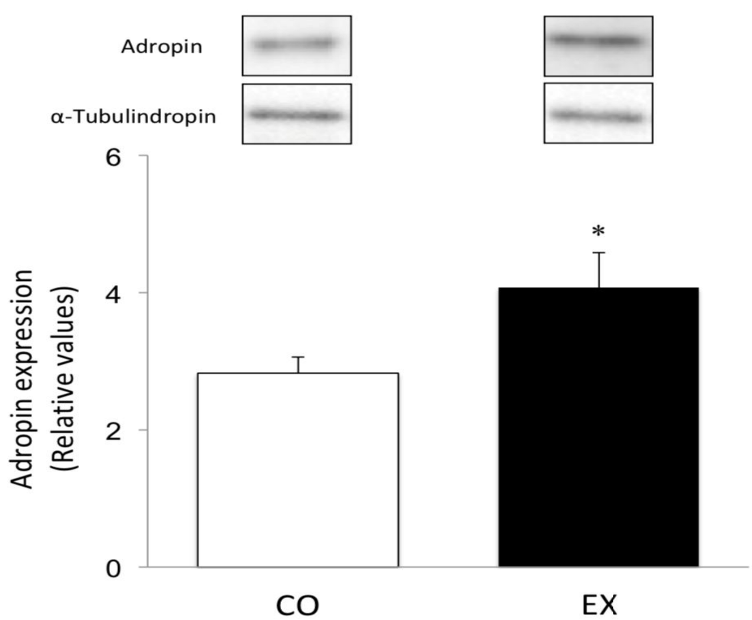

Figure 1. Comparison of Adropin Expression in the Heart Samples of CO and EX Groups. (CO, n = 7, EX, n = 7) *Significant difference vs. CO (P<0.05)

In the liver, adropin protein expression was not significantly different between the two groups (P<0.05, Figure 2).

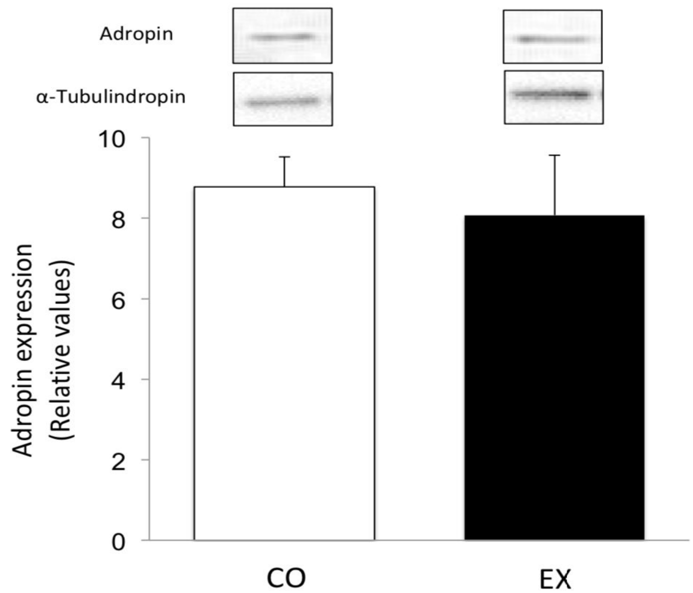

Figure 2. Comparison of Adropin Expression in the Liver Samples of CO and EX Groups. (CO, n = 7, EX, n = 7)

DISCUSSION

This study investigated the effect of short-term aerobic exercise training on adropin levels in male obese Zucker rats. After completion of a 6-wk aerobic exercise training program, enhanced adropin protein expression in the heart samples of the EX group was observed. Plasma adropin levels as well as adropin expression in the liver did not show significant differences in both groups. To the best of our knowledge, this is the first report to describe the effect of short-term aerobic exercise training on the tissue protein expression of adropin.

Adropin plays an important role in regulating energy homeostasis and insulin sensitivity (10). In a human study, serum adropin levels in obese participants were lower than that in participants with normal body weight (15). In contrast, our previous study showed that plasma adropin levels in obese Zucker rats were higher than that in lean Zucker rats (unpublished data). Our data were corroborated by Aydin and colleagues' study (2) that reported the serum adropin concentration in diabetic rats was higher than that in healthy rats. They suggested that increased adropin in diabetes may have a compensating role during the development of disease. Our obese Zucker rats showed very high insulin and glucose levels compared with lean Zucker rats (unpublished data), which might induce high plasma adropin levels.

Recently, Fujie et al. (6) and Zhang et al. (16) reported increased serum adropin concentration in their obese participants after 8 wks and 12 wks of aerobic exercise training, respectively. According to these studies, exercise training seems to increase adropin levels in blood. However, we did not find any difference in the plasma adropin levels between the CO and EX groups in the present study. Therefore, we suggest that either the short-term aerobic exercise training might not have been sufficient to change plasma adropin levels or that the increase in plasma adropin levels was leveled off because of high blood glucose, thus masking the effect of aerobic exercise training.

Adropin is expressed in the brain, liver, heart, kidneys, and pancreas (2). However, evidence that demonstrates the relationship between aerobic exercise training and adropin expression in various tissues is lacking. Therefore, we attempted to show the link between the two in this study by focusing on adropin expression in the heart and liver. This is because aerobic exercise affects the heart directly, and adropin expression is observed mainly in the liver (10). In this study, 6 wks of aerobic exercise training enhanced adropin expression in the heart but not in the liver. From these results, we inferred that the heart responds differently to aerobic exercise training compared with the liver and hence adropin expression also varied.

Recent studies showed that adropin has important effects on cardiac function. Reduced serum levels of adropin are associated with heart failure, coronary atherosclerosis, acute myocardial infarction, and cardiac syndrome X (15). Altamimi et al. (1) reported that adropin administration to mice increased cardiac output and coronary flow, which improved their cardiac function. Our results showed that aerobic exercise training increased adropin expression in the heart, which may contribute in improving cardiac function. However, the mechanism of how exercise training induced an increase in adropin expression in the heart remains unclear.

The highest amount of adropin expression is seen in the liver (2). In adult patients with non-alcoholic fatty liver disease, serum adropin levels were observed to be lower than that in healthy participants (11). Moreover, they found lower levels of serum adropin in patients with insulin resistance. There was no effect of short-term aerobic exercise training on the liver in our study. We also did not observe any change in plasma insulin and glucose levels after exercise program completion. Metz et al. (13) reported a significant decrease in plasma glucose and insulin levels after 10 wks of endurance exercise training. Hence, more long-term aerobic exercise training may affect the insulin and glucose levels, and it can lead to changes in adropin expression in the liver.

Limitations in this Study

This study has some limitations. First, our exercise training program might have been too short to affect glucose metabolism. Further studies are needed to clarify how exercise training improves glucose metabolism and how improved glucose metabolism in turn affects adropin expression in the liver. Second, we focused only on the heart and liver. It is important to consider the effect of exercise training on adropin expression in other tissues as well.

CONCLUSIONS

Short-term aerobic exercise training increased adropin expression in the heart, but not in the liver, and plasma adropin levels remained unaffected.

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