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Effects of Aerobic Exercise and Resveratrol Supplementation on Plasma Level and Liver Expression of Activin A and Follistatin in a Rats with Nonalcoholic Fatty liver Disease

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ABSTRACT

Background and objectives: Nonalcoholic fatty liver disease (NAFLD) is a chronic hepatic disease characterized by fat accumulation inside hepatocytes. The aim of this study was to evaluate effects of exercise training and resveratrol supplementation on activin A and follistatin levels in rats with NAFLD under a high-fat diet.

Methods: Fifty-six old (40-50 weeks) male Wistar rats were assigned to a healthy control group and seven experimental NAFLD groups: 1. high-fat diet, 2. saline, 3. resveratrol supplementation, 4. continuous exercise, 5. interval exercise, 6. continuous exercise+ resveratrol supplementation and 7. interval exercise+ resveratrol supplementation. Rats in the resveratrol supplementation groups were given 25 mg/kg of body weight intraperitoneal injection of resveratrol daily. Exercises were performed five days a week for eight weeks. Data were analyzed with SPSS (version 21) using one-way analysis of variance (ANOVA) and Tukey's post hoc test at significance of 0.05.

Results: Exercise training and resveratrol supplementation significantly decreased plasma activin A level and increased activin A expression ($P<0.05$). Plasma level of follistatin was significantly higher in rats under a high-fat diet compared to healthy control animals. All exercise and supplementation groups alone and combined lowered follistatin levels. However, follistatin mRNA expression increased significantly after resveratrol supplementation alone, continuous exercise+ resveratrol supplementation, and interval exercise+ resveratrol supplementation.

Conclusion: Resveratrol has a beneficial effect on activin A and follistatin levels in rats with NAFLD. In addition, resveratrol supplementation combined with exercise training may have greater health benefits for NAFLD patients compared to resveratrol supplementation or exercise training alone.

Keywords: Exercise, Resveratrol, Activin A, Follistatin, Nonalcoholic Fatty Liver Disease.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a leading cause of chronic liver disease worldwide, which is characterized by fat accumulation inside liver cells (1, 2). It ranges from simple nonalcoholic steatosis (SS) to nonalcoholic steatohepatitis (NASH) (1) and is a major risk factor for type 2 diabetes mellitus, cardiovascular disease and chronic kidney disease (2). NAFLD is a complex disease that may be caused by obesity, nutrient excess, hyperlipidemia and metabolic syndrome (3). The prevalence of NAFLD is approximately 25% worldwide (4) and30-35% in Iran(5).

Higher physical activity plays a key role in prevention and treatment of NAFLD (6,7). In addition, physical activity, especially regular exercise, can decrease the risk of developing type 2 diabetes mellitus, insulin resistance, impaired glucose tolerance, hyperlipidemia, metabolic syndrome and hypertension (7). Furthermore, it can increase fat oxidation and insulin sensitivity and decrease visceral fat content, triglyceride (TG) and accumulation of free fatty acids in the liver (8).

Dietary control is another effective intervention in treatment of NAFLD. In this regards, dietary supplements such as resveratrol have attracted a lot of attention. Some studies show that resveratrol supplementation can inhibit lipid metabolism and prevent progression of NAFLD (9,10) by reducing the level of alanine aminotransferase, aspartate transaminase, apolipoprotein B as well as blood glucose, total cholesterol, low density-lipoprotein-cholesterol, TG and body weight (11). In addition, resveratrol can improve insulin sensitivity and exert anti-inflammatory, anti-oxidative and anti-obesity effects (12,13). Activin A induces apoptosis of liver cells and termination of liver regeneration (14,15).

Follistatin is a natural antagonist of activin A, which is expressed in some tissues including the skeletal muscles and liver (16). As an activin antagonist, follistatin may be used to find potential therapeutic strategies for liver diseases (17).

The main purpose of this study was to evaluate effects of aerobic exercise and resveratrol supplementation on levels of activin A and follistatin in rats with NAFLD.

MATERIAL AND METHODS

Fifty-six old (40-50-week old) male Wistar rats (weighing 250 ± 15 g) were obtained from the Animal Centre of Islamic Azad University, Sari branch, Iran. The animals were housed in polypropylene cages (30 cm×15 cm) under controlled temperature (22±2 °C), humidity (50±5%) and 12-h dark/light cycle, with free access to water and standard rodent chow. Subjects were assigned to a control group and seven experimental NAFLD groups: 1. high-fat diet, 2. saline, 3. resveratrol supplementation, 4. continuous exercise, 5. interval exercise, 6. continuous exercise + resveratrol supplementation and 7. interval exercise + resveratrol supplementation. First, subjects were divided into two groups: healthy control group and NAFLD groups (with high fat diet). Rats in the control group were subjected to a standard diet (12% fat, 57% carbohydrate, 28% protein and 3% other compounds). To induce NAFLD, the experimental groups were subjected to a high-fat diet (22% fat, 2% cholesterol, 1% choline, 50% carbohydrate, 24% protein and 1% other compounds) for six weeks (18). Moreover, subjects in the resveratrol groups were given 25 mg/kg of body weight intraperitoneal injection of resveratrol daily .

The rats in the exercise groups were familiarized with treadmill running at 10 m/min, with a 0% incline, 5 min/day for five days. The main training program was performed for eight weeks. In addition, warm-up and cool-down (5 m/min) were performed at the beginning and at the end of each exercise session, respectively. In the continuous training group, subjects ran 60 min/day at 0% incline, five days a week. In the first week, the running speed was set at 15 m/min for 5 min/day. The running speed and duration gradually increase to 1-2 m/min and 1-2 min per day, respectively. By the end of the fourth week, the exercise intensity increased to 20 m/min for 60 min and continued in this manner until the end of the experiment. Interval training program consisted eight weeks of 10 running sets at 50% VO2 max, five days per week, with 2–min rest between each set. Initially, the running speed was set at 14 m/min but increased by 2 m/min every week, until it reached 28 m/min in the eight week. Obtained data were expressed as mean ± standard deviation. Normality of data was assessed using

the Shapiro–Wilk W-test. The data were analyzed using one-way analysis of variance (ANOVA) and Tukey's test. All statistical analyses were performed in SPSS (version 21)at significance of 0.05.

RESULTS

Figure 1 demonstrates activin A plasma and hepatic expression levels in all study groups. Exercise training in combination with resveratrol supplementation significantly decreased plasma levels of activin A ($P< 0.05$). Plasma activin A level was lower in the resveratrol, interval exercise, continuous exercise+ resveratrol, and interval exercise+ resveratrol groups compared with the high-fat diet and saline groups ($P< 0.05$). Resveratrol supplementation alone or in combination with continuous or interval training significantly increased activin A expression compared with the high-fat diet and saline groups ($P<0.05$).

Figure 2 shows the plasma and hepatic expression levels of follistatin in all study groups. The plasma level of follistatin was significantly higher in the high-fat diet animals than in the control animals, indicating that NAFLD can increase the follistatin level in plasma. Moreover, exercise training combined with resveratrol supplementation decreased plasma level of follistatin. All exercise trainings and supplementation interventions alone or combined significantly lowered follistatin levels when compared with the high-fat diet and saline groups (Figure 3) .

Follistatin mRNA expression increased significantly after resveratrol supplementation alone or combined with continuous or interval exercise compared with the high-fat diet and saline groups ($P< 0.05$), while follistatin expression was not affected significantly by continuous or interval training alone (Figure 4).

Figure 1. Serum levels of activin A in different study groups. P: high-fat diet, S: saline, C: control, RSV: resveratrol supplementation, CE: continuous exercise, IE: interval exercise, CE+RSV: continuous exercise + resveratrol supplementation, IE+RSV: interval exercise + resveratrol supplementation

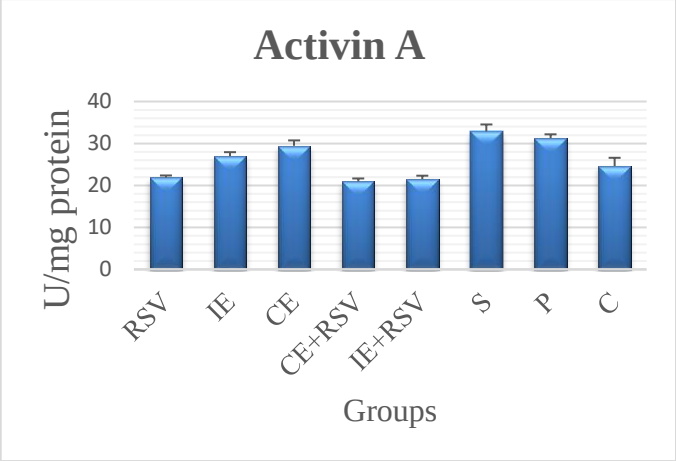


Figure 2. Activin A gene expression level in different study groups. P: high-fat diet, S: saline, C: control, RSV: resveratrol supplementation, CE: continuous exercise, IE: interval exercise, CE+RSV: continuous exercise + resveratrol supplementation, IE+RSV: interval exercise + resveratrol supplementation

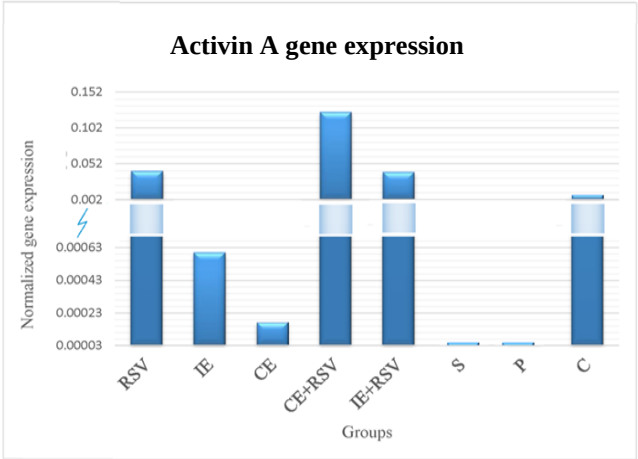


Figure 3. Serum levels of follistatin in different study groups. P: high-fat diet, S: saline, C: control, RSV: resveratrol supplementation, CE: continuous exercise, IE: interval exercise, CE+RSV: continuous exercise + resveratrol supplementation, IE+RSV: interval exercise + resveratrol supplementation

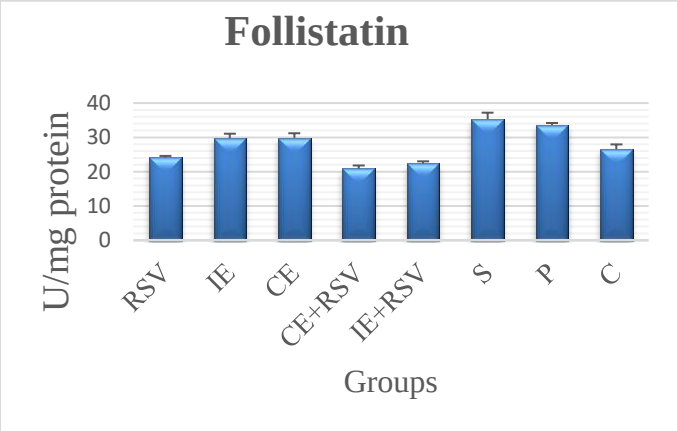
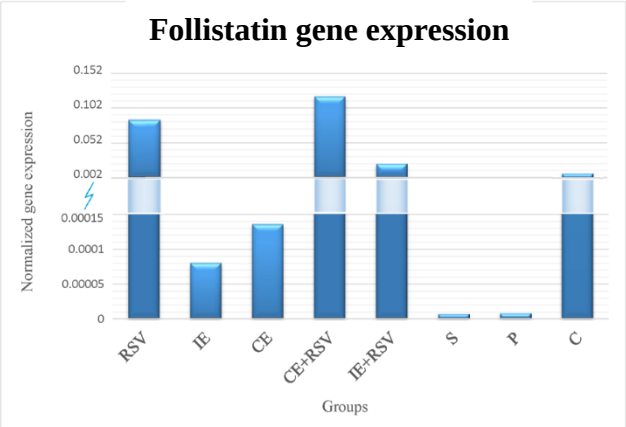


Figure 4. Follistatin gene expression in different study groups. P: high-fat diet, S: saline, C: control, RSV: resveratrol supplementation, CE: continuous exercise, IE: interval exercise, CE+RSV: continuous exercise + resveratrol supplementation, IE+RSV: interval exercise +



DISCUSSION

Lifestyle modification, with a focus on regular exercise and healthy eating, is considered as the main treatment for NAFLD (7,19,20). In a previous study, follistatin was higher in NAFLD (both SS and NASH) patients than in healthy control subjects (16). Growing evidence suggests that activin A could be involved in the pathogenesis of different liver disorders including certain hepatic malignancies, chronic viral hepatitis, acute liver injury and NAFLD (21). On the other hand, activin A inhibits hepatocyte growth, induces hepatocyte apoptosis and has a key role in hepatic fibrosis (22).

Follistatin is a binding protein of activin A that prevents its interaction with signaling receptors. It was shown that follistatin binds to activin A almost unchangeably, thereby inactivating activin A (16). This suggests that follistatin may constitute a negative loop to prevent activin A hyperactivity (23). At the stage of simple nonalcoholic steatosis, follistatin levels decrease in order to allow activin A to exert its anti-steatotic effects (16). The effects of exercise and resveratrol supplementation on activin A level in NAFLD patients have not been documented in detail (24,25). Chen et al. showed that resveratrol can improve insulin resistance as well as lipid and glucose metabolism (26). In the present study, for the first time, we demonstrated that resveratrol alone can decrease activin A level compared to a group under high-fat diet. This finding may indicate the preventive and protective effects of resveratrol on NAFLD. On the other hand, interval exercise alone and combined with resveratrol and continuous exercise significantly decreased activin A levels. Exercise can decrease intrahepatic triglyceride in NAFLD (27). Exercise also have many cardiometabolic benefits including increased insulin sensitivity, fitness and reduced blood pressure for NAFLD patients (28,29). These studies indicate that high-intensity exercise may induce greater benefits on intrahepatic triglyceride in individuals with NAFLD. Experimental studies (30) and observational research (31,19) indicate that high-intensity interval training may be more effective in decreasing fat accumulation and NAFLD progression compared to continuous moderate-intensity training .

Many functions of follistatin are facilitated through natural inhibition of other transforming growth factors (TGFs), such as activin and myostatin (19). Serum follistatin concentrations are elevated in patients with NAFLD or type 2 diabetes mellitus (17). However, circulating follistatin is increased following acute aerobic and resistance exercise (38). Exercise upregulates the induction of the follistatin in humans .

In our study, activin A and follistatin expression was significantly higher in rats subjected to resveratrol supplementation alone or combined with continuous or interval training compared to those treated with saline or a high-fat diet. In a study by Silva et al., showed that 4 weeks swimming training, 1.5 h/day, 5 days per week, could significantly increase activin bA subunit mRNA expression and decreased follistatin mRNA expression in control-swim and high fat-Swim groups than in either control-sedentary or high fat-sedentary animals (32). Hansen et al. also showed that intense swimming exercise can increase follistatin expression in the liver of rodents (34,38,39). The relationship between activin A and follistatin is a complex one that may be self-modulated at times and affected by the autocrine/paracrine signaling (33). Some studies have indicated potential mechanisms to describe the role of activin/follistatin in exercise-induced liver adaptation (37). As an anti- inflammatory cytokine, activin A can also have protective effects on both insulin resistance and NAFLD(34).

CONCLUSION

Combining resveratrol supplementation with exercise training has more beneficial effects on liver parameters compared to resveratrol supplementation or exercise training alone. Diet and interval training can significantly increase activin A and follistatin expression, which may be useful for treatment of NAFLD.

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CONFLICT OF INTEREST

All contributing authors declare no conflict of interest.

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