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Effect of regular exercise on the levels of subfatin and asprosin: a trial with different types of exercise

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Abstract. – OBJECTIVE: Subfatin (Metrnl) and asprosin are associated with metabolic diseases, such as obesity and diabetes. Exercise is among the most important regulators of health in humans and has been previously demonstrated to regulate these parameters. The present study aimed to investigate the effects of different types of regular exercises on levels of subfatin, asprosin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), uric acid, and glucose.

MATERIALS AND METHODS: The study included 120 young and healthy males, who participated in the study voluntarily. These participants were randomly divided into four groups, such as control (C), aerobic exercise (AE), intermittent (HIIT), and resistance exercise (RE) groups. Additionally, all the groups had equal numbers of participants. First, the subjects in the exercise group were made familiar with the exercise regime for two weeks. Then, they performed regular exercises, three days a week for eight weeks. Blood samples were collected from the participants at the beginning and end of the study. Subfatin and asprosin levels were analyzed using the ELISA method. AST, ALT, uric acid, and glucose levels were analyzed using the AutoAnalyzer.

RESULTS: No differences were observed in pretest values between the groups (*p*>0.05). Assessment of intragroup changes demonstrated no significant changes in the control group. In the comparisons, statistically significant changes were recorded in the levels of subfatin, asprosin, and glucose in all exercise groups. Particularly, differences were observed in the levels of AST and uric acid in the AE and HIIT groups while differences in ALT levels were observed only in the AE group (*p*<0.05).

CONCLUSIONS: In the conclusion of the study, different types of exercises caused significant changes in subfatin and asprosin levels. Thus, these results suggested that the parameters associated with metabolic diseases could be controlled with the aid of regular exercises.

Key Words:
Exercise, Metabolic diseases, Meteorin-like, Biochemistry, Glucose.

Introduction

Physical activity has many positive effects on the health of individuals[1]. In contrast, inactivity increases the risk of many health complications, including different types of cancer, diabetes, some cardiovascular disorders, obesity, and even death[2]. Exercise has been previously shown to regulate adipo-myokines[3]. Adipokines are generally known as peptides that signal the functional status of adipose tissue to targets in the brain, liver, pancreas, immune system, vascular system, muscle, and other tissues[4]. Furthermore, it is stated that adipokines have a potential for future pharmacological treatment strategies of obesity and metabolic diseases, as they are involved in the regulation of conditions, such as appetite and satiety, energy expenditure, blood pressure, insulin sensitivity, adipogenesis, fat distribution, and insulin secretion[5]. In general, adipose tissue releases adipokines that play important roles in metabolic and cardio-cerebrovascular homeostasis. Subfatin (Meteorin-like/

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Metrnl) is a newly discovered adipokine, which has a different tissue distribution from its homologue, meteorin, which is regulated by adipogenesis and obesity. Unlike meteorin, subfatin expression has been observed in adipose and brain tissues[6]. This protein, QQL, is highly expressed in white adipose tissue and plays an important role in neural development, browning of white fat, and insulin sensitivity[7]. Subfatin (Metrnl), which rises in peripheral tissues due to exercise-related increases in intramuscular, can further reduce obesity by improving metabolism in all body tissues[8]. Subfatin is associated with morphological and metabolic adaptations, and its levels acutely increase after resistance exercise. Moreover, resistance exercises have the potential to prevent metabolic diseases by introducing changes in white and brown adipose tissue and increasing plasma levels of subfatin[9]. Additionally, it might give different responses to exercises in water at different temperatures[10]. Asprosin is another adipokine involved in several processes, such as appetite, glucose metabolism, and insulin resistance[11]. In particular, asprosin was recently discovered as a hunger-induced protein that promotes hepatic glucose production. Furthermore, asprosin as an effective orexigenic hormone, was reported as a potential therapeutic for the treatment of both obesity and diabetes[12]. A study[13] was conducted to explore the same, and it was reported that eight weeks of regular aerobic exercises resulted in a decrease in the levels of asprosin in rats with type 1 diabetes. In another study[14], it was reported that the application of continuous and interval swimming training for eight weeks in rats with metabolic syndrome resulted in significant changes in asprosin levels. In a study[15] conducted on children, increased asprosin levels were reported in obese children as compared to the children with normal weight. In a review study[16] investigating the interaction between exercise and asprosin, it was emphasized that more in-depth and longer-term studies were required to understand this interaction further.

In addition to the aforementioned effects, exercise has been previously shown[17,18] to induce changes in several biochemical parameters in humans. In particular, uric acid levels have been shown to increase in obese individuals due to increasing triglyceride concentration[17]. In addition to this, it has previously been reported[20] that changes in glucose homeostasis can be associated with obesity and type 2 diabetes. High levels of alanine aminotransferase (ALT) are associated with metabolic syndrome and obesity[21]. Besides this, ALT is also used as an important predictor of diabetes[22]. Importantly, higher levels of liver enzymes (AST and ALT) have been reported[23] in obese individuals as compared to normal individuals.

Thus, all these biochemical parameters were included in the present study, based on the aforementioned information and reported interactions. In particular, asprosin and subfatin proteins have been shown to have a potential therapeutic role in metabolic diseases, such as obesity and diabetes, which further increases their importance. Several previous studies investigated the presence of the relationship between these proteins and exercise in a variety of animal models, making this subject even more interesting. Because of the protective and therapeutic effects of exercises, the response of these parameters to different exercise models in humans appears to be an interesting area for research.

In this study, it was hypothesized that different types of regular exercises would confer positive effects in young healthy individuals, particularly on subfatin, asprosin, and other biochemical parameters (AST, ALT, uric acid, and glucose). Therefore, the present study aimed to investigate the effects of the regular aerobic, interval, and resistance exercises on the levels of these parameters.

Materials and Methods

The presented experimental study was performed using the pretest and posttest model with the control group. The study was carried out following the declaration of Helsinki. All experiments included in the study were approved by the Local Ethics Committee at Firat University, Turkey. All subjects were pre-informed about the study and a voluntary consent form was obtained from each participant. Accordingly, the study involved 120 participants who did not exercise regularly (on a scheduled program), which were randomly divided into four groups with equal numbers of participants in each group. The participants were recruited as per the inclusion criteria: the individuals were required to be male, university students, not conducting regular exercises, and absent of health disorders or disability conditions. All the participants who were unwilling to continue, who were injured and had health problems excluded from the study. Blood samples were collected from the subjects twice, first at the baseline, and then, after 72 hours of the completion of exercise programs. For the blood sample collection, the participants were subjected to overnight fasting. All blood samples were divided into two tubes. For biochemical analysis, routine biochemistry tubes were used while aprotinin tubes with EDTA were used for the Enzyme-Linked Immunosorbent Assay (ELISA) method.

Study Groups and Exercises

First, the participants in all exercise models were subjected to a two-week adaptation period, which was followed by exercises three days a week for eight weeks. Before starting the exercises, the AE group performed 10 minutes of warmup and cooling exercises, while for other groups this duration was 15 minutes. Exercises were performed in the evening. The details of the exercise regime followed by different groups about the exercises are given in Table I.

Biochemical Analysis

The blood samples were centrifuged at 4000 rpm for 5 minutes in a centrifuge (Nüve Brand) and stored at -80°C until they were used for further analysis. Serum samples were analyzed using a Beckman Coulter AutoAnalyzer. Glucose and uric acid concentrations were specified in mg/dL, and AST while ALT were reported in U/L.

Analysis of Subfatin and Asprosin

Subfatin and asprosin levels were studied following the working procedures specified in the catalogs of the Human ELISA Kit (Metrnl: Sunred Bioscience, Catalog No. SRB-T-81862, Asprosin: Sunred Bioscience, Catalog no: 201-12-7193 Shanghai, China). For the Intra-Assay of the kit, the CV value was <10% while for the Inter-Assay the CV value was <12%. The assay range of the kit was 0.05 ng/mL - 15 ng/mL while the sensitivity was 0.042 ng/mL for Metrnl and assay range was 1 ng/mL - 300 ng/mL in addition to the sensitivity value of 0.756 ng/mL for Asprosin. Additionally, in the analyses, ChroMate Microplate Reader P4300 (Awareness Technology Instruments, USA) was used. Accordingly, the test results were reported in ng/mL.

Statistical Analysis

SPSS 22.0 (SPSS Inc., Armonk, NY, USA) was used for statistical analysis. The normality of the distribution for the recorded data was tested using the Kolmogorov-Smirnov test. In line with the obtained results, parametric tests were performed. The paired sample *t*-test was used to assess intra-group changes (pretest-posttest). The one-way variance (ANOVA) test was used to assess intergroup differences. The Tukey test was used as a post-hoc test to identify groups with differences. For all tests, the results were considered to be statistically significant for $p<0.05$.

Table I. Study groups and exercise programs.

Groups	Exercise Program	Age and BMI
Control Group (C)	The group that did not have any exercise program	Age: 20.60 ± 0.93 BMI: 23.67 ± 2.18
Aerobic Exercise (AE)	An exercise program with maximal heart rate of 60-75% (Karvonen method) with continuous running method was applied to the participants. Each exercise unit consists of a main phase of 40 minutes	Age: 20.43 ± 0.81 BMI: 22.50 ± 2.19
High Intensity Interval Training (HIIT)	HIIT program (20 seconds ultra-intensive loading followed by 10 seconds rest, 8 repetitions) was applied to the participants[24] as running.	Age: 21.19 ± 1.29 BMI: 23.87 ± 2.39
Resistance Exercise (RE)	A program was implemented for upper and lower extremity muscle groups with weights of 65-75% of the participants' 1RM (Maximum repetition) values. The program consisted of 10 exercises (separate for each exercise unit). Day 1: Chest and forearm, Day 2: Back and back arm, Day 3: Shoulder and leg. Each exercise was applied in 3 sets and 8-10 repetitions.	Age: 20.93 ± 2.27 BMI: 23.48 ± 3.97

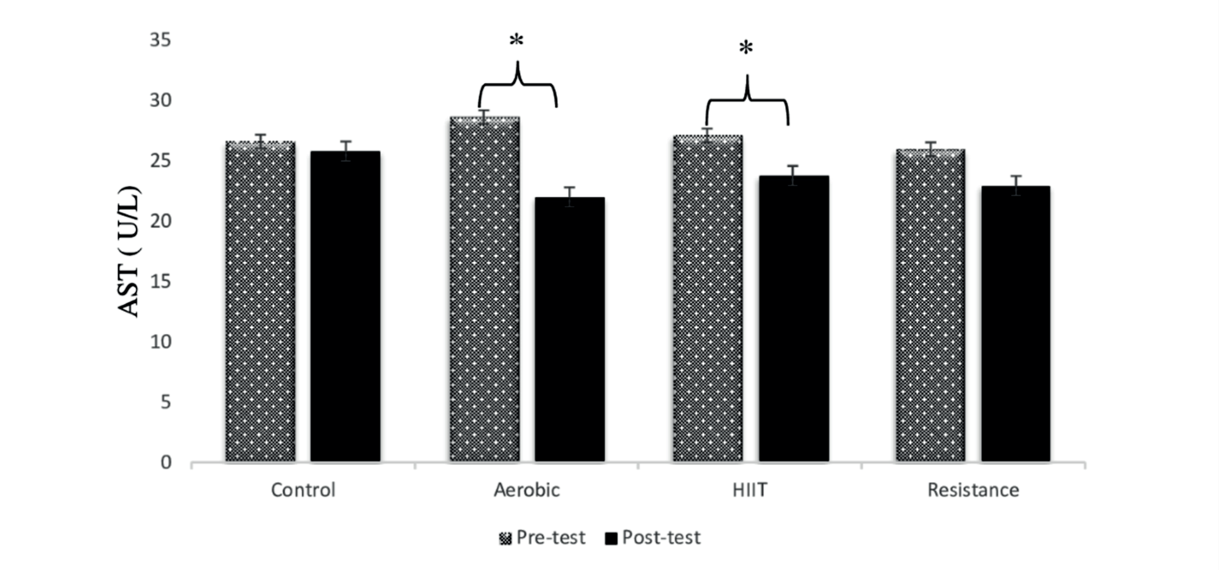

Figure 1. Changes in AST levels in different exercise groups. *There is a statistically significant difference between the measurements (p<0.05). AST: Aspartate aminotransferase.

Results

Evaluation of the changes within the group showed that there was no difference in AST levels in terms of control and resistance exercise groups after eight weeks of exercise (p>0.05) (Figure 1). However, significant changes were recorded in the aerobic exercise group (-23.15%) and the HIIT group (-12.32%).

Evaluation of the changes within the group showed no difference in ALT levels in control, HIIT, and resistance exercise groups after eight weeks of exercise (p>0.05) (Figure 2). However, significant changes were recorded for the aerobic exercise group (-15.07%).

Assessment of changes within the group demonstrated no difference in uric acid levels after eight weeks in terms of control and resistance exercise groups (p>0.05) (Figure 3). However, significant differences were recorded in the aerobic exercise group (-12.05%) and the HIIT group (-10.70%) (p<0.05). The comparison of posttest

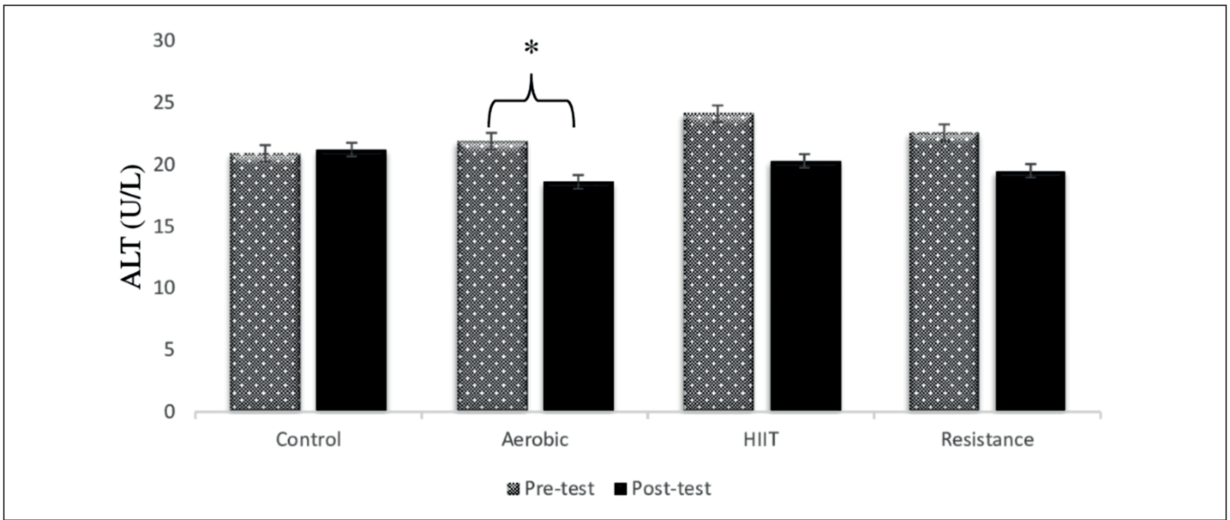

Figure 2. Changes in ALT levels in different exercise groups. *There is a statistically significant difference between the measurements (p<0.05). ALT: Alanine aminotransferase.

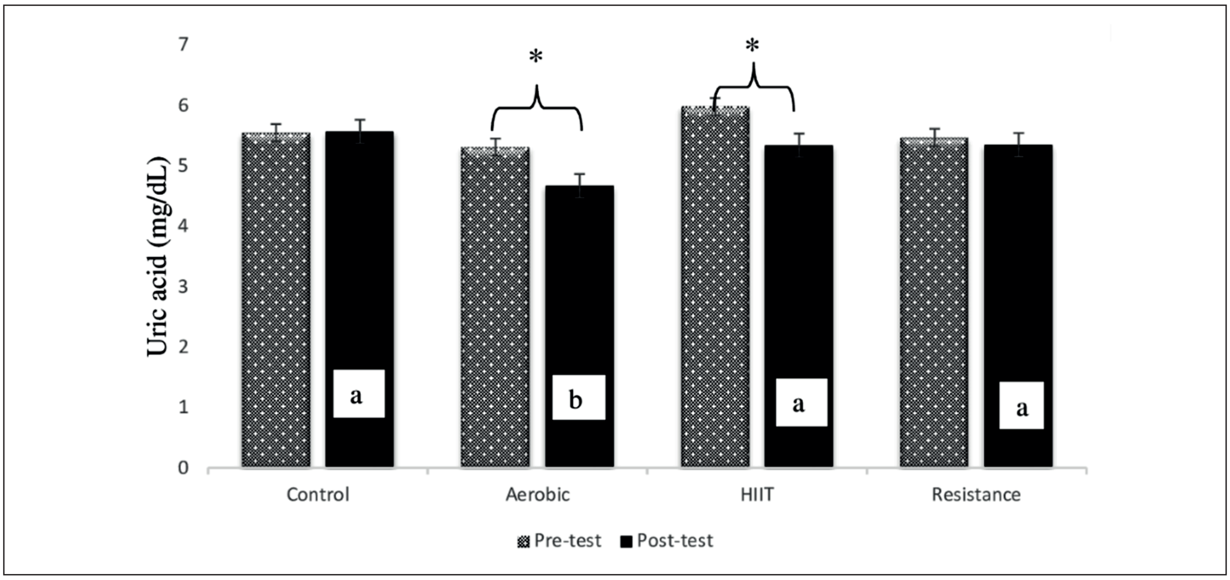

Figure 3. Changes in uric acid levels among different exercise groups. *There is a statistically significant difference between the measurements ($p<0.05$). **a-b,** The difference between groups with different letters is important ($p<0.05$).

values between the groups showed that there were significant differences between the groups and these differences were caused by the aerobic exercise group ($p<0.05$) (Figure 3).

Evaluation of the changes within the group showed that there was no difference in glucose levels in the control group after eight weeks ($p>0.05$) (Figure 4). However, significant changes were recorded in the aerobic exercise group (-8.79%), HIIT group (-9.19%), and resistance exercise group (-5.90%).

Assessment of the changes within the group demonstrated no differences in subfatin levels after eight weeks in terms of the control group. However, significant differences were observed in terms of the aerobic exercise group (66.43%), HIIT

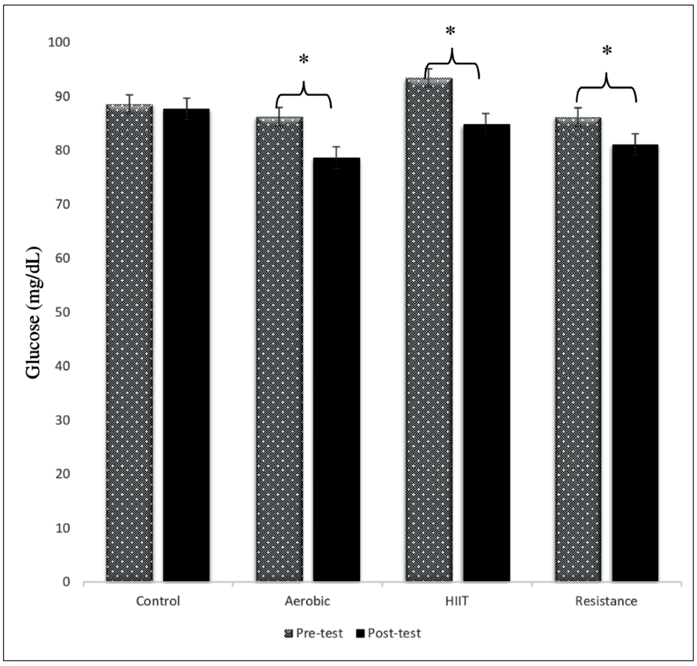

Figure 4. Changes in glucose levels according to different exercise groups. *There is a statistically significant difference between the measurements ($p<0.05$).

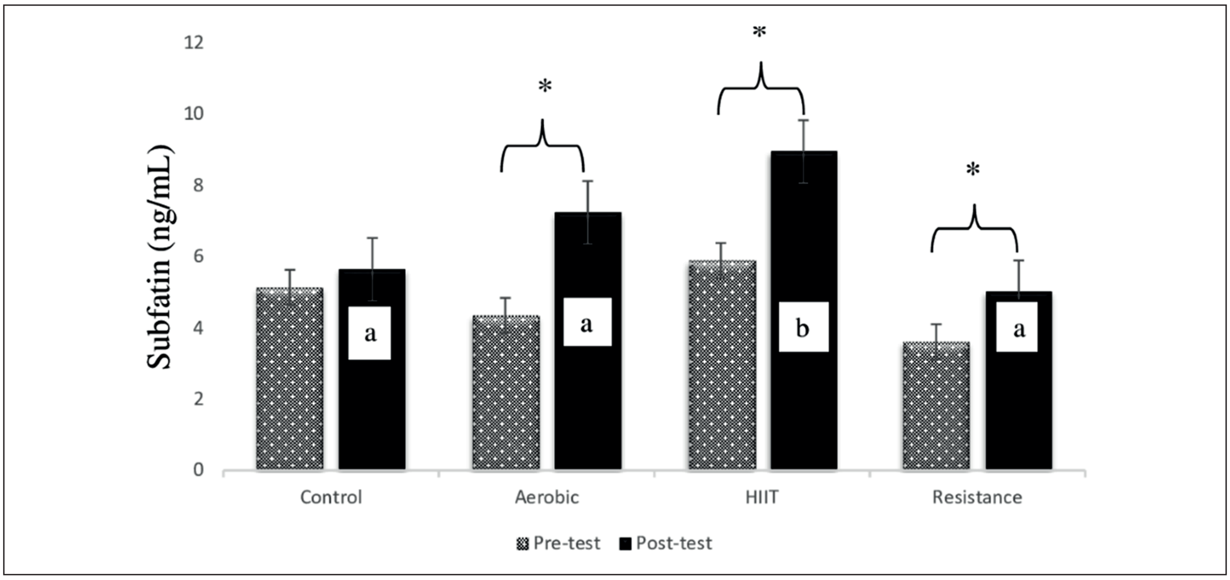

Figure 5. Changes in subfatin levels according to different exercise groups. *There is a statistically significant difference between the measurements (p<0.05). ab: The difference between groups with different letters is important (p<0.05).

(51.95%), and resistance exercise group (38.78%) (p<0.05). When posttest values were compared between the groups, significant differences were recorded between the groups which were caused by the HIIT group (p<0.05) (Figure 5).

Assessment of changes within the group reported no difference in the control group after eight weeks (p>0.05) (Figure 6). However, significant changes were observed in the aerobic exercise group (-26.81%), HIIT (-15.78%), and resistance exercise group (-24.19%).

Discussion

The present study examined the effects of different exercise methods on subfatin, asprosin, and some of the biochemical parameters. Subfatin and asprosin are known to be closely related to certain metabolic diseases. The response of these proteins to regular exercises was found to be remarkable. It was observed that both these parameters changed significantly in all exercise groups. Specifically, the most important changes were

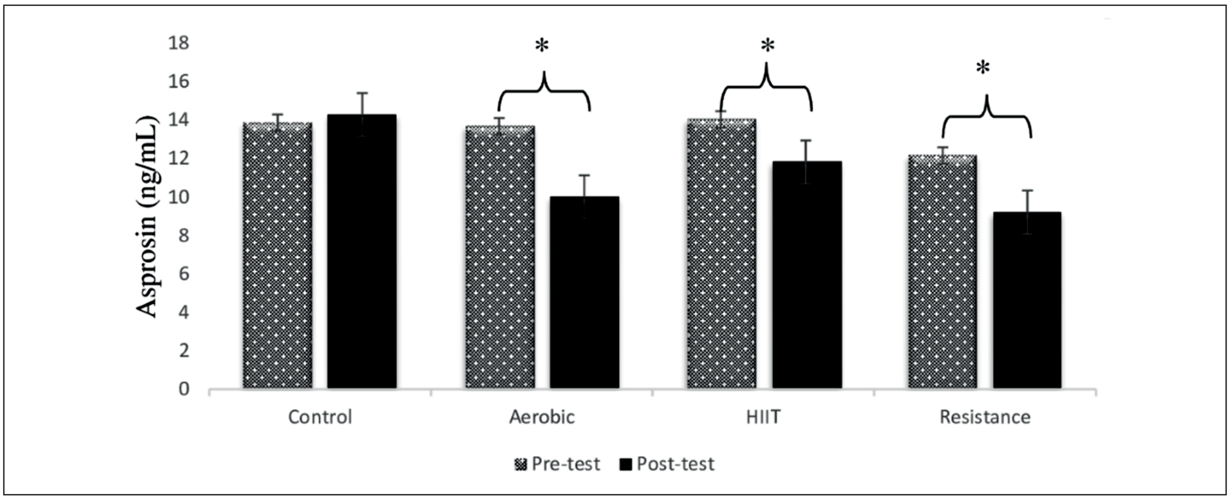

Figure 6. Changes in asprosin levels according to different exercise groups. *There is a statistically significant difference between the measurements (p<0.05).

observed in the aerobic exercise group which was expressed in terms of the percentage change. Moreover, it can be stated that the changes in other biochemical parameters occurred similarly. Previous studies have shown that regular exercises induced changes in levels of liver enzymes[25-27], glucose[18,28,29], and uric acid[30-32]. The present study also reported some changes in these parameters. However, these changes differed according to the type of exercise performed. In a previous study[33] evaluating the relationship between subfatin and exercise in overweight adolescents, the application of interval sprint training for 3 days a week for 6 weeks resulted in a significant increase in the levels of subfatin. The results obtained for the interval training group in the present study were in concordance with this research.

In another study, it has been emphasized that intramuscular Metrnl, which is regulated with regular aerobic exercises, can be effectively utilized to suppress obesity, diabetes, and insulin resistance mediated *via* improvement of metabolism in almost all body tissues[34]. In a separate study, it was found that the application of aerobic exercise and green coffee supplementation in obese females for 8 weeks for 5 days a week, resulted in statistically significant differences in Metrnl levels[35]. In a study conducted with healthy males (mean age 20.5±1.5), a significant increase was recorded in the levels of Metrnl mRNA both after a single high-intensity interval exercise and after a short-term (20 days, 2 training days) interval training period. It has also been emphasized that future studies are required to confirm this result at the protein level[36].

The present study reported similar results for protein secretion as a result of interval training. A study[37] supporting these results showed that the application of running sprint interval training for 3 days a week for 6 weeks in sedentary overweight adolescents (mean age 18.0±1.5) increased serum subfatin levels. One of the studies aimed at exploring the interaction between asprosin and exercise assessed the effects of combined aerobic resistance exercises on asprosin levels in males with type 2 diabetes. Post application of aerobic resistance regularly exercised for 12 weeks, 3 days a week, resulted in significant decreases in asprosin levels[38]. In a study[39] conducted on experimental animals, the application of 8 weeks of treadmill exercise, 60 minutes/day for 4 days a week, in rats with type 1 diabetes decreased the levels of asprosin.

Conclusions

Despite the number of studies on this topic, the results of the present study clearly showed that exercise resulted in a decrease in the levels of asprosin in young healthy individuals. However, since the evidence is limited, there is a need to conduct more in-depth studies to establish these results in the future. Age, gender, not exercising regularly, and being healthy were limitations of the study. In addition to these, energy balance control acted as a critical limitation of the current study. Despite these limitations, the present study highlighted the need to conduct studies in different types of exercise, duration, intensities, and sample groups in the future. Importantly, the results of the present study are remarkable, especially in terms of the levels of subfatin and asprosin. Thus, it could be possibly inferred that all these parameters, which are closely associated with diabetes and obesity, could be controlled by regular exercises, and thus regular exercise could confer protective effects against chronic and metabolic diseases. Altogether, the present study suggested that acquiring exercise in daily routine, before the development of health disorders or diseases might play a key role in ensuring a healthy life.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Ethics Approval and Consent to Participate

Study was carried out in accordance with the declaration of Helsinki. The approval of the local ethics committee was obtained from Firat University.

Authors' Contribution

T.A. and V.C conceived and designed the research; T.A. performed the exercises; K.U, Z.K.K, M.Y., and S.A. performed ELISA and biochemical analysis, T.A. and V.C. analyzed the data; T.A. and V.C. wrote the paper. T.A, K.U, Z.K.K, M.Y, S.A did the last reading and corrections.

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