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Original article

Evaluation of Aerobic Exercise Induced Metabolic Stress on Serum Asprosin Levels: Comparison of Fitness Status

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Summary. We comparatively evaluated effects of aerobic exercise performed morning and afternoon on asprosin levels in young trained, sedentary males. A total of fifty male subjects (trained n=25, sedentary n=25) participated in morning and afternoon aerobic running exercises to approximately 70% of the subject's maximal heart rate for about 45 min. Pre- and post-exercise venous blood samples were taken and analysed for asprosin using ELISA. Serum CK and MDA levels were determined by measuring with an autoanalysers and a HPLC, respectively. Asprosin, CK and MDA levels increased significantly at the end of both morning ($p<0.05$), and afternoon ($p<0.05$) exercises in the trained and sedentary group. At the end of the acute running exercise in the trained group in the morning, the asprosine, MDA and CK levels increased by 24%, 29% and 32%; while they increased by 24%, 30%, and 40% in the afternoon, respectively. In the sedentary group, asprosin, MDA and CK levels increased by 31%, 38% and 31% after the acute running exercise in the morning, while the asprosin, MDA and CK levels increased by 34%, 58% and 42% after the acute running exercise in the afternoon, respectively. Asprosin levels increased in all subjects in both aerobic exercise tests without correlating increase of MDA and CK. Altered asprosin levels could be related other factors rather than metabolic and muscular stress parameters.

Key words: Asprosin, Creatine Kinase, Exercise, Malondialdehyde, Metabolism

Introduction

Maintaining energy homeostasis requires a delicate balance between energy intakes to consumption ratio that can be influenced many factors (1). Excess energy due to the imbalance of energy homeostasis may cause serious metabolic disease including diabetes and obesity (2). In clinical medicine and sports science, controlling energy intake and/or increased energy consumption is the main approach to maintain energy homeostasis (3, 4). Thus, exercise induces increased metabolic activity, which causes negative energy balance, is a widely applied procedure for treatment of obesity and metabolic disorders (5-7).

Importantly, it should be taken into the consideration that exercise may have also great influence on energy homeostasis by affecting the levels of some energy regulatory hormones, in addition to its mechanical effects (8-10). In literature, there are many studies focusing on exercise and altered levels of some energy regulatory hormones including leptin, ghrelin, nesfatin-1 and irisin (8, 9). Despite the contradictions between the results of studies, the main outcome is support for the beneficial effects of exercise on energy balance by affecting levels of energy regulatory hormones (8, 9).

In today's scientific world, researchers reveal the existence of new energy regulating hormones in their

studies. Recently a new hormone secreted by white adipose tissue and also skeletal muscle called as asprosin was introduced (11). Asprosin is an orexigenic agent that reported to have important effects on glucose metabolism and appetite regulation (11-13). The studies generally focused on the expectative therapeutic effects of asprosin in metabolic disease including diabetes and obesity (14). Thus, asprosin response to the exercise induced increased muscle activity becomes an important issue for metabolic regulation. There are a few conflicting data concerning exercise and its effects on asprosin. The investigators studied the response of asprosin levels to various types of exercise performed in subjects with obesity (15) or different genders (16). There is no data concerning exercise induced increased factors, that reflects metabolic or muscular stress, on response of asprosin secretion.

It is generally accepted that exercise is an effective stress factor on exercising muscle and metabolic systems activity that enhanced both malondialdehid (MDA) levels reflecting increased lipid peroxidation (17, 18) and creatine kinase (CK) enzyme levels reflecting increased muscle injury (19, 20).

In this study, we comparatively evaluated effects of aerobic exercise performed morning and afternoon on asprosin levels in young trained and sedentary males.

Materials and Methods

The study procedures described in this study that conducted according to the Declaration of Helsinki were approved by our Local Research Ethics Committee.

Participants

A total of fifty male subjects (n=25 trained and n=25 sedentary) gave a signed informed consent forms before participating to this study. The subject's physical characteristics and body compositions are given in Table 1.

The subjects' body mass index (BMI) should be in normal range (18.5 kg/m^2 - 25 kg/m^2) and aged between 18 years to 25 years. The body composition of the subjects was measured using foot-to-foot bioelectrical impedance analysis method with controlling hydration status to avoid inaccurate measurement of body composition (Tanita Body Fat Analyser, Tokyo, Japan, TBF 300) (21). All of the subjects were free of any diseases, including cardiorespiratory, muscular or metabolic, non-smokers, taking no alcohol. The sedentary subjects were active but they did not perform exercise regularly. The trained subjects should be exercising regularly for 3 days a week for at least 5 years. The all subjects were informed not to change their eating behaviour during the study and not to take additional vitamins or antioxidant supplements. To avoid exercise induced metabolic stress and muscle damage on CK and other parameters, the subjects were advised to not exercise one week before the study (22).

Exercise Protocol

The subjects were performed randomly two aerobic running exercises between 08:00 and 09:00 in the morning and between 17:00 and 18:00 in the afternoon after eating a light meal at least 3 hours before exercise. The exercise intensity was estimated using hear rate parameter with 70% of their estimated maximal heart rate for approximately 45 minutes (23). There were seven day intervals between two tests. To ensure that exercise intensity remained at the specified level, the heart rate of each subject was monitored during each 45-minute session using a heart rate monitor.

Table 1. Mean (±S) values of the subjects age, height weight, body mass index (BMI), fat mass (FM) and fat free mass (FFM)

	Age (year)	Height (cm)	Weight (kg)	BMI (kg/m^2)	FM (kg)	FFM (kg)
Trained	19.2 ± 0.9	173 ± 7	61.5 ± 6.6	20.5 ± 1.7	6.22 ± 1.61*	55.27 ± 5.42
Sedentary	19.3 ± 1.2	174 ± 4	63.1 ± 7.1	20.7 ± 1.9	7.43 ± 2.41	55.63± 5.06

*Statistically significant differences.

Blood Collection and Biochemical Analysis

Venous blood samples were collected from the antecubital vein in tubes containing aprotinin to avoid protein denaturation one before exercise and other immediately after exercise. Plasma samples were separated after being centrifuged at 4500 rpm for 5 minutes at 4 °C. Samples were stored at -80 °C until analysis.

Enzyme Linked Immunosorbent Assay for Detection of Asprosin

The kit is a sandwich enzyme immunoassay for the in vitro quantitative measurement of asprosin (SunRed. Biological Technology Co., Ltd., Shanghai, China, Catalog No: 201-12-7691). Each well of the supplied microtiter plate was pre-coated with a target specific capture antibody. Standards and samples are added to the wells and the target antigen binds to the asprosin antibody labelled with biotin. Afterwards, a Streptavidin -HRP conjugate, Chromogen A and B substrate, and a stop solution are added to terminate the colour development reaction and then the optical density of the well is measured at a wavelength of 450 nm ± 2 nm using automated optical densitometry (Intra-assay CV <10%; Inter-assay CV <12%; Assay range:1 ng/ml-300 ng/ml; Sensitivity: 0.765 ng/ml). Each sample was run in duplicate, and the mean value was used for analysis. This assay has high sensitivity and excellent specificity for detection of human asprosin. No significant cross-reactivity or interference between human asprosin and analogues was observed.

MDA and CK Analysis

Serum CK level was determined by measuring with auto analysers (Siemens Advia 1200). Serum MDA values were evaluated using a high performance liquid chromatography method with a commercial kit (Immu Chrom GmbH Tiergartenstr. 7 D 64646 Heppenheim IC 1900). The intra and inter-assay of variation and sensitivity for MDA were 9% (0.86 µmol/L) - 6.4% (2.55 µmol/L), 10.9% (0.89 µmol/L) - 7.5% (2.5 µmol/L), respectively (18).

Statistical analysis

Data are expressed as mean (± Standard Deviation (SD)). A paired t-test was used to analyse the significance of within-group comparisons of the data. The statistical analyses of between-group data were performed using an independent t-test. Linear regression analysis was used to analyse the correlation of body weight and fat mass with baseline hormone levels. A value of $p<0.05$ was accepted as statistically significant.

Results

Asprosin Levels

Asprosin levels before and after morning and afternoon exercises in trained and sedentary groups are shown in Figure 1. All subjects showed systematic increase in asprosin levels in both exercise. The mean percent increase in asprosin levels was significantly higher in sedentary subjects compared to trained subjects in morning (31% vs 24%, $p < 0.05$) and afternoon (34% vs 24%, $p < 0.05$), respectively.

There was significantly differences in basal asprosin levels between morning and afternoon exercises in the trained ($p < 0.05$) and sedentary groups ($p < 0.05$) (Figure 1). The basal asprosin level was found to be significantly lower in the trained group compared to sedentary groups ($p < 0.05$) (Figure 1).

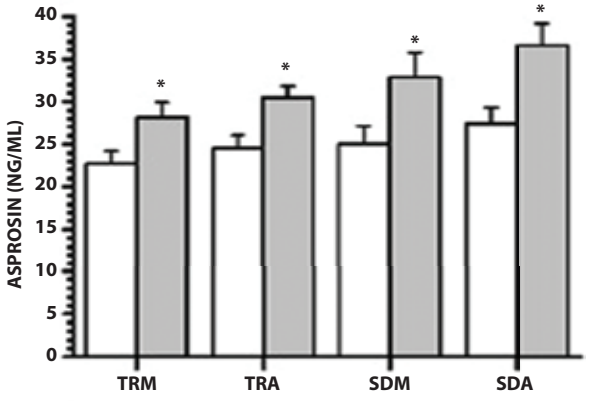

Figure 1. Asprosin levels (Mean±SD) response to the exercise performed in morning (M) and afternoon (A) in trained (Tr) and sedentary (Sd) subjects.
*reflects statistically significant compared to baseline values.

The linear regression analysis showed no significant relationships between bod fat mass, body weight and baseline asprosin levels in both groups in both exercises. The percent increase of asprosin during both exercise sessions also did not correlate statistically significant with MDA and CK changes in both groups.

CK Levels

There was significant increase in CK levels at the end of morning (from 119.6 ± 29 U/L to 158.7 ± 42 U/L, $p < 0.05$), and afternoon (from 155.5 ± 49 U/L to 217.2 ± 76 U/L, $p < 0.05$) exercise in the trained group. The acute aerobic running exercise in morning and afternoon caused marked increase in CK levels averaged at 32%, and 40% in the trained group, respectively.

In the sedentary group, CK increased significantly from 168.5 ± 65 U/L to 218.1 ± 80 U/L, (31% of increase) ($p < 0.05$) in the morning exercise and from 228.2 ± 123 U/L to 322.2 ± 168 U/L, (42% of increase) ($p < 0.05$) afternoon exercise.

There was a statistically significant difference between basal CK levels measured before morning and afternoon exercises in the trained ($p < 0.05$) and sedentary groups ($p < 0.05$). Basal CK levels were significantly lower in morning compared to afternoon values in both the trained ($p < 0.05$) and sedentary groups ($p < 0.05$). In addition, increases of CK levels were found to be higher in afternoon exercise compared to morning in both groups ($p < 0.05$).

MDA levels

MDA levels were increased significant from 0.636 ± 0.11 μmol/L to 0.816 ± 0.13 μmol/L, (29 % of increase) ($p < 0.05$), in morning exercise and from 0.813 ± 0.11 μmol/L to 1.052 ± 0.11 μmol/L, (30 % of increase) ($p < 0.05$) in afternoon exercise in the trained group. MDA levels were increased significant from 0.842 ± 0.11 μmol/L to 1.153 ± 0.12 μmol/L, (38 % of increase) ($p < 0.05$), in morning exercise and from 0.957 ± 0.08 μmol/L to 1.503 ± 0.15 μmol/L, (58 % of increase) ($p < 0.05$) in afternoon exercise in the sedentary group.

Discussion

There is increasing interest among the investigators concerning exercise induced energy regulatory hormones on body metabolic system homeostasis. There is still no consensus on exact hormonal mechanisms that cause exercise to regulate energy intake. To our knowledge, this is the first study investigating whether there is a variation in response of asprosin levels in the subjects with different fitness status as a result of aerobic exercise performed different times of days, i.e. morning and afternoon. Actually, information with regarding asprosin and its essential role in action of body system functions are not yet well documented. In addition, response of asprosin in pathophysiological condition remains as an interesting topic in scientific investigations (11, 13).

In addition, aerobic exercise induced metabolic stress determined by an increased in MDA and muscular stress determined by CK on metabolic regulatory hormone of asprosin has been examined. The exercise protocol used in this study is associated with the concept of anaerobic threshold (24). Anaerobic threshold reflects moderate to high exercise intensity and can be used effectively in clinical medicine and sport science training and therapeutic purposes (4, 24). It is well known that exercise is an essential way in the management of diabetes (25) and obesity (4).

We have found that aerobic exercise caused statistically significant increase in serum asprosin levels in trained and sedentary groups in morning and afternoon exercise (Figure 1). Since first introduction of asprosin in 2016 (11), there is no wide range of studies conducted on asprosin in response to the exercise as much as other well-known energy regulatory hormones, e.g. leptin, nesfatin-1, irisin and ghrelin. In contrast to our findings, a decrease in asprosin levels has been reported following aerobic exercise performed in overweight and normal body mass index in morning and evening (15). Aerobic exercise training has been shown to be reduce hepatic asprosin levels in diabetic rats (26). However, increases in asprosin levels with closely related to the blood glucose following anaerobic exercise in women has been (16).

A higher circulation asprosin levels have been reported in patients with diabetes (11) and obesity

(27). Interestingly, other study reported decrease in asprosin levels in obese children compared to normal weight children (28). Thus, the interaction of asprosin with other glycogenic and energy regulatory hormones in regulating of glucose, insulin resistance and energy intake has not been clearly identified (13). It has been reported that orexigenic peptide of asprosin could be able to cross the blood-brain barrier and may stimulate food intake through activation of AgRP neurons in the hypothalamus (29). Aerobic exercise induced increase of asproin levels could be expected to have stimulatory effects on appetite and hunger situations. However, exercise has also great influence on body energy metabolic system homeostasis by enhancing the levels of irisin, leptin and nesfatin-1 levels in normal subjects or patients with metabolic disorders (8, 9).

It should be emphasised that widely variation in metabolic and physiological response of each individual can be often seen during exercise (30). Exercise induced increase in serum CK and MDA levels is the consequences of metabolic and/or mechanical effects of damaged muscle tissues (18, 31). In the present study, there were higher levels of MDA and CK in sedentary groups reflecting higher lipid peroxidation and muscle injury compared to trained groups (18, 20, 32). The observation of higher basal levels and also higher percent increase of asprosin during exercise in sedentary groups may be related with its protective effects against higher metabolic and muscular stress (33). In addition, the study performed in females showed significant impact of training status on asprosin concentration which is observed higher in sedentary group compared to trained group (34). However, we found no significant correlation between fat mass, body weight, metabolic stress as determined by MDA and mechanical mass activity stress determined by CK and asprosin levels in sedentary and trained subjects. The increased asprosin levels during exercise may have a role in hepatic glucose secretion (11). The other important finding obtained in this study higher basal asprosin levels in afternoon compared to morning which could be related circadian effects or other factor based on daily metabolic stress. A higher percent of increase in asprosin levels have also been observed in afternoon exercise compared to morning exercise in both groups.

In the present study we have found no statistically significant correlation between exercise induced increased CK, MDA levels and change in asprosin levels. However, observation obtained from this study point at new questions concerning asprosin response to the various type of exercise intensity and duration on metabolic and oxidative stress parameters in subjects with various levels of metabolic impairments.

In our study have some limitations. This study has resting and exercise period data and does not contain post exercise response of asprosin. Considering increased asprosin level and its stimulatory effects of food intake, post exercise response in following hours become important issue. The other issue is clarifying the interaction between asprosin and other energy regulatory hormone during exercise. Further research is required on effects of exercise with different intensity on the asprosin levels and its interaction of metabolic stress factors in subjects with different body compositions.

Conclusions

In conclusion, long term aerobic exercise enhances serum asprosin levels. Increase of asprosin level is markedly higher in sedentary subjects compared to trained subjects. Basal asprosin levels show differences with regarding time of day and also training status of the subjects. Exercise also causes significant alteration of CK and MDA levels but it did not correlate with increase of asprosin levels.

Exercise may stimulate increase of asprosin levels but it is not directly related to the stress enforced with increased metabolic or mechanic activation of exercising muscles. Exercise induced alteration in body energy (especially carbohydrate) stores could be likely to occurs during aerobic exercise. The carbohydrate regulatory hormones of asprosin may be increased at the end of exercise either to replace the reduced carbohydrate stores. Further studies should be performed in subjects with carbohydrate or fat metabolic imbalance to obtain elucidative information.

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Conflict of Interest: We declare that there is no conflict of interest.

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