Effect of Regular Exercise on Fat Metabolism in Rats Fed on Zinc Picolinate Supplemented Diet

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A B S T R A C T

The aim of this study was to determine the effect of exercise on levels of fatty acid synthesis (FAS), zinc-α2-glycoprotein (ZAG), adipose triglyceride lipase (ATGL), glucose transporter-4 (GLUT-4) and insulin receptor substrate-1 (IRS-1) in adipose tissue of rats fed on zinc picolinate supplement diet. A total of 42 male 8-week-old Wistar albino rats were randomly divided into 6 groups, each of seven; Control (C), Zinc picolinate (ZP), Chronic exercise (CE), Chronic exercise+Zinc picolinate (CE+ZP), Acute exercise (AE), Acute exercise+Zinc picolinate (AE+ZP). Rats were subjected to a 30-min running test 5 days a week for 6 weeks after dietary zinc picolinate (6 mg Zn / kg) was started. An exhaustion exercise was performed on the last day for acute exercise. It was observed that ATGL, GLUT-4, IRS-1 and ZAG levels increased and FAS level decreased in the ZP group compared to the control group. Compared to the control group, the exercise groups were found to have a decrease in the FAS level with both AE and CE, and an increase in the GLUT-4 and ZAG parameters. There was no change in ATGL and IRS-1 with AE, but an increase in ATGL IRS-1 level with CE. In zinc picolinate plus exercise groups. ATGL, GLUT-4, IRS-1 and ZAG increased with both acute and chronic exercises and FAS level decreased. It was determined that the biggest change in all parameters occurred in the zinc picolinate plus chronic exercise group. Regular exercise and zinc picolinate supplementation appear to have positive results on ATGL, ZAG and GLUT-4, which are markers of glucose metabolism. Besides, it has been determined that regular exercise and zinc picolinate supplementation have also important effects on FAS and IRS-1 values, which represent fat metabolism.

INTRODUCTION

Nowadays, obesity has become an increasing and serious public health problem. Obesity causes the development of many complications in the body directly or indirectly, and causes an increase in the risk of other non-communicable diseases and death (Beaglehole and Yach, 2003). In addition to genetic factors in the formation of obesity, a sedentary lifestyle and the amount of calories taken exceeding the amount of calories spent are also important reasons. These excess calories are stored as fat in the body and obesity occurs (Kasch et al., 2017). Exercise and special diet practices are among the most effective methods in the treatment of obesity (McQueen, 2009). It is known that regular exercises have a positive effect on performance, as well as their protection and therapeutic properties against obesity-related chronic diseases and reduce body fat (Mikami et al., 2020). However, the biochemical mechanisms mediating the beneficial effects of exercise on the organism have not been fully clarified. Researchers generally follow two paths in studies where they examine the relationship between exercise and weight loss. The first of these; They try to examine their physiological changes by giving nutritional supplements with different content to those who participate in physical activity, and secondly, to determine the effects of physical activity on metabolism. In recent years, it can be said that the interest in studies conducted by giving minerals and elements or other different supplements along with exercise has increased (Briffa et al., 2013; Axelsson and Stenvinkel, 2008; Jia et al., 2012). All trace elements found in the body are involved in many physiological reactions. They are particularly effective in carbohydrate, fat and protein metabolism. In this context, it is important to determine the level at which exercises are effective in the functions of trace elements in the organism (Cinar, 2012). One of the important trace elements in the body is zinc. Zinc, in addition to its role in energy metabolism and immune system, is also a mineral with antioxidant properties. Zinc is part of protein complexes in the musculoskeletal system, providing the enzymatic functions and structural stability of metallo enzymes such as lactate dehydrogenase, superoxide dismutase, and carbonic anhydrase. It is also necessary for nucleic acid and protein synthesis, differentiation by cellular division, for the use of glucose and insulin secretion (Torlak and Torlak, 2017). There are enzymes and hormones that are...
effective in the use and regulation of glucose (GLUT-4, IRS-1) and lipid (ZAG, FAS, ATGL) metabolism in the organism. Especially, GLUT-4 is a carrier protein responsible for the transport and regulation of glucose hemostasis in the organism (Huang and Czech, 2007), and IRS-1 is a basic molecule responsible for the control and coordination of the hormone insulin, which is responsible for glucose metabolism (Gorgisen, 2018). In addition, ZAG, which prevents the development of lipid metabolism and obesity in the organism, and FAS and ATGL, which are effective in the synthesis of triglycerides and lipids, are enzymes that are effective in the protection of energy hemostasis in the body (Ceperuelo-Mallafre et al., 2009; Zimmermann et al., 2009; Leibundgut et al., 2008). In general, it is known that supplementation intake with regular exercises positively affects glucose and lipid metabolism.

For this reason, zinc is a biological element that is necessary for not only sports performance and general health and athlete health and should be taken in certain amounts every day. In addition to the effect of zinc on exercise and performance, it is known that exercise has an important effect on zinc metabolism, too. In this study, the effect of regular exercise program with zinc picolinate supplement, which is a type of zinc, on fat metabolism in rats was investigated.

**MATERIALS AND METHODS**

**Animals and treadmill exercises**

This study was carried out in accordance with the ethical rules after the approval of Firat University Animal Experiments Ethics Committee Board of Firat University (2018/09/109). The rats used in the study were supplied by FÜDAM (Firat University Experimental Research Center). The experiment was conducted in Firat University Experimental Research Center (FÜDAM). Rats used in the study were subjected to 12 h lighting in a ventilated environment with a temperature of 22±2°C, a humidity of 55±5%. The animals were regularly given pellet feed and water ad libitum 7 days a week.

A total of 42 male Wistar albion rats aged 8 weeks, divided into 6 groups, 7 in each, were used. For treadmill exercises, initially, the rats started to run at 10 m/ min on the treadmill and reached a speed of 30 m/ min (speed can be changed) at the end of the two-week acclimatization period with controlled increments. In order to keep the rats running continuously, 100 millivolts of electricity was given at 10 second intervals. The rats were subjected to a running test five days a week for six weeks, and exhaustion exercise was performed on the last day for acute exercise. The inclination of the treadmill was adjustable between 0° and 15°. Running tests were performed between the hours of the morning (10:00-12:00) (to ignore the effectiveness of basal glyocorticoids). The rats in the control group were only kept on the treadmill. Rats were subjected to a 30-min running test daily.

**Experimental design**

Six groups of rate, each of seven were maintained as follows: (i) control (C) fed on a standard diet, (ii) zinc picolinate (ZP) fed on a standard diet with 6 mg Zn/kg of ZP added. (iii) Chronic exercise (CE) fed on a standard diet and exercised for 5 days a week for 6 weeks. (iv) CE+ZP group fed on a diet with 6 mg Zn/kg ZP added and exercised 5 days a week for 6 weeks. (v) Acute exercise (AE) were fed on a standard diet and exercised 5 days a week for 6 weeks and underwent exhaustion exercise on the last day and (vi) AE+ZP group fed on a diet containing 6 mg Zn/kg zinc picolinate and exercised 5 days a week for 6 weeks and underwent exhaustion exercise on the last day.

At the end of experiments, after 12-h starvation, the animals were decapitated by cervical dislocation under anesthesia and fat tissue samples were taken and immediately frozen on dry ice. Samples were transferred to tared eppendorf tubes. The weights of the samples taken were determined on a precision balance. The samples taken were stored at -80 ° C in the freezer until the analysis of fatty acid synthesis (FAS), glucose transporter-4 (GLUT-4), insulin receptor substrates (IRS1), adipose triglyceride lipase (ATGL) and zinc-a2-glycoprotein (ZAG) levels.

**Western blot analysis**

Western blot analysis was performed with minor modifications (Orhan et al., 2021) for the separation of FAS, GLUT-4, IRS1, ATGL and ZAG levels in adipose tissue. Tissue samples collected and homogenized from animals in the same group were treated with a buffer with a protease and phosphatase inhibitor mixture. After drawing the total protein content (Invitrogen, Life Technologies Corporation, Carlsbad, CA, USA), gel electrophoresis (Bio-Rad, Life Sciences Research) was applied on the proteins for fractionation and then transferred to a nitrocellulose membrane. Membranes were blocked with TBS-T and incubated overnight with antibodies FAS, GLUT-4, IRS1, ATGL and ZAG (Abcam, Cambridge, UK). Western blotting was repeated at least three times to verify results for all proteins. Finally, the membranes were scanned and transferred to Image J software (National Institutes of Health, USA) for densitometric analysis. Specific binding between primary and secondary antibodies was visualized using diaminobenzidine to control protein loading.

**Data analysis**

IBM SPSS (version 22) package program was used...
to analyze the data. For the normality analysis of the data, Shapiro Wilk, Histogram, Kurtosis and Perpendicularity values were looked at and parametric tests were applied for the analysis of the research to the data determined to show normal distribution. One Way Anova was used for comparisons between groups, and Tukey Post Hoc test was used to determine differences between groups. Data were given as group means and standard deviation. Statistical significance in the data was considered significant for values with probability values less than $P < 0.05$.

**RESULTS AND DISCUSSION**

Figure 1 shows the effect of exercise on ATGL, FAS, GLUT4, IRS-1 and ZAG in rats fed on zinc picolinate supplemented diet.

**Adipose triglyceride lipase (ATGL)**

It was found that there was no statistically significant difference between ATGL of ZP, CE, CE+ZP groups ($p > 0.05$). The AE+ZP group differed from all groups ($p < 0.05$). Mikloš et al. (2019) have shown that acute exercises applied at different intensities and durations increased ATGL levels. This result is similar to the acute exercise group results in our research group. Dashti et al. (2018) examined the effects of an eight-week exercise program on serum iris, glucose and ATGL levels in animals in which they developed diabetes. As a result of the study, they found that high intensity exercise increased ATGL levels more than the diabetes group, healthy control group and other low intensity exercise groups. Riis et al. (2019), on the other hand, determined that there was no change in ATGL levels of the participants in the exercise program they applied three days a week for four weeks. Showing difference with our research findings, this study stands out for its exercise model applied and difference of the subjects used in the study. This result makes us think that short-term exercises do not affect ATGL levels in humans.

**Fatty acid synthesis (FAS)**

The FAS level of the control group was higher ($P < 0.05$) than all groups. It was observed that there was no difference between PAS of CE and CE+ZP groups ($p > 0.05$), but differed from all other groups ($p < 0.05$). The AE group differed ($p < 0.05$) from all groups but there was no significant difference ($p > 0.05$) between ZP group and AE+ZP groups. Uchiyama et al. (2020) investigated the effect of a twelve-week regular physical activity program on FAS and micro albumin-creatinine levels in individuals with kidney disease. As a result, it has been determined that regular exercises, as in the present research findings, lead to a significant decrease especially in FAS levels. In a similar study, So et al. (2020) examined the effects of an acute exercise on PGC-1α and FAS levels in rats. As a result of the study, it was found that acute exercises decreased PGC-1α levels in humans. In a similar study, Musial et al. (2019) determined the effect of a six-week exercise program on FAS levels with some components in liver metabolism in animals. When the research findings were evaluated, they found that the FAS levels of the diet + exercise group were lower than the FAS levels of the control group, placebo group, and exercise group. Musial et al. (2019) stated that in the exercise program they applied, the lowest FAS level in obese rats was in the exercise group.

**Glucose transporter-4 (GLUT-4)**

The GLUT-4 in the control group was statistically at a lower level ($P < 0.05$) than all other groups and there was no difference between CE and CE+ZP groups ($p > 0.05$).
However, there was a difference when compared with the other groups (p < 0.05) and no difference between ZP, AE and AE+ZP groups (p > 0.05). Pala et al. (2018) examined the effect of L-carnitine supplementation along with exercise on oxidative stress, glucose transporters and biochemical parameters in rats. According to the findings obtained as a result of the research, it was determined that GLUT-4 levels were higher than GLUT-4 levels of the exercise + supplement group and the GLUT-4 levels of the control group. In another recent study, Wang et al. (2020) examined the effect of exercise on GLUT-4, PGC-1α and PPARα levels in rats in a sixteen week low-intensity exercise program. As a result of the study, they found that the GLUT-4 levels of the exercise group were higher than the GLUT-4 levels of the diabetes group and the control group. On the other hand, Pala et al. (2020) applied an exercise program of 30 min / day for five days a week for six weeks and found that both acute and regular exercise led to an increase in GLUT-4 levels in their study, which investigated the effect of chromium picolinate supplementation on glucose and lipid metabolism in rats. In addition, chromium picolinate supplementation also appears to produce results similar to the application of zinc picolinate in current research. In another study that supports our study results; Pataky et al. (2019) stated that acute exercise they applied in male rats increased GLUT-4 levels.

**Insulin receptor substrate-1 (IRS-1)**

The IRS-1 levels, in the control group did not differ from the AE group, but was different from all other groups (p<0.05). ZP, AE and AE+ZP groups did not differ (p<0.05), but differed when compared to other groups (p<0.05). As in the GLUT-4 variable, it was found that there was no difference between CE and CE+ZP groups in IRS-1 variable (p>0.05), however, it was determined that it differed with all groups (p < 0.05). Turgut et al. (2018) examined the effect of a thirty-minute regular exercise program, five days a week for six weeks, on IRS-1 levels. They found that the highest value in IRS-1 levels after the exercise program was in the chronic exercise + chromium histidinate + biotin group. Tan and Guo (2019) also examined the effect of swimming exercises four days a week for three months on IRS-1 and GLUT-4 levels in patients with metabolic syndrome. They found that swimming exercises increased the levels of IRS-1 and GLUT-4 in patients with metabolic syndrome. Similarly, Rattanavichit et al. (2018) found that the IRS-1 levels of the exercise group were higher than the control group’s IRS-1 levels in their study in which they examined the effect of a six-week exercise program on IRS-1 levels in rats. In a study in which results supporting the previous research results were obtained, Kuga et al. (2018) determined that the IRS-1 levels of the exercise group in middle-aged rats were lower than the IRS-1 levels of the control group in their exercise program.

**Zinc-a2-glycoprotein (ZAG)**

Looking at the ZAG values, as in most of the other parameters, it was observed that all application groups differed from the control group (p < 0.05). It was found that the AE group was different from all other groups (p < 0.05), and there was no difference between the groups of ZP, CE+ZP and AE+ZP (p > 0.05). It was determined that there was a statistically significant difference between the CE and AE groups (p < 0.05). In addition, it was seen that the AE group was different from all groups (p < 0.05).

Kon and Suzuki (2019) found a significant increase in ZAG concentration as well as improvement in insulin sensitivity and energy metabolism of resistance exercises consisting of 70% vigorous 90 seconds training, 90 seconds rest, five sets and ten repetitions in healthy individuals. Soori et al. (2019) applied an eight-week high intensity exercise program to determine ZAG levels in obese animals. As a result of the research, it was determined that ZAG levels in the high intensity exercise group increased more than the control and exercise + obese groups. Lin et al. (2019) determined in their study that the ZAG levels of the control group in rats were higher compared to the other groups.

The limitations of this study are that it was studied with a healthy male animal group and a limited number of parameters effective in adipose tissue. It can be said that in future studies, addressing similar research topics by using different disease models and genders as well as applying different nutritional supplements will provide significant contributions to making glucose and fat metabolism improved.

**CONCLUSIONS**

The acute exercise did not affect the parameters that are effective in glucose and fat metabolism, while chronic exercise caused an increase in ATGL, ZAG, GLUT-4 and IRS-1 levels and a decrease in FAS levels. In this context, in addition to using the current application to improve fat and glucose metabolism, we argue that it can have positive effects on community health, considering that it will have positive results on exercise groups and risk groups.

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Statement of conflict of interest
The authors declare that there is no conflict of interest.

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