

ADAPTATION OF THE EFFECT OF CHROMIUM MINERAL SUPPLEMENT ON SERUM IRISIN, LEPTIN AND GHRELIN HORMONE LEVELS TO EXERCISE TRAININGS

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Abstract

This work; It is aimed to adapt the Effect of Chromium Mineral Supplement on Serum Irisin, Leptin and Ghrelin Hormone Levels to Exercise Trainings. For this purpose; A total of 28 male rats of eight weeks old Wistar Albino were included in the study and randomly divided into four groups. The control group (n=7) who continued to be fed with a daily diet and waited for eight weeks without any supplementation; The exercise group in which the treadmill exercise was performed at a speed of 15 m/min on the treadmill for five days for eight weeks (n=7); supplementation group (n=7) given chromium picolinate by gavage method (8 µg / kg/day) once a day for eight weeks in addition to daily dietary nutrition; In addition to dietary nutrition, a single dose (8 µg/kg/day) chromium picolinate was given by gavage for eight weeks and supplemented + exercise group (n=7) in which 20 min/day treadmill exercise was performed for eight weeks. At the end of the studies, Irisin, leptin and ghrelin analyzes in blood samples were studied by preparing them according to the kit procedure with the elisa method. According to the data we obtained, the normality homogeneity test was applied and it was determined that the data were normally distributed. As a result, one way anova test and paired sample t test were applied from parametric tests (p<0.05 statistically significant difference was accepted) and tukey test was applied to determine the difference between groups. SPSS-21 package program was used in the analysis of the data (p< 0.05 was considered a statistically significant difference). Correlation test was used to determine the relationship between variables. As a result, treadmill exercise applied at a speed of 20 minutes, 15 km/h, 5 days a week for eight weeks, and chromium mineral supplementation decreased the hormone level of ghrelin, thus reducing the need for food intake and less energy consumption. It was found that the use of chromium picolinate alone had a lowering effect on the leptin hormone, and that it also reduced the leptin hormone level in long-term exercise, that the combination of chromium picolinate supplement and exercise further reduced the already low leptin level compared to the chromium group and exercise group. We found that the level was significantly reduced.

Keywords: chromium picolinate, obesity, exercise, leptin, ghrelin, irisin.

INTRODUCTION

It is known that mechanisms responsible for increased cardiovascular diseases and morbidity and mortality due to other causes emerge with metabolic syndrome. (Solymoss et al., 2003; Davidson, 2005). The most important reasons for the increase in the incidence of metabolic syndrome are obesity and physical inactivity. Obesity is a complex, multifactorial chronic disease resulting from the interaction of genetic and environmental factors. The use of physical activity as a treatment modality in people with metabolic syndrome has not been clearly established in randomized controlled trials (Yu et al., 2022; Nicklas et al., 1997).

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Cross-sectionally, several studies have shown a higher prevalence of metabolic syndrome in people with low physical fitness. (Suire et al., 2022; Erdogan et al., 2022; Smith et al., 2022; Lakka et al., 2003).

Except for the relationship between physical activity and obesity, chromium is an essential mineral element for human beings. (Heffernan et al., 2019; Lukaski, 1999). Trivalent chromium (Cr(III)) is associated with the metabolism of carbohydrates, lipids and proteins in animals, in addition, chromium is also called the "glucose tolerance factor (GTF)" because it regulates the metabolic action of insulin. (Chang et al., 2020). Suboptimal dietary chromium intake is associated with increased risk factors associated with diabetes and cardiovascular disease. In the last five years, chromium has been shown to improve glucose and related variables in subjects with glucose intolerance and type 1, type 2, gestational and steroid-induced diabetes. Chromium increases insulin binding to cells, increases the number of insulin receptors, and activates insulin receptor kinase, resulting in increased insulin sensitivity. (Anderson, 2000). Chromium plays a role in carbohydrate and lipid metabolism and glucose homeostasis by increasing the effect of insulin (Haymes, 2022; Anderson et al., 1988; Lane et al., 1989). Chromium is an essential building mineral involved in the metabolism of carbohydrates, fats and proteins and combines with picolinic acid to increase absorption. Promotes insulin action, has been shown to improve glycemic control in people with diabetes and improve body composition by supporting the maintenance of body fat percentage (Suksomboon et al., 2014; Eckerson, 2015). It has been reported that chromium picolinate supplementation has an antioxidant role in diabetic patients and prevents the detrimental effects of oxidative stress. (Ali et al., 2011).

In addition, many researchers are trying to identify the mechanisms that pursue the imbalance between energy intake and energy expenditure. Body weight is regulated by a complex system that includes both peripheral and central factors. Two of the hormones known to play an important role in the regulation of food intake and body weight are leptin and ghrelin. These two hormones originate from the periphery and signal the brain, especially the hypothalamus, in different ways (Frederich et al., 1995; Sahu, 2004).

Considering all this information, the mechanism of action of chromium on glucose and lipid metabolism is not fully understood. Considering the positive effects of the above-mentioned exercise and the mechanisms that the chromium mineral affects, in our study to examine its relationship with health factors from a different perspective; In this study, it was aimed to examine the effects of chromium mineral supplementation on serum irisin, leptin and ghrelin hormone levels, which are associated with obesity and body fat ratio, and also to adapt to exercise training.

MATERIALS AND METHODS

Ethics committee approval was obtained from Van Yüzüncü Yıl University Animal Experiments and Local Ethics Committee before starting this study, which aims to examine the effects of long-term exercise combined with chromium mineral supplementation on serum irisin, leptin and ghrelin levels in rats. (With the decision number 2021/02-13 dated 25.02.2021)

Supply and Care of Animals

During our study, the care of the experimental animals was carried out in the Experimental Animals Laboratory of Van Yüzüncü Yıl University, and all the experimental animals we used in the study were kept in transparent cages in rooms with 12-12 light/dark lighting, 22 ± 2 degrees temperature and 55% humidity.

Creation Of Groups

A total of 28 8 weeks old Wistar Albino male rats, 220-350 gr. After they were taken in weight and fed with standard pellet feed for 8 weeks, they were randomly divided into 4 groups as 7 rats in each cage as follows.

Group 1 (n=7): Positive control group.

Group 2 (n=7): Long-term exercise group.

Group 3 (n=7): Chromium mineral supplement group.

Group 4 (n=7): Long-term exercise and chromium mineral supplementation

After the rats were divided into groups, the 8-week exercise program was applied to Group 2 on a special treadmill 5 days a week, at 15 km/h speed, for 20 minutes. The exercised rats were started to run at a speed of 5 m/min at the beginning on the treadmill and were subjected to jogging for 2 weeks, 5-10 minutes daily, before the exercise protocol to ensure adaptation to the exercise. Commercially available Ocean brand chromium picolinate component in Group 3 was dissolved in water at a dose of 8 µg/kg/day (Büyükleblebici, 2009) and given by gavage method for 8 weeks. Group 4 was given chromium mineral supplementation in addition to the 8-week exercise program. During the experiment, all rats continued to be kept in light/dark rooms for 12 hours with controlled temperature and humidity levels. At the end of the study, which lasted for a total of 10 weeks, 5 ml blood samples were taken from all rats under anesthesia using the intracardiac method with the help of an injector. The blood samples taken were analyzed by ELISA method according to the kit procedure.

Sample Collection and Analysis

Immediately after the end of the experimental process, blood was taken from all animals in aprotinin tubes to be used in the necessary analyzes by decapitation between 09:00 and 10:00 in the morning. Blood samples were taken at 4000 rpm for 10 min. The serum samples obtained after centrifugation were aliquoted and stored at -80 degrees until the study day.

İrisin Analysis

The irisin levels in the samples obtained (Sunred Biological Technology Co., Ltd., Shanghai, CHINA) were studied by ELISA method in accordance with the working procedures specified in the kit catalogues. Measuring range of rat irisin ELISA kit: 0.25-72 ng/mL sensitivity 0.247 ng/mL, Intra-Assay: CV Microplate Reader P4300 devices (Awareness Technology Instruments, USA) were used and test results were stated as ng/mL.

Ghrelin Analysis

Analyzes were performed at Van Yüzüncü Yıl University Experimental Medicine Application and Research Center. Spi-Bio brand ghrelin (Mouse/rat) eia kit was used for ghrelin analysis and analyzes were performed in accordance with the procedures. Plasma samples were diluted 1/5 with enzyme immunometric assay (EIA) buffer. EIA buffer was reconstituted with distilled water in a 50 ml vial. A rat acylated ghrelin standard was prepared, which was diluted with 1 ml of distilled water to obtain S1. Then, 500 µl of EIA buffer was added to 7 pieces of ependorpha. 500 µl of S1 dilution was taken and transferred to the first tube and serial dilution was made. Quality control was diluted with 1 ml of distilled water. Anti-acylated ghrelin-acetylcholinesterase (AChE) tracer was reconstituted with 10 ml of EIA buffer. It was prepared by diluting 1 ml of wash buffer with 400 ml of distilled water, adding 200 µl of tween 20 and mixing in a magnetic stirrer. Elman's Reagent was prepared 5 minutes before use by diluting with 49 ml of distilled water and 1 ml of concentrated wash buffer and mixing well. Plates were washed 5 times with 300 µl wash buffer and dried thoroughly before use. Cavity wells were left empty. 100 µl of EIA buffer was added to the non-specific binding (NSB) wells. Rat acylated ghrelin standards were studied in duplicate and 100 µl were added to each well, respectively, and the addition was started at a low concentration. Quality control and samples were run in duplicate and 100 µl was added. Except for the antiacylated ghrelin ache tracer blank, 100 µl was added to the wells and incubated for 3 hours at room temperature. Each well (300µl/well) was washed 5 times and after the final wash it was gently shaken with a shaker for 5 minutes without spilling the contents. Then it was washed again (300 µl/well) 5 times and dried. 200 µl of Ellman's Reagent was added to all wells. It was incubated at room temperature and in the dark with an orbital shaker. In the short reaction, reading was taken at 405 nm between 30-60 minutes. Average absorbances were calculated for each NSB, standard, and sample. A standard curve was created according to known standard concentrations and absorbances. Using this standard curve, the leptin concentrations of the samples were calculated in pg/ml.

Leptin Analysis

Analyzes were performed at Van Yüzüncü Yıl University Experimental Medicine Application and Research Center. Sigma Rat Leptin Elisa Kit was used for leptin analysis and analysis was performed in accordance with the procedures.

Blood samples were taken to room temperature (18-25°C) before analysis and analysis of all samples was repeated twice. Standards were prepared and blood samples were placed. The placed blood samples were covered and incubated at room temperature for 2.5 hours with a Heidolph Tiramax 1000 orbital shaker with gentle shaking. The solution was removed and washed 4 times with 1X wash solution. Each well was washed by filling with wash buffer (300 µl) using a multiple pipette. Complete conveyance of liquid is ensured for best performance at all stages. Biotinylated antibodies were prepared and 100 µl of these prepared antibodies was added to each well. It was incubated for 1 hour at room temperature with gentle shaking. The solution was removed and washed 4 times with 1X wash solution. Each well was washed by filling with wash buffer (300 µl) using a multiple pipette. Each well was washed by filling with wash buffer (300 µl) using a multiple pipette. Streptavidin solution was prepared and 100 µl of these prepared solutions was added to each well. The solution was removed and washed 4 times with 1X wash solution. Each well was washed by filling with wash buffer (300 µl) using a multiple pipette. 100 µl of TMB one-step substrate composition was added to each well. It was incubated at room temperature and in the dark for 30 minutes with gentle shaking. 50 µl of stop solution was added to each well and read immediately at 450 nm. The mean absorbance of each data was calculated. A standard curve was drawn with absorbance values corresponding to standard leptin concentrations. Using this standard curve, the leptin concentrations of the samples were calculated in pg/ml.

Applied Treadmill Exercise

The rats in the running group were exercised on a special treadmill 5 days a week for eight weeks. The rats subjected to exercise were started to run at a speed of 5 m/min initially on the treadmill and the intensity was increased according to the situation of the rats on the band. At the end of the 2-week acclimatization period, a speed of 15 m/min was reached for 20 minutes. Exercise practices were performed between 09:00 and 11:00 in the morning. In order to provide compulsory exercise continuity in the motivation of the animals for running, electrical stimulus was applied and a gradual electric shock between 1 and 6 mA was used. The treadmill used in the study is the "May Time 0804 Animal Treadmill" model treadmill with 6 lanes, obtained from Van Yüzüncü Yıl University Experimental Medicine Application and Research Center, with international CE certificate.

Statistical Analysis

SPSS-21 package program was used for data analysis. The distribution of the data was examined with the normality homogeneity test and it was found that it showed normal distribution. As a result, the One Way Anova Test and Paired Sample T Test, which are parametric tests, were applied, and the Tukey Test was applied to determine the difference between the groups. Pearson correlation test was used to

determine the relationship between variables. Significance levels were evaluated according to (p<0.05).

RESULTS

The results obtained from our study and the statistical analyzes of these results are presented in the table below.

Table 1 Ghlerin, Irisin and Leptin Levels in All Groups

Parameters	Control Group (Mean±Standard Deviation, n:7)	Chromium Group (Mean±Standard Deviation, n:7)	Exercise Group (Mean±Standard Deviation, n:7)	Exercise+Chromium Grubu (Mean±Standard Deviation, n:7)
Ghreltin (mg/dL)	1125,41±206,54 ^{ab}	915,25±205,05 ^a	1241,01±173,61 ^b	580,90±107,52 ^c
Irisin (mg/dL)	5,38±1,35 ^a	4,19±1,23 ^a	4,29±1,26 ^a	2,32±0,67 ^b
Leptin (mg/dL)	195,70±19,33 ^a	168,40±30,09 ^{ab}	173,20±34,70 ^{ab}	143,86±46,38 ^b

abc: The difference between different letters on the same line is statistically significant (p<0.05).

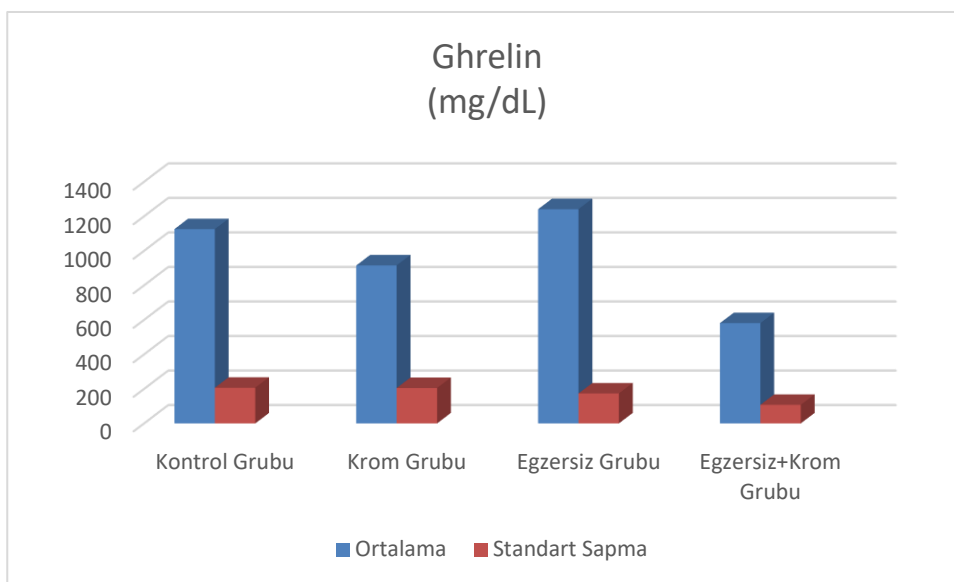


Figure 1. Ghreltin Levels in All Groups.

Exercise+Chromium Group Ghreltin values; Ghlerin levels

were statistically lower in the control group, Chromium group and Exercise group.

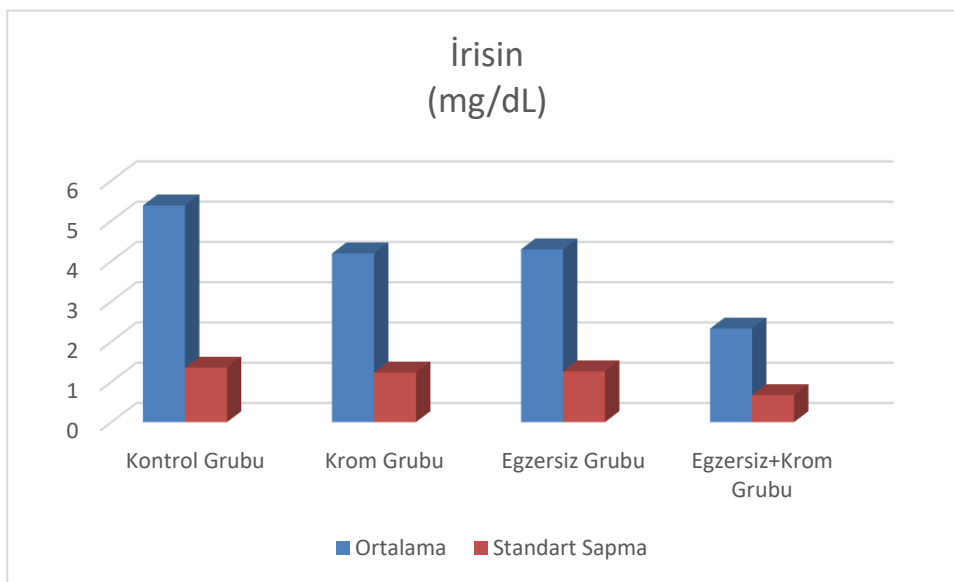


Figure 2. Irisin Levels in All Groups

It was observed that the irisin levels of the Exercise + Chromium Group were statistically ($p < 0.05$) significantly decreased compared to the Control, Chromium group and

Exercise groups. No statistically significant difference was found in the comparison between the control group, chromium group and exercise groups ($p > 0.05$).

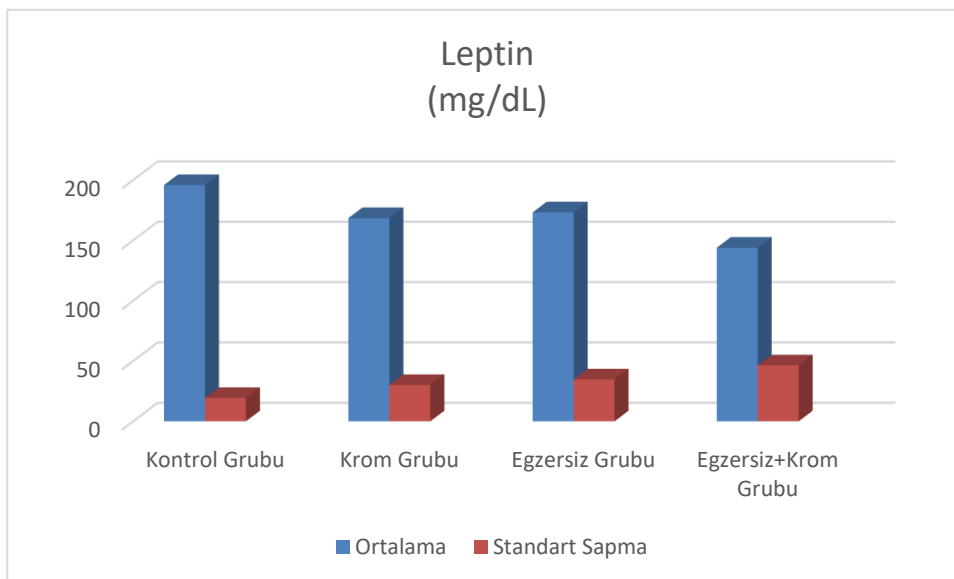


Figure 3. Leptin Levels in All Groups

While there was no statistically significant difference in Leptin levels between the Chromium group and the Exercise group; Leptin levels of these two groups were found to be significantly ($p < 0.05$) decreased compared to the control

group.

Leptin level of the Exercise + Chromium group; It was determined that it was statistically decreased compared to the control group, the chromium group and the Exercise group.

Table 2 Correlation Level Between Ghrelin (mg/dL), Irisin (mg/dL) and Leptin (mg/dL) Levels of All Groups

Variables	Ghrelin (mg/dL)	Leptin (mg/dL)	Irisin (mg/dL)
Ghrelin (mg/dL)	1	0,424*	0,684**
Leptin (mg/dL)		1	0,535**
Irisin (mg/dL)			1

*The correlation level is statistically significant ($p < 0.05$).

**The correlation level is statistically significant ($p < 0.01$).

A statistically significant correlation was found between ghrelin levels and both Leptin (mg/dL) ($p < 0.05$) and Irisin (mg/dL) levels ($p < 0.01$). Statistically significant ($p < 0.01$) was found in the correlation between leptin (mg/dL) and irisin (mg/dL) levels.

DISCUSSION AND CONCLUSION

The effects of chromium mineral supplementation on serum irisin, leptin and ghrelin hormone levels were investigated and the following results were obtained:

1. Exercise + Chromium Group Ghrelin values; Ghrelin levels were statistically lower in the control group, chromium group and exercise group.
2. Exercise + Chromium Group Irisin levels were found to be statistically ($p < 0.05$) significantly decreased compared to the control, chromium group and exercise groups. No statistically significant difference was found in the comparison between the control group, chromium group and exercise groups ($p > 0.05$).
3. While there was no statistically significant difference in leptin levels between the chromium group and the exercise group; It was observed that the leptin levels of these two groups decreased significantly ($p < 0.05$) compared to the control group.
4. Leptin level of the Exercise + Chromium group; It was determined that it decreased statistically compared to the control group, the chromium group and the exercise group.
5. Ghrelin level was statistically significantly correlated with Leptin (mg/dL) level ($p < 0.05$) and Irisin (mg/dL) level ($p < 0.01$). A statistically significant ($p < 0.01$) correlation was found between leptin (mg/dL) levels and irisin (mg/dL) levels.

Cho et al., (2017), Jones et al., (2009), Yu et al., (2018), Sartorio et al., (2008), Plinta et al., (2012), Heden et al., (2013), Beer et al., (2020), Matos et al., (2020), Toshinai et al., (2007), Howe et al., (2016), Holliday and Blannin, (2017), R. Rämson et al., (2012), our study, in which chromium supplementation combined with long-term exercise, decreased the level of ghrelin hormone, showed parallelism with the results obtained by the researchers mentioned above. However, Martin et al., (2010), Bowyer et al., (2019), Jürimäe et al., (2009), Russel et al., (2009), Erdmann et al., (2007), Hedayati et al., (2012), their studies showed the opposite of our results and found an increase in ghrelin concentration. Leidy et al., (2004), Guelfi et al., (2013) reported both an increase and a decrease in ghrelin concentration in their studies.

Among the studies conducted to examine the effect of acute exercise on leptin hormone concentration, we found that

chromium picolinate supplement taken with exercise reduces serum leptin concentration; Sari et al., (2007), Jürimäe J. and Jürimäe T., (2005), Legakis, (2004), Zafeiridis et al., (2003), Bouassida et al., (2004), Zaccaria et al., (2002), Duclos et al., (1999), Olive and Miller, (2001), reported supportive reductions. Among the studies conducted to examine the effect of chronic exercise on leptin hormone concentration; Ozcelik et al., (2004), Polak et al., (2006), Miyatake et al., (2004) found data supporting the leptin hormone concentration result we obtained. Desgorces et al., (2004) reported that the chronic exercise protocol used in their study both increased and decreased the leptin concentration of the subjects. This study showed that the relationship between leptin and physical activity is not the main determinant of whether the activity is short- or long-term, and that the duration of activity per unit time is also valuable for significant differences in leptin levels.

Among the studies conducted to examine the effect of chronic exercise on serum irisin hormone concentration; Norhein et al., (2014) reported supportive results. However, from the studies conducted to examine the effect of chronic exercise on serum irisin hormone concentration; Bluher et al., (2014) obtained findings in the opposite direction of our study. To examine the effects of acute exercise on irisin concentration; Aydin et al., (2013), Norhein et al., (2017), Löffler et al., (2015), Winn et al., (2017) reported increases in irisin concentration, contrary to our findings. Likewise, Huh et al., (2015) applied different types of acute exercise in their studies and investigated whether these types affect irisin concentration, as a result, it was concluded that irisin concentration increased more in the group that applied resistance exercise compared to the high-intensity intermittent exercise and continuous moderate exercise groups. Pekkala et al., (2013), Kurdiova et al., (2014), Fukushima et al., (2016) reported that chronic exercise did not affect irisin concentration.

In this study, which we conducted to investigate the effect of long-term exercise and chromium picolinate mineral supplement combination on hormone levels, we determined that the ghrelin hormone level decreased with long-term exercise and chromium picolinate supplementation, thus reducing the need for food intake and less energy consumption. The use of chromium picolinate alone has a lowering effect on the leptin hormone, it also reduces the leptin hormone level in long-term exercise, the combination of chromium picolinate supplement and exercise further reduces the leptin level, which is already low, compared to the chromium group and the exercise group, and that this decrease reduces the sugar rate in the blood due to the increased energy need and Physiologically, we think that glucagon corrects blood sugar level and thus the satiety center in the brain is not stimulated. We also found that chromium picolinate supplementation taken with exercise significantly reduced irisin hormone levels.

A sedentary lifestyle and unhealthy diet cause a decrease in the quality of life of individuals and shorten their life span.

Excess weight causes physiological and psychological negative effects on the organism, causing individuals to lose their daily life energies, self-confidence and motivation. This work; In line with the results obtained, important information and recommendations should be made to the experts in the exercise programs and diets that will be adapted to humans, and for this purpose, first of all, positive and negative effects were determined on rats, and then adaptations were made to apply and work on humans. Recommendations will be made to athletes, obese and obese people regarding the positive or negative effects of these supplements.

Weight gain due to inactive life after injury in athletes brings with it many negative situations. The excess body fat percentage in the athletes causes a loss of time in returning to the current condition before the injury. With our study, it is a promising study in terms of eliminating the disadvantages and accelerating the return of the athletes to their former productivity by integrating the fat burning process of the athletes with the chromium mineral supplement and exercise, which are effective on the metabolism, insulin, blood sugar parameters, and integrating them into the exercise programs of the athletes to the maximum extent. In addition to the negative effects of obesity on both physical and psychological health, the high cost of surgical treatment and the need for a long-term diet and exercise program, people suffering from obesity are looking for quick, easy and low-cost ways to get rid of this situation. They find salvation in buying harmful slimming teas and medicines sold in the market. The negative products used turn the existing bad state of the person into an unsolvable mess, let alone stop it. A much more effective and healthy weight loss can be achieved by adopting the right diet, additional minerals, exercise, lifestyle changes and habits, instead of the stated unrecommended weight loss efforts.

We aimed to be a glimmer of hope for public health problems such as type II diabetes, insulin resistance, metabolic syndrome, and obesity, which many people experience and are adversely affected by. Since the researches in this field are limited, we think that the findings we obtained in our study will provide important information to those who will study in the literature on a similar subject area.

The next study will be more comprehensive by establishing obesity and diabetes because it is a question of what effect it will have on obese and diabetic rats.

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