

Resveratrol Supplementation is Associated with Elemental Metabolism in Some Tissues of Acute Swimming Rats*

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Research Article

Received: 08/03/2023

Accepted: 05/07/2023

Published: 31/12/2023

Abstract

The aim of this study was to investigate how resveratrol administration affects element metabolism in some tissues in rats undergoing acute swimming exercise. In the study, 28 adult male Wistar rats were used and the animals were divided into 4 groups in equal numbers. Group 1, Control; Group 2, Swimming: The group fed a standard diet and had 30 minutes of acute swimming exercise. Group 3, Resveratrol: The group receiving 10 mg/kg resveratrol supplement for four weeks. Group 4, Resveratrol + Swimming: The group that received 10 mg/kg resveratrol supplement for four weeks and also had 30 minutes of acute swimming exercise. At the end of the four-weeks diet, group 2 and group 4 animals were given 30 minutes of acute swimming exercise. Animals were sacrificed immediately after swimming practices, and testis, kidney, heart and liver tissue samples were taken. Elemental analyzes in tissue samples were determined by atomic emission method. Elements measured in testicular tissue were not affected by exercise and resveratrol applications. Both resveratrol administration and acute swimming exercise resulted in changes in zinc, iron, magnesium, selenium, molybdenum levels in kidney tissue, iron and calcium levels in heart tissue, and zinc, iron and molybdenum levels in liver tissue ($p<0.05$). The results of study, which revealed that resveratrol administration caused changes in element metabolism in some tissues of rats both during and independently of exercise, can be presented as the first and original finding based on med-line scans.

Keywords: Acute swimming exercise, Element metabolism, Resveratrol application, Rat

Resveratrol Desteği Akut Yüzme Egzersizi Yaptırılan Sıçanların Bazı Dokularında Element Metabolizmasıyla İlişkilidir

Öz

Bu çalışmanın amacı, akut yüzme egzersizi yaptırılan sıçanlarda resveratrol uygulamasının bazı dokulardaki element metabolizmasını nasıl etkilediğini araştırmaktır. Wistar cinsi 28 adet erişkin erkek sıçan kullanılan araştırmada hayvanlar eşit sayıda 4 gruba ayrıldı. Grup 1, Kontrol; Grup 2, Yüzme: Standart diyetle beslenen ve 30 dakika akut yüzme egzersizi yaptırılan grup. Grup 3, Resveratrol: Dört hafta boyunca 10 mg/kg resveratrol desteği alan grup. Grup 4, Resveratrol + Yüzme: Dört hafta boyunca 10 mg/kg resveratrol desteği alan ve 30 dakika akut yüzme egzersizi yaptırılan grup. Dört haftalık diyet uygulamalarının bitiminde grup 2 ve grup 4 hayvanlarına 30 dakika akut yüzme egzersizi yaptırıldı. Yüzme uygulamalarından hemen sonra hayvanlar sakrifiye edilerek testis, böbrek, kalp ve karaciğer doku örnekleri alındı. Alınan doku örneklerinde element analizleri atomik emisyon yöntemiyle tayin edildi. Testis dokusundaki ölçümü yapılan elementler egzersiz ve resveratrol uygulamalarından etkilenmedi. Hem resveratrol uygulaması hem de akut yüzme egzersizi böbrek dokusunda çinko, demir, magnezyum, selenyum, molibden, kalp dokusunda demir, kalsiyum ve karaciğer dokusunda ise çinko, demir, molibden seviyelerinde değişiklikle sonuçlandı ($p<0.05$). Resveratrol uygulamasının hem egzersizde, hem de egzersizden bağımsız olarak sıçanların bazı dokularında element metabolizmasında değişikliklere yol açtığını ortaya koyan çalışmamızın bulguları med-line taramalardan yola çıkarak ilk ve orijinal bulgu olarak takdim edilebilir.

Anahtar Kelimeler: Akut yüzme egzersizi, Element metabolizması, Resveratrol uygulaması, Rat

* This study was presented as an oral presentation at the International Balkan Sports Sciences Congress, held in Bursa between 21-23 May 2017.

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INTRODUCTION

Athlete nutrition is critical in maintaining athletic performance. For this reason, the relationship between nutrition and performance in athletes is the focus of attention of researchers. Two methods are frequently used to determine the interaction between physical activity and nutrition. The first of these is to examine the physiological and performance responses by giving different foods to the participants in physical activity, and the other is to determine the effects of physical activity on nutrition.

Trace elements are involved in many vital biological processes. Dietary deficiency of these elements in sports nutrition may adversely affect athlete performance (Heffernan et al., 2019). The vast majority of athletes consume these trace elements, a type of micronutrient, to improve their performance (Heffernan et al., 2019). For this reason, the relationship between performance and element metabolism in athletes is being investigated.

Sports, exercise and physical activity are defined as the whole of movements that improve the health status of people and ensure the continuation of this good condition (Sharman et al., 2015). Despite all its beneficial effects on health, the relationship between sports and exercise and oxidant stress is extremely complex. This relationship is closely related to the duration and intensity of exercise. Especially in sports branches that require acute exercise and endurance training, there is an increase in tissue damage and thus oxidative stress (Pingitore et al., 2015). For this reason, it is accepted that oral supplementation of antioxidant elements and natural antioxidants to athletes is beneficial in preventing oxidative stress and increasing sportive performance (Pingitore et al., 2015).

Attention is drawn to the healing effect of resveratrol supplement, which is a powerful antioxidant among these natural compounds, on tissue damage that occurs during exercise (Jo et al, 2019). Resveratrol is a polyphenol produced in response to oxidative stress in plants such as peanuts, blackberries and blueberries, mainly in grape skin (Jo et al., 2019). Its potent capacity to prevent biological stresses in plants has led to the identification of resveratrol as an important antioxidant (Jo et al., 2019). Although resveratrol has been shown to have antioxidant, anticancer, estrogenic, antiplatelet, ischemia-reperfusion injury, anti-inflammatory and antimicrobial activities (Baltaci et al., 2016), publications investigating its relationship with exercise, performance and element metabolism are quite limited. The aim of this study was to investigate how resveratrol administration affects element metabolism in blood and tissues in rats undergoing acute swimming exercise.

METHODS

Experimental Design

This study was carried out on 28 male Wistar rats obtained from Necmettin Erbakan University Kombassan Experimental Medicine Application and Research Center. The study protocol was approved by the ethics committee of the same center (2013-183). The animals were divided into 4 equal groups:

Group 1, Control: The group in which no application was made and fed with standard rat chow. Group 2, Swimming: The group fed a standard diet and had 30 minutes of acute swimming exercise. Group 3, Resveratrol: The group given resveratrol (10 mg/kg) in drinking

water for four weeks in addition to the daily standard diet. Group 4, Resveratrol + Swimming: In addition to the daily standard diet, resveratrol (10 mg/kg) was given in drinking water for four weeks and 30 minutes of acute swimming exercise was performed.

After 24 hours after the end of the resveratrol applications, which lasted for four weeks, the animals in groups 2 and 4 were given acute swimming exercise for 30 minutes in a single session. The idea of 30-minute swimming exercise was born from the recognition that stress factors are minimized in animals (McDonald et al, 1986). Swimming exercises were performed in a heat resistant (polyethylene) swimming pool. Experimental animals were sacrificed immediately after swimming exercise, and testicular, kidney, heart and liver tissue samples were taken and stored at -35°C until the time of analysis. Zinc, iron, calcium, magnesium, copper, selenium, molybdenum and boron levels were then determined in tissue samples by atomic emission method (ICP-AES; Varian Australia Pty LTD, Australia). International standards, which are valid criteria, were taken into account in analysis determinations (NIST, 1996).

Statistical Evaluations

The statistical evaluation of the findings was made with the SPSS 21.0 computer package program, and the arithmetic mean and standard deviation of all parameters were calculated. According to the Shapiro-Wilks test applied to the data, it was determined that the data showed a normal distribution. One-way Analysis of Variance was used to detect intergroup differences, and Least Significant Difference (LSD) test was used to determine which group the difference originated from. Differences at the $P < 0.05$ level were considered significant.

RESULTS

Element Levels in Testicular Tissue of Study Groups

In this study, no significant difference was found between the zinc, iron, calcium, magnesium, copper, selenium, molybdenum and boron values in the testicular tissue of the study groups (Figures, 1-2).

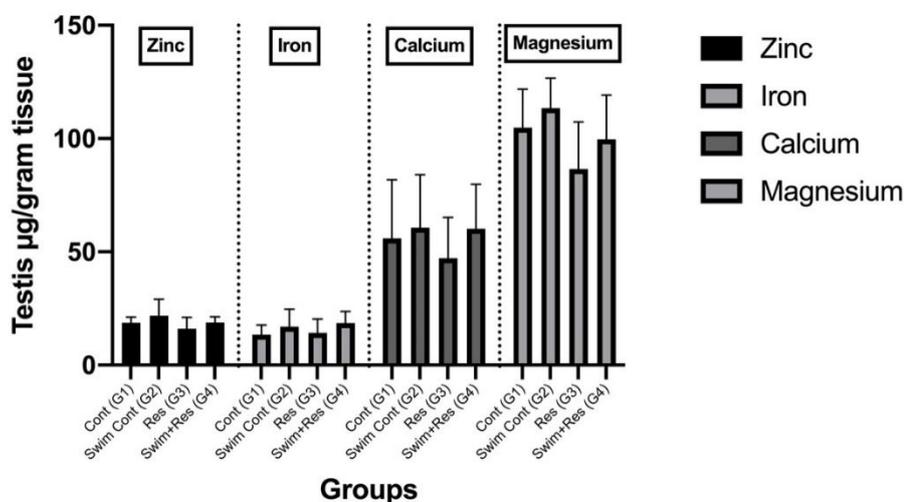


Figure 1. Zinc, iron, calcium and magnesium values in testicular tissue of groups

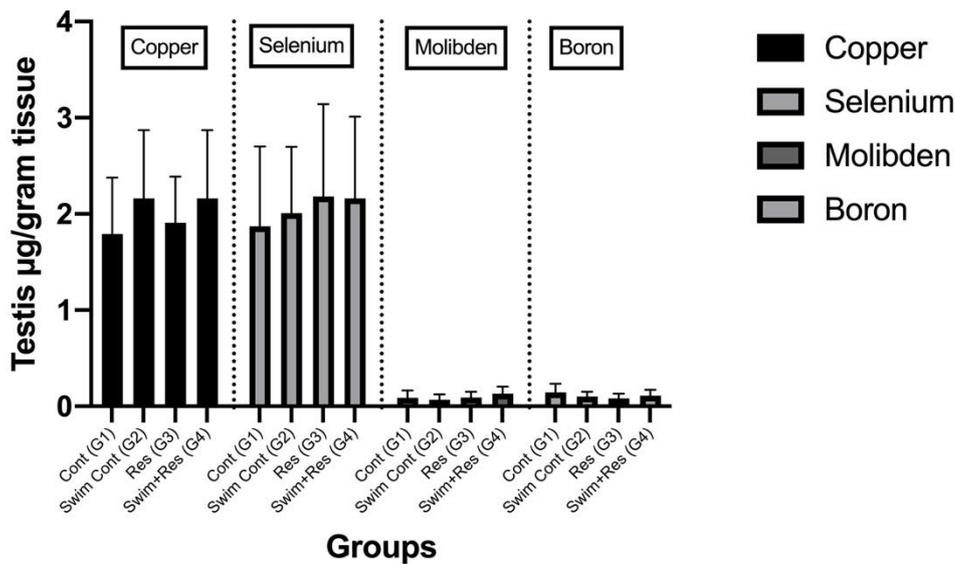


Figure 2. Copper, selenium, molibden and boron values in testicular tissue of groups

Element Levels in Kidney Tissue of Study Groups

The highest zinc values in kidney tissue were obtained in Group 1 (control) ($p < 0.05$). Kidney iron levels were higher in Group 1 (control) than all other groups ($p < 0.05$), while it was lowest in the resveratrol-administered swimming group (G4) ($p < 0.05$). The highest magnesium values were obtained in the control group (G1) ($p < 0.05$). The kidney magnesium levels of the swimming control group (G2) were found to be higher than the swimming group (G4) administered resveratrol and Group 3 receiving only resveratrol supplementation ($p < 0.05$). No significant difference was found between the calcium levels in the kidney tissue of the study groups (Figure 3).

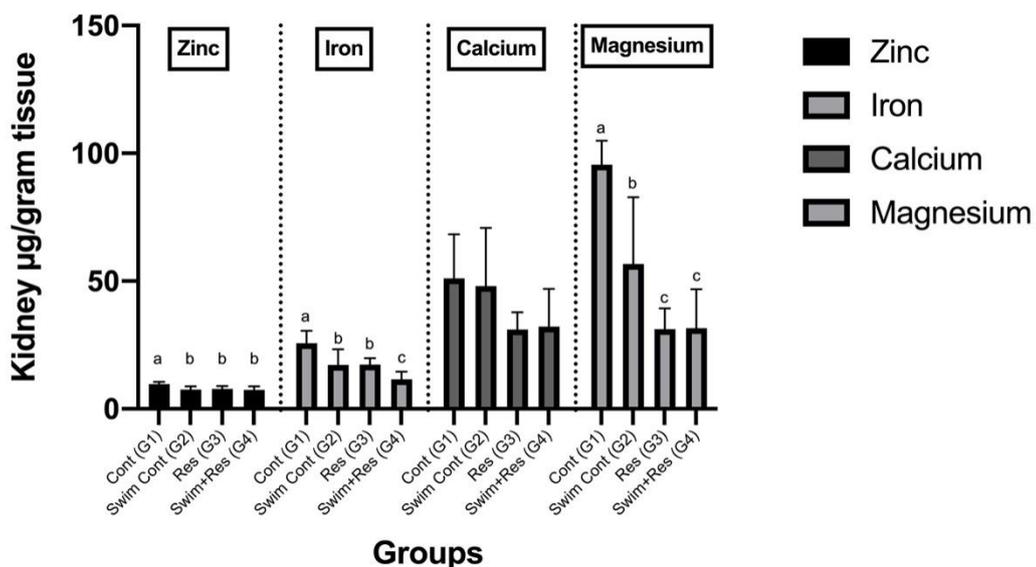


Figure 3. Zinc, iron, calcium and magnesium values in kidney tissue of groups

*Means with different superscripted letters in the same column are statistically significant $a > b > c$ ($p < 0.05$).

The lowest selenium levels in the kidney tissue were obtained in the swimming group (G4) treated with resveratrol ($p < 0.05$). The highest molybdenum levels in kidney tissue were obtained in swimming control (G2) ($p < 0.05$), and the lowest molybdenum values in resveratrol administered (G3) group ($p < 0.05$). Kidney copper and boron values of the study groups were not different from each other (Figure 4).

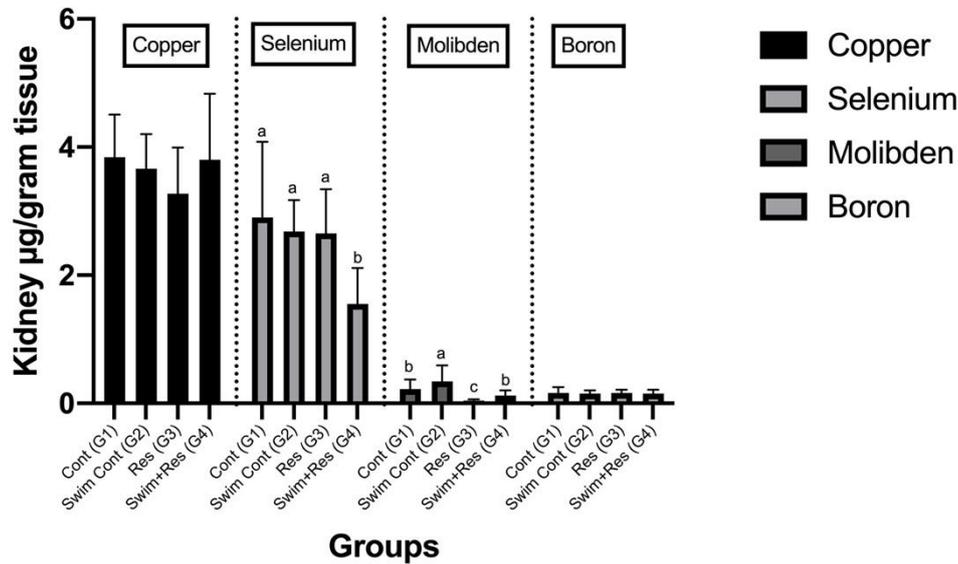


Figure 4. Copper, selenium, molibden and boron values in kidney tissue of groups
*Means with different superscripted letters in the same column are statistically significant $a > b > c$ ($p < 0.05$).

Element Levels of Study Groups in Heart Tissue

The highest iron parameter in the heart tissue was obtained in the swimming group (G4) ($P < 0.05$), in which resveratrol was applied, and the highest calcium values were obtained in the swimming control (G2) group ($p < 0.05$). Zinc and magnesium values in the heart tissue of the groups were not different from each other (Figure 5).

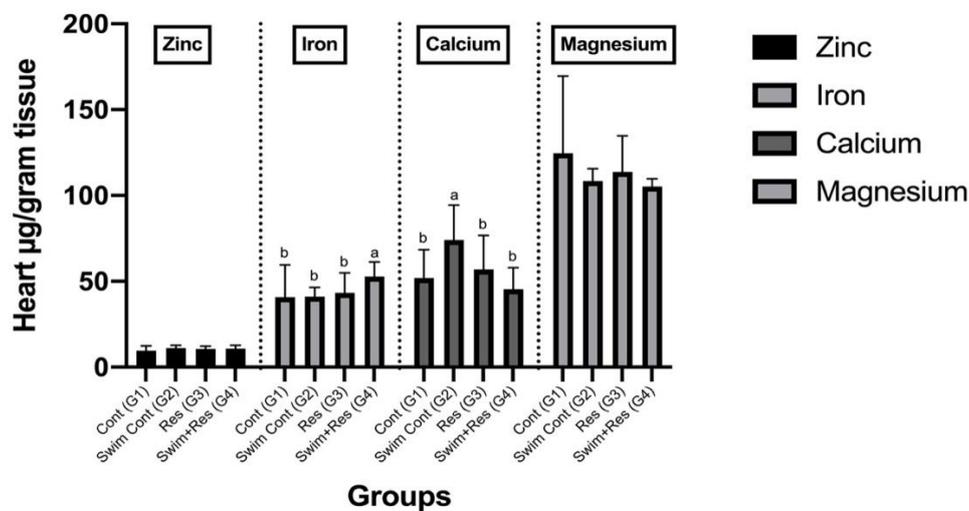


Figure 5. Zinc, iron, calcium and magnesium values in heart tissue of groups
*Means with different superscripted letters in the same column are statistically significant $a > b$ ($p < 0.05$).

Copper, selenium, molybdenum and boron values in the heart tissue of the groups were not different from each other (Figure 6).

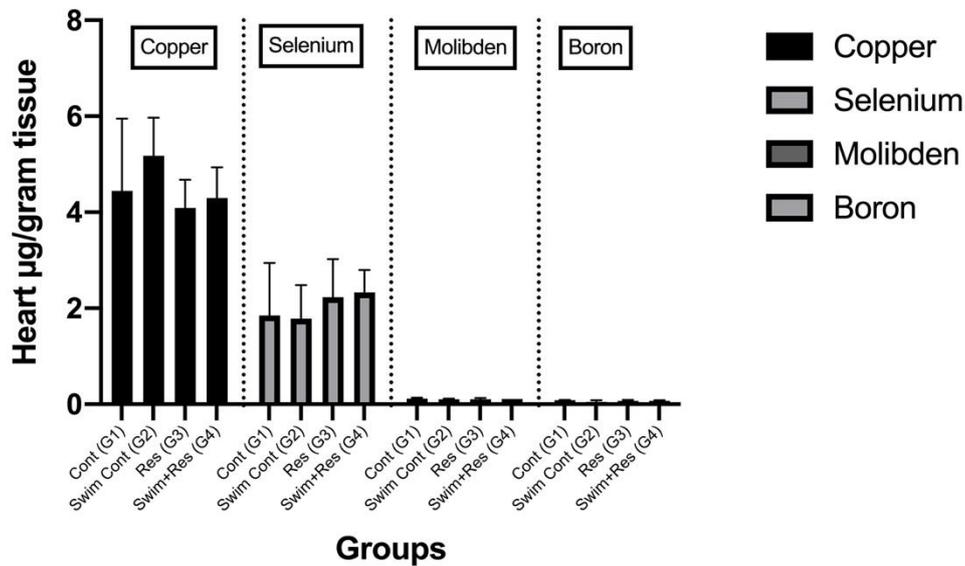


Figure 6. Copper, selenium, molibden and boron values in heart tissue of groups

Element Levels in Liver Tissue of Study Groups

The highest zinc levels in liver tissue were obtained in the resveratrol administered group (G3) ($p < 0.05$). The liver iron values of the resveratrol-administered swimming (G4) and resveratrol-only group (G3) were significantly higher than the control (G1) and swimming control (G2) groups ($p < 0.05$). There was no significant difference between the calcium and magnesium values in the liver tissue of the study groups (Figure 7).

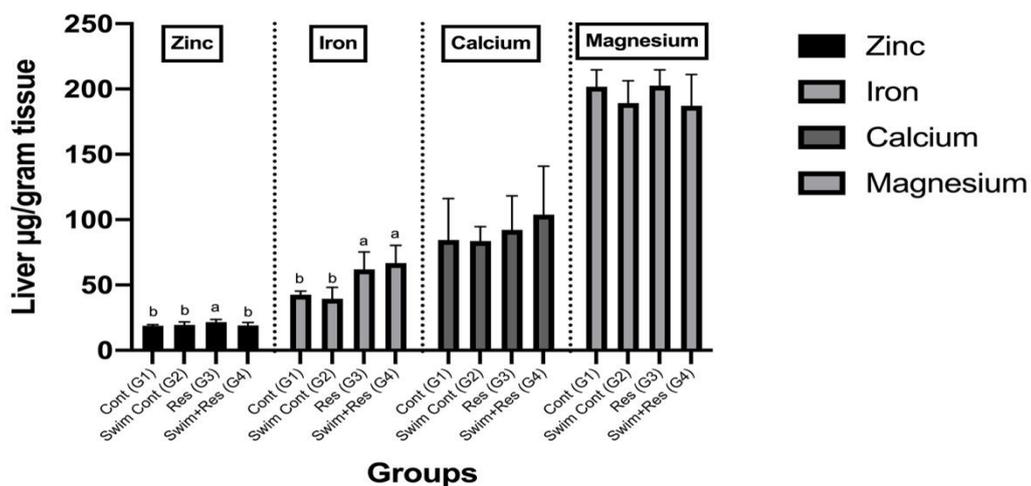


Figure 7. Zinc, iron, calcium and magnesium values in liver tissue of groups

*Means with different superscripted letters in the same column are statistically significant $a > b$ ($p < 0.05$).

While the molybdenum levels in the liver tissue of the resveratrol-administered (G3) group were higher than the other groups ($p < 0.05$), there was no significant difference between the copper, selenium and boron values in the liver tissue of the study groups (Figure 8).

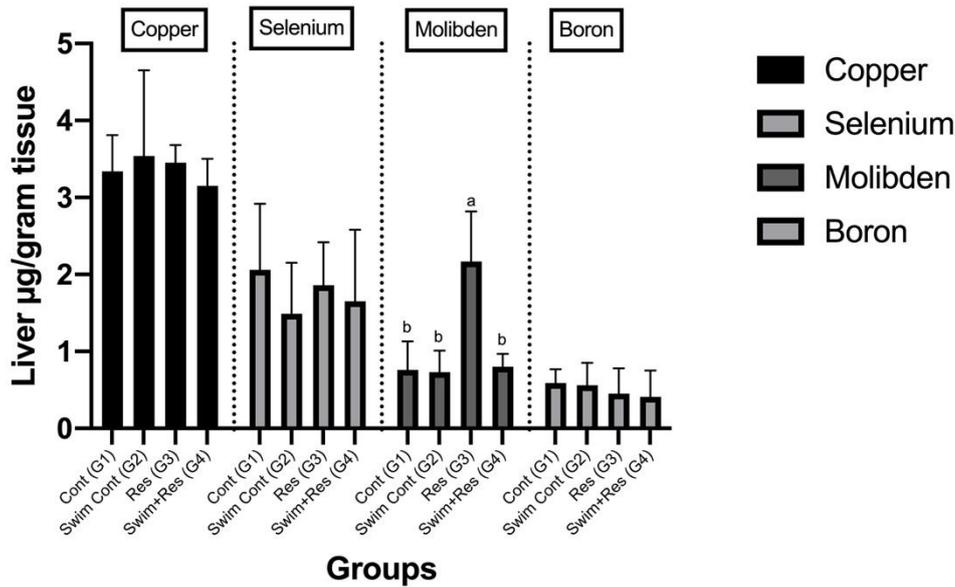


Figure 8. Copper, selenium, molibden and boron values in liver tissue of groups
 *Means with different superscripted letters in the same column are statistically significant a>b (p<0.05).

DISCUSSION and CONCLUSION

Discussion of Element Levels in Testicular Tissue of Study Groups

In this study, no significant difference was found between the zinc, iron, calcium, magnesium, copper, selenium, molybdenum and boron values in the testicular tissue of the study groups. The balance of elements in blood and tissues can be affected by many factors. Physical activity and exercise are one of these factors (Ghio et al., 2021). However, it is not clear how element metabolism in testicular tissue is affected by exercise. Various dietary practices have been shown to affect elemental metabolism in testicular tissue. It has been shown that the testicular tissue is significantly affected by the application of melatonin and zinc, and as a result, changes in the element levels in the testis tissue occur (Kaya et al., 2006; Ozturk et al., 2003). In this study, it can say that the levels of the above-mentioned elements in the testicular tissue are not affected by both exercise and resveratrol administration.

Discussion of Element Levels in Kidney Tissue of Study Groups

In this study, zinc levels of the application groups (swimming control, resveratrol applied swimming and resveratrol group) were found to be significantly lower than the control group. Exercise caused an increase in zinc levels in the kidney tissue of diabetic rats (Cordova, 1994; Sivrikaya et al., 2012). It has been reported that zinc levels in the kidney tissue of non-diabetic rats undergoing acute swimming exercise were not different from controls (Sivrikaya et al., 2012). The results of the above-mentioned reports do not support the low zinc values we obtained in the treatment groups in our study. However, the reporting that a one-year swimming

training resulted in a decrease in zinc levels in kidney tissue in aged rats (Kuru et al., 2003) is consistent with the low zinc values we obtained in the treatment groups. In our study, we obtained the lowest kidney iron levels in the swimming group treated with resveratrol. The iron levels in the kidney tissue of the swimming control and resveratrol-only groups were higher than the resveratrol-administered swimming group, but significantly lower than the control group. The fact that Navas et al., (2000) showed that exercise performed until fatigue in rats reduces iron concentrations in the kidney tissue is an important report supporting the low iron levels we achieved in the treatment groups compared to the control group in our study. Renal selenium levels of the swimming group treated with resveratrol were significantly lower than all other groups. It has been suggested that increased urinary excretion of selenium during exercise may result in a performance-limiting effect (Sivrikaya et al., 2012). Considering this aspect, it can be said that resveratrol application reduces the selenium levels in the kidney tissue and prevents urinary excretion of selenium, and as a result, it can be beneficial for sportive performance. Despite the reports stating that the kidney magnesium levels did not change in swimming exercise (Kaptanoglu et al., 2003; Sivrikaya et al., 2012), the kidney magnesium values of the applied groups were found to be significantly lower than the control group. Interestingly, the magnesium levels in the kidney tissue of the swimming control group were higher than the resveratrol-administered groups with or without exercise. This result shows that resveratrol administration can decrease the urinary excretion of magnesium by decreasing the magnesium values in the kidney tissue. In the deficiency of magnesium, which plays an important role in mitochondrial energy production and mitochondrial oxidative metabolisms and protein synthesis, the tendency to oxidative damage and cardiovascular diseases increases (Kharb & Sing, 2000). Therefore, there is an important relationship between magnesium and physical activity. In the absence of magnesium, which plays a role in the activities of enzymes involved in energy metabolism, the activities of related enzymes decrease (Wells, 2008) and as a result, physical performance is adversely affected. The decrease in urinary excretion of magnesium in resveratrol administered groups can be considered as an effect in favor of exercise, and this result we obtained suggests that resveratrol has a performance-enhancing effect in exercise (Mercken et al., 2012; Schrauwen & Timmers, 2014). In this study, it was obtained the highest kidney molybdenum levels in swimming control and the lowest molybdenum values in sedentary rats administered only resveratrol. Renal molybdenum levels of the swimming group treated with resveratrol were not different from the control group. Publications on the relationship between molybdenum and exercise are very limited. It has been shown that exercise performed until fatigue at night causes a significant increase in serum molybdenum values (Patlar et al., 2014). This finding indirectly supports the high molybdenum values get obtained during exercise in kidney tissue. However, it was determined that only resveratrol administration significantly reduced molybdenum values in kidney tissue. There was no any studies investigating the relationship between resveratrol and molybdenum in Med-line searches. However, this study shows that there may be a relationship between resveratrol and molybdenum in kidney tissue, and that only resveratrol application reduces the molybdenum level in kidney tissue and turns the molybdenum values that increase with exercise into control values.

Discussion of Element Levels in Heart Tissue of Study Groups

In this study, it was found the highest iron levels in the heart tissue in the swimming group to which resveratrol was applied, and the highest calcium values in the swimming control group. Apart from this, all of the measured parameters did not differ between the groups. It has

been reported that exercises of various intensity affect the distribution of iron in the blood and tissues, while moderate exercise increases the absorption of iron in the small intestine, while high-intensity exercise may have negative effects on both body iron and absorption of iron from the digestive system (Liu et al., 2006). In the study that carried out, obtained the highest iron values in the heart tissue in the swimming group in which resveratrol was applied. Studies investigating the relationship between resveratrol and iron are limited and mostly focus on iron concentration in tumoral events. Skrajnowska et al., (2013) found a decrease in iron levels in rats in which DMBA developed breast cancer, in the groups with or without resveratrol administration, while they obtained higher iron levels in the control groups than in the cancerous groups. Skrajnowska et al., (2013) show that resveratrol administration has no effect on iron levels in the blood. However, the above-mentioned study deals with serum iron levels in animals with breast cancer and is quite different from our study. This study shows that resveratrol administration increases iron levels in heart tissue, at least in rats undergoing acute swimming exercise. In this study, it was obtained the highest calcium values in the heart tissue in the swimming control group. The results of studies on the effect of exercise on calcium levels are inconsistent. Considering the exercise-calcium relationship, the findings of Maughan (1999), who pointed out that very vigorous exercise may increase calcium loss, do not support the high calcium values got obtained in the heart tissue. However, the high calcium values obtained in both an exercise until fatigue at night (Patlar et al., 2014) and acute swimming exercise (Sivrikaya et al., 2012) are consistent with present study findings.

Element Levels in Liver Tissue of Study Groups

In the liver tissue analyzes of this study, the highest zinc and molybdenum values were obtained only in the resveratrol administered (G4) group. It could not find studies on the effect of resveratrol application on zinc and molybdenum values in liver tissue in Med-line scans. Skrajnowska et al., (2013) showed that the combined application of resveratrol and copper significantly suppressed serum zinc levels in rats fed a standard diet in which they developed breast cancer by applying 7,12-dimethyl-1,2-benz[a]anthracin (DMBA). In their study, Skrajnowska et al. (2013) applied resveratrol not alone, but in combination with copper. While providing dietary element support, attention is drawn to the balance between zinc and copper (Cordova & Navas, 1998). It has been reported that high-dose zinc in the diet may impair copper absorption (Lukaski, 1989), while high-dose copper may impair zinc absorption (Fischer et al., 1984). The low zinc values obtained in the study of Skrajnowska et al., (2013) probably appear as a result of copper application rather than resveratrol application. There are very few publications investigating the relationship between molybdenum and exercise, and they are mostly related to the blood values of this element. It has been shown that exercise performed until fatigue at night causes an increase in molybdenum levels (Patlar et al., 2014). In this study, only resveratrol application increased molybdenum levels in liver tissue. Patlar et al., (2014) report that exercise causes an increase in molybdenum levels is partially consistent with our results. At least, the results of this study show that resveratrol administration causes an increase in liver zinc and molybdenum levels. This finding it was obtained can be considered as an original result, especially in the relationship between resveratrol and zinc.

The highest iron values in the liver tissue were detected in swimming (G3) and only resveratrol-administered (G4) groups. Many researchers have reported that an acute exercise causes an increase in iron values in liver tissue (Bicer et al., 2015; Navas & Cordova, 2000;

Sivrikaya et al., 2013). The increased liver iron get obtained in the swimming group treated with resveratrol in this study is consistent with the findings of the researchers whose reports were presented above. In this study, it was also showed that only resveratrol administration caused an increase in liver iron levels. Bobrowska-Korczak et al., (2012) showed that the combined application of resveratrol and zinc in rats that developed breast cancer by applying DMBA could not prevent increased iron levels in the mammary tissue. Although the findings of Bobrowska-Korczak et al., (2012) reflect a study in a tumoral event, they may be important, at least in that they demonstrate that the combination of resveratrol and zinc does not affect increased iron levels in breast tissue. However, the most important problem that hinders the comparison with this study here is that resveratrol application is not applied alone but together with zinc application. Zhao et al., (2015) reported that resveratrol administration may have a protective effect on iron losses in bone tissue in osteoporosis. In this study, the high iron levels that we obtained with only resveratrol application in the liver tissue are partially compatible with the findings of Zhao et al., (2015).

CONCLUSION

The findings of this study, which revealed that resveratrol administration caused changes in element metabolism in some tissues of rats both during and independently of exercise, can be presented as the first and original finding based on med-line scans. Based on the findings obtained in the current study, it can be said that resveratrol supplementation may have beneficial effects on bone metabolism and performance for both sedentary individuals and athletes.

Conflict of interest: The authors declare that they have no potential conflicts of interest to disclose.

Author contributions: DA, SBB, OU made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work; RM, and AKB drafted the work or revised it critically for important intellectual content, approved the version to be published. The authors declare that all data were generated in-house and that no paper mill was used.

Research Data Policy and Data Availability Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Funding: This study was supported by the Scientific Research Projects Coordinatorship of Selcuk University (SUBAPK; project no. 1420132013).

Compliance with Ethical Standards: This study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Experimental Animals Ethics Board of Necmettin Erbakan University's Kombassan Experimental Medicine Research and Application Center (2013-183). This research was performed on the animals (rat).

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