

Original Research

Effect of resveratrol administration on muscle glycogen levels in rats subjected to acute swimming exercise

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Abstract: The present study aims to examine how resveratrol administration affects muscle glycogen levels in rats subjected to an acute swimming exercise bout. The study was conducted on adult male rats of Wistar-Albino. The 28 rats used in the study were equally divided among four groups: Group 1, Control Group: The group fed on a standard diet and not subjected to any procedure. Group 2, Control Swimming Group: The group fed on a standard diet and subjected to an acute swimming exercise bout for 30 minutes at the end of the study. Group 3, Resveratrol Group: The group fed on a standard diet and given (10 mg/kg) resveratrol in drinking water for four weeks. Group 4, Resveratrol + Swimming Group: The group fed on a standard diet, given (10 mg/kg) resveratrol in drinking water for four weeks and subjected to a 30-minute acute swimming exercise at the end of the study. At the end of the four weeks, the animals were decapitated, muscle glycogen levels using immunohistochemical method. The highest muscle glycogen levels were obtained in the resveratrol-administered Group 3 and the lowest levels in group 2 (swimming group) ($p < 0.05$). The results of the study demonstrate that resveratrol support had a protective and/or regulatory effect on muscle glycogen in both exercised and not-exercised rats.

Key words: Swimming exercise; Resveratrol; Leptin; Glycogen; Rat.

Introduction

Resveratrol (3,4,5-trihydroxystilbene) is a natural antioxidant in the form of polyphenol, which is abundant in grape grains (1). Clinical and experimental studies have shown that resveratrol inhibits platelet aggregation (1, 2), and protects tissues from harmful effects of ischemia (2). Although resveratrol has been shown to have antioxidant, anticancer, estrogenic, antiplatelet, protective for ischemia-reperfusion injury, antiinflammatory and antimicrobial activities (1-5), studies investigating its relationship between exercise and performance are quite limited.

Attention is drawn to the fact that resveratrol can improve muscle performance in physical activity (6). It has been reported that resveratrol administration has positive effects on renewal of liver cell and so this application may also affect hepatic glycogen positively (7). In the study investigating the relation of resveratrol with muscle fatigue and performance by Wu et al; it has been pointed out that resveratrol administration has a regulatory effect on glucose metabolism and it may delay fatigue by reducing blood lactate levels (8). It has also been pointed out that resveratrol administration decrease lactate levels (9) and delays muscle fatigue in elderly rats which were subjected to swimming exercise (10). Similarly, when compared with the control group, 15 mg / kg / day of resveratrol administration for 4 weeks in 18-month-old rats was found to suppress

oxidative stress parameters in the gastrocnemius muscle and reduce blood lactate levels (11).

In many cases, studies on experimental animals show that resveratrol may result in a performance enhancing effect by activating the molecular pathways during exercise (12,13,14). Conversely, human studies suggest that resveratrol may prevent beneficial effects of exercise on certain patient groups (15,16). In conclusion, the effects of resveratrol on exercise are controversial and require investigation (17). For this reason, it can be said that there is an increasing interest in the research of resveratrol and exercise relation (17). The purpose of this study is to investigate how resveratrol affects muscle glycogen levels in rats subjected to acute swimming exercise.

Materials and Methods

Animal material and groups

This study was carried out at Necmettin Erbakan University, Experimental Medical Application and Research Center, and the rats were obtained from the same center. The study protocol was approved by the ethics committee of the same center. A total of 28 Wistar-Albino genius rats were divided into 4 groups of equal number.

Group 1, (n:7) Control group: The group fed on a standard diet and not subjected to any procedure.

Group 2, (n:7) Swimming Group: The group fed on a standard diet and subjected to an acute swimming exer-

cise bout for 30 minutes at the end of the study.

Group 3, (n:7) Resveratrol Group: The group fed on a standard diet and given (10 mg/kg) resveratrol in drinking water for four weeks.

Group 4, (n:7) Resveratrol+Swimming Group: The group fed on a standard diet, given (10 mg/kg) resveratrol in drinking water for four weeks and subjected to a 30-minute acute swimming exercise at the end of the study.

Experimental animals

Experimental animals were fed in special steel cages which were cleaned daily by washing. The animals were fed about 10 g of food per 100 g of body weight each day. Resveratrol administrations were performed on rats forming groups 3 and 4 in drinking water as 10 mg / kg / day per rat. The study lasted four weeks and at the end of the study, muscle tissue specimens from decapitated animals were taken between 09:00 and 10:00 in the morning.

Swimming exercise

The exercise was carried out in a heat-resistant (polyethylene) swimming pool with a length of 80 cm and a height and width of 50 cm. Exercises were performed after 24 hours by the end of resveratrol administration, in groups of two for 30 minutes.

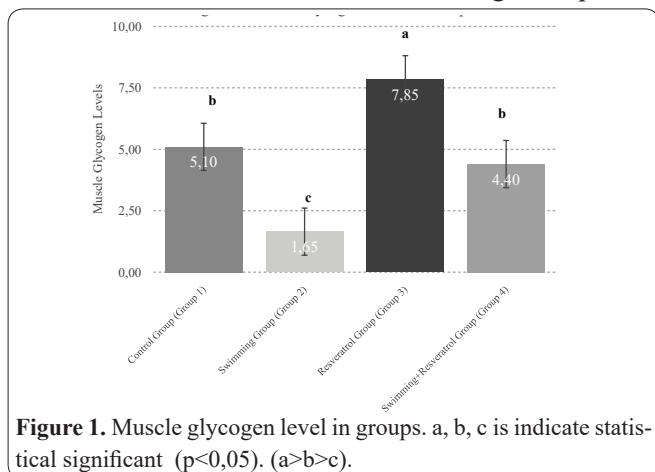
Analysis

Immunohistochemical analysis of glycogen in muscle tissue

Muscle tissue samples were fixed in 95% ethyl alcohol. Following auto-technicon analysis, the tissue samples were buried into paraffin, from which 5 μm cross-sections were obtained and stained with PAS. Stained preparations were evaluated under a Nikon Eclipse 400 light microscope. Using the Nikon Coolpix 5000 digital camera, digital images of relevant sites were obtained. In order to ensure calibration during photographing, Nikon Stage Micrometer images were also produced at the same microscopic magnifications. All images were transferred to a PC and evaluated using Clemex PE 3.5 image analysis software. With the image analysis software, areas of 0.1 mm^2 were chosen. Muscle cells containing glycogen (positively stained with PAS) at these sites were tagged and automatically counted.

Statistical evaluations

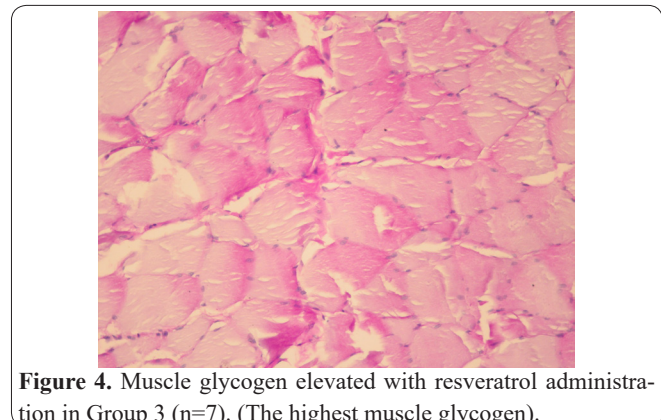
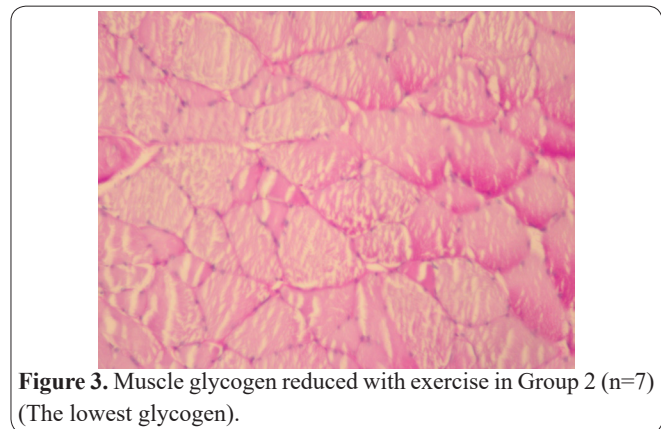
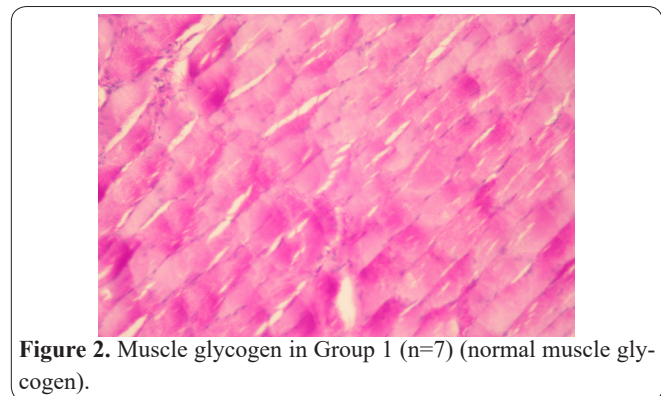
The statistical evaluation of the findings was performed



med with 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 normal distribution. One-way ANOVA was used to determine group differences, and Least Significant Difference (LSD) test was used to determine which group lead the difference. Differences in $p < 0,05$ were considered significant.

Results

Mean muscle glycogen levels for groups control, swimming, resveratrol, swimming+resveratrol were $5,10 \pm 0,96$, $1,65 \pm 0,03$, $7,85 \pm 1,80$, $4,40 \pm 1,20$ (Figure 1). In our study, the highest muscle glycogen levels were obtained in resveratrol group (group 3) ($p < 0,05$) and the lowest muscle glycogen levels were obtained in group 2 (swimming group). There was no significant difference in muscle glycogen levels between control group (group 1) and resveratrol+ swimming group (group 4) (Figures 2, 3, 4,5).



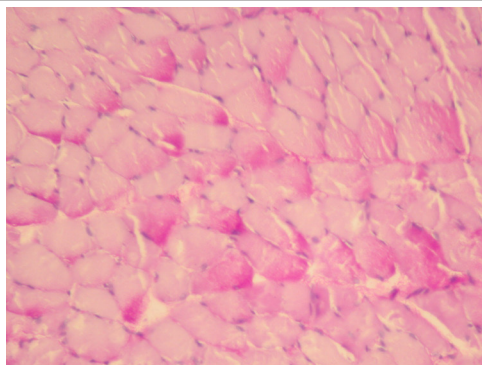


Figure 5. Muscle glycogen protected by resveratrol administration in exercise Group 4 (n=7).

Discussion

Even though limitedly, it has been reported that resveratrol administration can alter normal training response, including glycogen content, in people who are exercised (6). It has also been suggested that resveratrol should be regarded as a pharmacological agent which may prevent fatigue especially in physical activity (8). This information is interesting. If so resveratrol administration may be critical to improve physical performance.

In our study, the lowest muscle glycogen levels were obtained in group 2 (swimming group). Muscle tissues require considerable amount of energy (ATP) for contraction function during physical activity (17). For this reason, decreased muscle glycogen levels in Group 2 (swimming control) can be considered as a consequence of acute swimming exercise. The important result here is that the muscle glycogen levels of the rats subjected to resveratrol administration (group 4) were higher than those of group 2 (swimming group). This finding suggests that resveratrol administration may protect muscle glycogen deposits which reducing during swimming exercise. The required energy during physical activity (ATP) can be produced by muscle mitochondria with oxidative phosphorylation. In fact, one of the muscle's useful adaptations for training is increased mitochondrial function and content (18, 19). Similar to exercise, resveratrol provides mitochondrial biogenesis in skeletal muscle, cardiac tissues and endothelial cells (20, 21, 22). Based on the reports above, we can say that resveratrol supplementation in rats subjected to swimming exercise has preserved and / or regulated muscle glycogen levels when compared with swimming control rats. Baltaci *et al.* (2107) investigated how resveratrol administration affected the metabolism of elements in blood and brain tissue on rats subjected to acute swimming exercise. In that study it has been shown that both the swimming exercise and the resveratrol-applied swimming exercise lead to changes in the distribution of elements in the blood and tissues of the rats, and it has been concluded that resveratrol regulate element metabolism in exercise.

In the study we performed, the highest muscle glycogen was obtained in the resveratrol group (group 3). This finding suggests that, independently from exercise, resveratrol administration may contribute to the regulation of glycogen in muscle tissue. One of the limited studies on the relationship between resveratrol and exercise in terms of resemblance to our study is

the study of Duran *et al.* (2015), which is published as a summary of the results, on how resveratrol administration affects plasma leptin and liver glycogen levels in rats subjected to acute swimming exercise. Resveratrol administration in rats with and without exercise did not lead to a change in plasma leptin levels, and resveratrol administration in rats subjected to acute swimming exercise prevented reduction in liver glycogen. This finding suggests that resveratrol has a protective effect on liver glycogen. This study shows a strong parallelism with the findings we obtained in our investigation of the relationship between resveratrol and muscle glycogen.

In conclusion; the findings of the present study demonstrates that resveratrol administration may have protective and regulatory influence on muscle glycogen in rats (both exercising and non-exercising). The results of our study were the first to investigate the relation of resveratrol-muscle glycogen and exercise in rats.

Ethical guidelines

Research meets ethical guidelines is a required field.

Conflict of interest

Authors declare no conflict of interest.

Acknowledgments

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