# EXPERIMENTAL STUDY

# The effect of selenium supplementation on elements distribution in liver of rats subject to strenuous swimming

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Abstract: The present study aims to explore how selenium supplementation affects the element distribution in the liver tissue of rats subjected to strenuous swimming exercise. Thirty-two Spraque-Dawley male rats were equally divided into the four groups: Group 1, normal control group. Group 2, selenium-supplemented, nonswimming (0.6 mg/kg/day sodium selenite) group. Group 3, swimming, no supplementation group. Group 4, swimming, selenium-supplemented (0.6 mg/kg/day sodium selenite) group. After one month, the animals were decapitated and liver tissue samples were collected to determine the levels of lead, cobalt, boron, molybdenum, chromium, sulfur, magnesium, sodium, potassium, phosphorus, copper, iron, zinc and selenium. The chromium, molybdenum, iron, sodium and potassium values were higher in the swimming groups, relative to controls. Group 3 had significantly lower lead levels (p<0.001). The highest cobalt levels were obtained in the Group 1 and that of the Group 2 was higher than in the Groups 3 and 4. The boron values in the Group 3 were higher than those in all other groups. The copper and magnesium levels were higher in the Groups 3 and 4, compared to the Groups 1 and 2. The highest phosphorus levels were found in the Group 1. The highest selenium and zinc values were obtained in the Group 2 and those of the Group 4 were higher than in the Groups 1 and 3. Group 1 had higher selenium and zinc levels than the Group 3. The results of the present study demonstrated that selenium-supplemented rats subjected to strenuous swimming exercise had distinct elements distribution in liver tissue. Also, selenium supplementation offsets the decrease in zinc levels in rats subjected to vigorous swimming (Tab. 3, Ref. 20). Full Text in PDF www.elis.sk.

Key words: selenium, exercise, liver, elements distribution, rat.

Selenium (Se) is an essential trace element that is required for normal development of human and animal organisms (1,2). Our knowledge of the short- and long-term functional roles of selenium in human health is growing daily (3). Selenium is known to be necessary for metabolic processes, including immune functions, plays a role in thyroid hormone metabolism, and is protective against oxidative stress (3). An adult human body contains about 20 mg Se, concentrated particularly in muscle tissue, liver and kidneys (4). The daily amount of selenium intake, recommended by health organizations, is 70 µg for adult males and 55 µg for females. The daily selenium needs increase during childhood and pregnancy (5).

Changes in body Se concentration directly influence glutathione peroxidase, a selenium-containing enzyme that binds about 12 % of the total Se in plasma and is involved in antioxidant mechanisms that prevent oxidative damage (6-9).

Exhaustive physical exercise is known to lead to oxidative damage, probably by stimulating free radical production in many tissues, including the muscle, liver, heart and lungs in animals (10). Many researchers have focused on the relationship between nutrition and on development and maintaining performance. Two methods are commonly used to determine the correlation between physical activity and nutrition. The first is to administer various foods to subjects engaged in physical activity and then examine their physiological and performance responses. A second approach consists in establishing the effects of physical activity on nutrition (11, 12).

The interest in researching the relationships between exercise and major- and trace element status continues to grow (13). In the present study we report the effect of selenium supplementation on the distribution of several elements in the liver tissue of rats subjected to acute swimming exercise.

## Materials and methods

#### Animal material and groups

This study was conducted on 32 Spraque-Dawley type adult male rats obtained from the Experimental Medicine Application and Research Center of Selcuk University and performed at the Experimental Animals Unit of Selcuk University, Faculty of Veterinary Medicine. The Ethics Committee of Selcuk University approved the study protocol.

The experimental animals used in the study were equally divided into four groups, as follows:

Group 1: General control group where no procedure was applied. Group 2: Selenium-supplemented control group. The rats were

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1	lab.	1. (	Throm	um,	moly	bdenun	, iron,	, sodium	and	potassium	levels*	(mg/l	) in live	er tissue	e of rat	ts in the	e four stu	idy gro	oups (n =	= 8 each)	•
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Groups	Chromium	Molybdenum	Iron	Sodium	Potassium
1 – Controls	0.38±0.12b	0.14±0.09b	81.09±11.95 <sup>b</sup>	334.0±96.7b	91.5±76.4 <sup>b</sup>
2 - Selenium Supplemented, Non-Swimming	0.32±0.14b	0.18±0.12 <sup>b</sup>	87.54±14.20 <sup>b</sup>	337.8±98.7 <sup>b</sup>	107.1±89.8 <sup>b</sup>
3 - Swimming Controls	1.71±0.27 <sup>a</sup>	0.85±0.22ª	188.19±46.44 <sup>a</sup>	893.0±172.4ª	734.9±171.1ª
4 - Selenium Supplemented Swimming	$1.38{\pm}0.35^{a}$	$0.77 \pm 0.18^{a}$	$197.61 \pm 51.50^{a}$	868.4±151.3ª	$873.2 \pm 187.7^{a}$

\*Means with different superscripted letters in the same column are significantly different (p<0.001).

Гаb. 2.	Levels of	f lead,	, cobalt, p	hospho	orus and	sulf	fur (mg	/l) iı	1 liver	tissue o	f rats	in tl	he f	iour stuc	ly groups (	(n = 8	8 each	ı).
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Groups	Lead	Cobalt	Phosphorus	Sulfur
1 – Controls	2.66±1.25ª	1.85±0.46 <sup>a</sup>	935±129ª	1138±135
2 - Selenium Supplemented, Non-Swimming	2.79±1.34ª	1.30±0.41 <sup>b</sup>	455±130 <sup>b</sup>	1196±142
3 – Swimming Controls	1.50±0.82 <sup>b</sup>	0.95±0.29°	469±145 <sup>b</sup>	1250±155
4 - Selenium Supplemented Swimming	2.98±1.85ª	0.98±0.23°	461±155 <sup>b</sup>	1132±166

\*Means with different superscripted letters in the same column are significantly different (p<0.001).

#### Tab. 3. Levels of copper, magnesium, boron, zinc and selenium (mg/l) in liver tissue of rats in the four study groups (n = 8 each).

Groups	Copper	Magnesium	Boron	Zinc	Selenium
1 – Controls	$0.54{\pm}0.08^{b}$	110.45±28.47 <sup>b</sup>	0.39±0.18b	3.19±0.56°	0.94±0.15°
2 - Selenium Supplemented, Non-Swimming	0.62±0.11b	102.60±25.50b	0.45±0.12b	5.35±0.80ª	2.18±0.36 <sup>a</sup>
3 – Swimming Controls	3.56±0.49ª	227.49±45.85ª	1.30±0.55ª	2.11±0.32 <sup>d</sup>	0.37±0.11 <sup>d</sup>
4 - Selenium Supplemented Swimming	$3.50{\pm}0.37^{a}$	229.27±48.37ª	$0.40{\pm}0.17^{b}$	4.18±0.75 <sup>b</sup>	1.56±0.24 <sup>b</sup>

\*Means with different superscripted letters in the same column are significantly different (p<0.001).

supplemented with 0.6 mg/kg intraperitoneal sodium selenite/day for 4 weeks.

Group 3: Swimming control group. The rats were subjected to a 30-minute acute swimming exercise.

Group 4: Selenium-supplemented swimming group. Rats that were subjected to 30-min strenuous swimming and treated with Se as described for group 2.

The rats were fed with standard rat pellet (10 g pellet per 100 g body weight) and allowed to drink tap water, ad libitum. They were kept in an environment with 12 hour/12 hour dark cycles and standard room temperature ( $21\pm1$  °C). All the injections to rats in the Groups 2 and 4 were given at 9.00 a.m. for four weeks. At the end of the experimental period, all animals were decapitated under ether anesthesia to collect liver samples to be used for determination of the chromium, molybdenum, iron, sodium, potassium, lead, cobalt, phosphorus, sulfur, copper, magnesium, boron, zinc and selenium levels.

## Experimental procedures

Sodium selenite supplementation

Sodium selenite (Natriumselenite-Pentahydrate, Merck KGaA 64271 Dramstadt, Germany), was dissolved in distilled water and diluted in saline so that a 0.5 ml intraperitoneal injection would provide 0.6 mg/kg sodium selenite/day to each rat in the Groups 2 and 4.

#### Swimming exercise

The exercise was performed in a heat-resistant glass swimming pool, 50 cm in length and width, provided with a thermostat that maintained the temperature fixed at 37 °C. The rats swam in pairs for 30 minutes only one time and then were immediately decapitated.

The concentrations of chromium, molybdenum, iron, sodium, potassium, lead, cobalt, phosphorus, sulfur, copper, magnesium, boron, zinc and selenium were determined in the liver tissue. In preparation for the assay, the liver tissue samples were put into capped, polyethylene tubes previously washed with  $HNO_3$  and rinsed several times with deionized water to prevent contamination, and stored at -35 °C until the analysis day.

For the analysis, the liver tissue was pounded into powder in a mortar, and its wet weight was recorded. The tissue was then digested with a 1:10 mixture of sulfuric and nitric acids in a closed system microwave oven (CEM – Marsx5) at 1.2 MPa pressure and 200 °C temperature for 20 minutes. Then the volume of each sample was adjusted to 25 ml with deionized water and the samples were read after waiting for 30-min using a ICP-AES located in the Department of Soil, Faculty of Agriculture, Selcuk University.

#### Statistical evaluations

The SPSS computer program was used for the statistical evaluation of the results. The results are reported as the arithmetic means and standard errors. Variance analysis was used to establish the differences between the groups. The Least Significant Difference Test was used to compare group means that were found to be statistically significant in the variance analysis. Differences, for which p<0.05, were considered as significant.

#### Results

The chromium, molybdenum, iron, sodium and potassium values in the liver tissues of the Groups 3 and 4 (swimming controls and supplemented swimming, respectively) were higher than in the non-swimming Groups 1 and 2 (p<0.001) (Tab. 1). The swimming controls (Group 3) had the lowest liver lead level (p<0.001). The highest cobalt and phosphorus levels were seen in the Group 1 (p<0.001), and cobalt was higher in the Group 2, relative to the Groups 3 and 4 (p<0.001). There were no significant differences for sulfur levels among any of the groups included in the study (Tab. 2).

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Group 3 had the highest boron values (p<0.001). The levels of copper and magnesium in swimming rats (Groups 3 and 4) were higher than in non-swimming Groups 1 and 2 (p<0.001). The highest selenium and zinc levels were observed in the Group 2 (p<0.001). Selenium was higher in the Group 4, followed by Groups 1 and 3 (p<0.001) (Tab. 3).

# Discussion

The levels of chromium, molybdenum, iron, sodium, potassium, copper and magnesium in the liver tissues of swimming groups were higher than in the non-swimming groups. These elevations might have resulted from the acute swimming exercise, but not selenium supplementation because the values were higher in swimming rats with or without administration of selenium supplements.

Physical activity is a known factor that influences the distribution of elements in the body (14). Intense and acute exercise is known to affect the distribution of elements in the body, possibly due to an increase in post-exercise element excretion through perspiration and urine and/or a change in the distribution of elements in the liver (15,16), which explains the elevation we found in the liver values of the elements mentioned above.

The levels of boron and lead in the liver were significantly lower in the swimming, untreated controls in comparison to all other groups. This may be a consequence of the distribution of elements between body stores, blood and tissue, following acute exercise (17). However, more important here is the absence of any changes in the selenium-supplemented swimming group, suggesting that selenium supplementation prevented the decrease in the levels of these elements.

The non-swimming groups showed higher cobalt levels than the two swimming groups and the general controls had higher levels of phosphorus than all the other groups. Similarly, phosphorus levels were higher in the Group 1, relative to all other groups. This result indicated that swimming resulted in lowering the levels of cobalt and perhaps also of phosphorous.

In the present study, the lowest selenium and zinc levels in the liver tissue seen in the swimming controls, were consistent with previous observations that both selenium and zinc in blood and tissues were decreased after an intense exercise (18, 19) and that joint supplementation of zinc and selenium to athletes (20) could contribute to their health and performance. However, the most salient result of our study is the elevations of zinc and selenium in the selenium-supplemented swimming group relative to the swimming controls. This result demonstrated that selenium supplementation to rats subjected to an acute swimming exercise brought about the increase not only of selenium, but also of zinc levels. An online search of the relevant literature did not produce any study about how selenium supplementation affects zinc levels. Thus, the elevated zinc levels we found in the selenium-supplemented group may prove to be an original finding.

In conclusion, the results of the present study indicated that an acute swimming exercise and selenium supplementation significantly change the element distribution in the liver tissue of rats. An important result of our study was that selenium supplementation to rats subjected to acute swimming exercise prevented the decrease of zinc levels.

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