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Effects of Zinc Deficiency and Supplementation on the Glycogen Contents of Liver and Plasma Lactate and Leptin Levels of Rats Performing Acute Exercise

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ABSTRACT

The aim of the present study was to investigate how zinc (Zn) deficiency and supplementation affect glycogen content of the liver and plasma lactate and leptin levels of rats performing acute swimming exercise just before the blood samples were obtained. Four sets of 10 rats each served as the (1) Zn-deficient group, (2) Zn-supplemented group, (3) swimming controls, and (4) normal controls. Plasma lactate levels of Zn-deficient animals were significantly higher than those in the other three groups ($p<0.01$), and those in the swimming controls (group 3) were significantly higher than in the Zn-supplemented animals, group 2 ($p<0.01$). The plasma glucose of the Zn-deficient group was significantly higher than all other groups ($p<0.01$) and that of group 2 was significantly lower than group 4 ($p<0.01$). Glycogen levels in liver of the Zn-deficient animals was significantly lower than groups 2 and 4 ($p<0.01$), and, in turn, were higher than for group 3 ($p<0.01$). The plasma leptin and Zn levels of group 1 were significantly lower than in all other groups ($p<0.01$). These results suggest that Zn deficiency exerts a negative influence in the above-mentioned parameters and that Zn supplementation has the opposite effect.

Index Entries: Exercise; glycogen; lactate; leptin; swimming; zinc.

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INTRODUCTION

Despite the well-known metabolic role of zinc (Zn), its effects on physical performance have not been fully established. Studies involving Zn and exercise are mainly concentrated on its distribution in tissues as a response to exercise *(1,2).* There are reports about decreases in plasma Zn following exercise *(3–5).* Low plasma Zn levels may result in a decrease of muscle Zn concentrations. Because several enzymes that are involved in energy metabolism require Zn as an active participant, a decrease in muscle Zn levels may result in diminished muscular endurance or performance *(6).* These results suggest that Zn may be a stimulating factor for functional changes in systems and tissues that are involved in pathogenesis of fatigue *(7).*

Leptin is a hormone synthesized and secreted by adipose tissues. It has a well-known role in the regulation of energy balance *(8).* It has been established that there is a positive correlation between Zn and leptin *(9–11).* Although there are some conflicting results, it appears that physical activity might change the secretion of leptin *(12–14).*

The present study was designed to investigate the effects of Zn deficiency and supplementation on plasma glucose, leptin, lactate, and liver glycogen levels in rats performing acute swimming exercise just before samples were obtained.

MATERIALS AND METHODS

The local ethics committee for animal experimentation approved the protocol of this study. A total of 40 male adult Spraque–Dawley rats were obtained from the Selcuk University Experimental Medicine Research Centre, where all experiments were performed. The animals were randomly divided into 4 groups of 10 animals each, as follows:

Group 1: The group of Zn-deficient rats was fed a diet containing 0.65 ppm Zn/g for 4 wk. They were subjected to 30-min acute swimming just before they were sacrificed to obtain samples of liver and blood *(15).*

Group 2: The Zn-supplemented group was fed a normal diet containing 96.95 mg Zn/kg and received daily intraperitoneal injections equivalent to 3 mg zinc sulfate/kg weight. After the 4-wk experimental period, they were treated in the same manner as group 1 *(16).*

Group 3: This group of animals was fed a normal diet and subjected to the 30 min of acute swimming at the end of the 4-wk study period. They constituted the swimming controls.

Group 4: The animals in this group were fed the same diet as group 3 but did not swim prior to sampling.

At the end of the study period, the rats from groups 1–3 were placed, two from each group at a time, in a 50-cm-deep, temperature-controlled glass water bath maintained at 37°C, where they were allowed to swim in single 30-min sessions and then they were killed by decapitation; the liver and blood samples were obtained immediately.

Analytical Techniques

Glucose in Plasma

The blood samples were centrifuged at 3000 rpm for 5-min and the plasma glucose level was determined by means of an Olympus AU 560 automated analyzer. The results were expressed as milligrams per deciliter.

Plasma Lactate

Two milliliters of blood were placed in test tubes containing fluoride oxalate as anticoagulant, placed in a dry ice pack, transferred to the laboratory within 15 min, and centrifuged at 3000 rpm at 4°C to obtain the plasma. The lactate levels were determined at the Biochemistry Laboratory of the Medical School of Selcuk University using a Technicon RA-XT automated calorimetric analyzer. The method uses a diagnostic lactate kit from Sigma. The results are expressed as milligrams per deciliter.

Plasma Zinc

The plasma Zn level was determined using a Shimatsu ASC-600 atomic absorption spectrometer. The results are given in micrograms per deciliter as the average of two runs per sample.

Plasma Leptin

The plasma leptin analysis was performed with a rat leptin RIA kit from LINCO (cat. no. RL-83 K). The results are expressed as nanograms per milliliter.

Histological Procedure

The livers were removed immediately after sacrificing the animals and fixed in Rosmann's mixture at 4°C for 24 h before histological examination *(17).* The tissue was placed in 96% alcohol until the color of picric acid was removed. Two slices 5 μm thick were then obtained from each sample by means of a paraffin microtome. One of the slices was treated with α-amylase (Sigma) at 37°C for 1 h to be used as negative control and then both were stained by the technique of Best's Carmine *(18).* The slides were examined using a Leitz Laborlux-12 microscope; photographs were taken of selected samples. Histological findings were classified as non-

Table 1
Mean Body Weight of Animals in All Study Groups[1]

Group[2]	Mean body weight, before experiment (g)	Mean body weight, after experiment (g)
1 Zinc deficient, swimming	230.56 ± 16.29	211.67 ± 15.00 [b]
2 Zinc supplemented, swimming	231.00 ± 17.29	259.50 ± 9.56 [a]
3 Swimming controls	233.75 ± 19.41	261.88 ± 11.00 [a]
4 Non-Swimming Controls	231.75 ± 14.93	258.75 ± 12.50 [a]

[1] Different letters in the same column indicate significant differences, a>b, $p<0.01$.
[2] $n = 10$, each group.

staining cell (0), 25% staining of cell (1), 50% staining (2), >50% staining (3), and total staining (4).

Statistical Analysis

The statistical analysis was performed using the SPSS computer program for Windows. The results are expressed as mean ± standard deviation. The Kruskal–Wallis analysis of variance was used for comparison between groups and the Mann–Whitney *U*-test was used for those with $p<0.05$.

RESULTS

There were no significant differences in the weight of the animals selected for the study. After the 4-wk experimental period, the mean body weight was significantly lower for the animals in group 1 than for all of the other groups ($p<0.01$), with the least significant difference (LSD) = of 12.39. These results are shown in Table 1.

Table 2 shows the results for plasma glucose, leptin, lactate, and Zn levels. The glucose and lactate levels were significantly higher for the Zn-deficient animals ($p<0.01$), which also had the lowest leptin levels ($p<0.01$). The LSDs were 27.54, 0.40, and 20.47, respectively. The glucose was significantly lower for the Zn-supplemented group than for groups 1 and 4 ($p<0.01$, LSD = 27.54). Plasma leptin was higher in the Zn-supplemented animals and in the nonswimming controls when compared to the swimming controls ($p<0.01$, LSD = 0.40). The Zn-supplemented group also showed significantly higher lactate levels when compared to the Zn-deficient and swimming control groups ($p<0.01$, LSD = 20.47).

Table 2
Plasma Glucose, Lactate, Zn, and Leptin Values Determined After Acute Swimming[1]

Group	Glucose (mg/dl)	Lactate (mg/dl)	Zinc (μg/dl)	Leptin (ng/ml)
1	183.89 ± 35.91^{a}	65.01 ± 29.06^{a}	50.78 ± 6.28^{c}	0.10 ± 0.06^{c}
2	119.90 ± 17.87^{c}	13.18 ± 6.56^{c}	186.80 ± 4.52^{a}	1.97 ± 0.56^{a}
3	$144.63 \pm 29.64^{b,c}$	40.51 ± 22.63^{b}	108.00 ± 15.46^{b}	1.21 ± 0.27^{b}
4	148.50 ± 6.35^{b}	$24.52 \pm 2.73^{b,c}$	111.75 ± 12.55^{b}	2.03 ± 0.40^{a}

Note: Different letters in the same column indicate significant differences, a>b>c, $p<0.01$.
[1] Except group 4, normal controls.

Table 3
Comparison of Liver Glycogen Content

Groups	Median Value of Liver Glycogen[1]
1	0.50^{a}
2	3.00^{b}
3	1.00^{a}
4	3.00^{b}

Note: Different letters indicate significant differences, a>b, $p<0.01$.
[1] As Best's Carmine paint H.E. 450× scores.

As expected, the plasma zinc levels of the deficient animals were the lowest, and those of the Zn-supplemented animals was the highest ($p<0.01$, LSD = 12.39).

As shown in Table 3 and in Figs. 1–4, the glycogen content in liver of groups 1 and 3 was significantly lower than those of groups 2 and 4 ($p<0.01$).

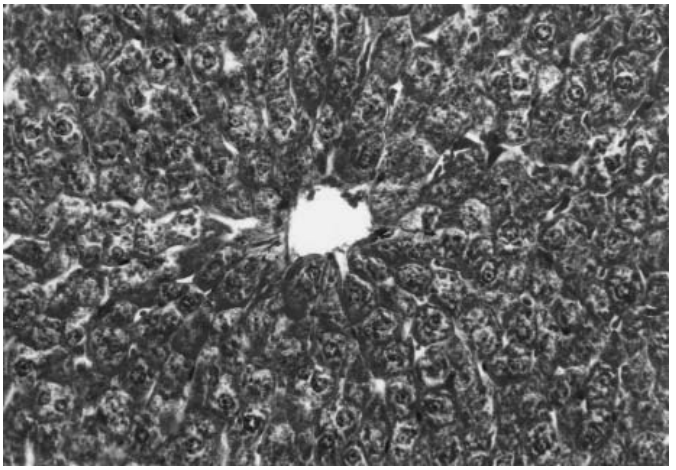

Fig. 1. Glycogen distribution in liver epithelial cells of the Zn-deficient animals (group 1, score 0.5, Best's Carmine paint, H.E. 450×).

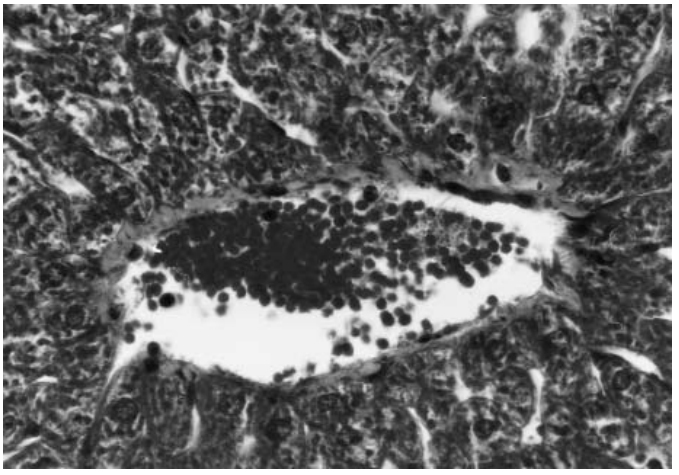

Fig. 2. Glycogen distribution in liver epithelial cells of the Zn-supplemented animals (group 2, score 3, Best's Carmine paint, H.E. 450×).

DISCUSSION

At the end of experimental period, the mean body weight of the Zn-deficient group was significantly lower than the other groups. This result is consistent with previous studies where weight loss induced by Zn-deficiency has been reported *(9,11,19).* The weight loss has been attributed to decreased food intake, which is considered to be a typical symptom of Zn deficiency *(20).*

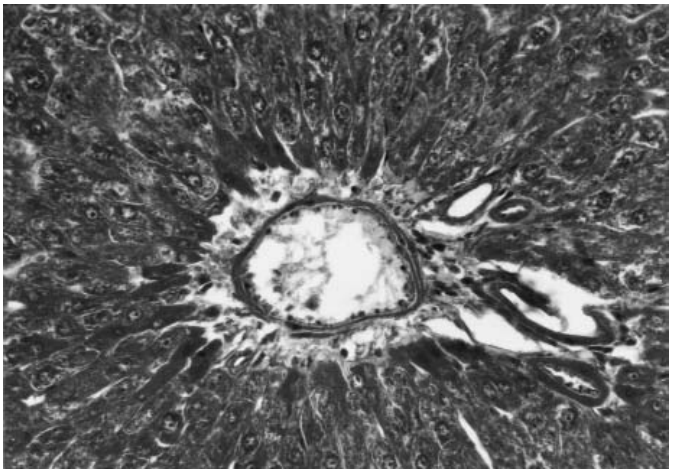

Fig. 3. Glycogen distribution in liver epithelial cells of the swimming controls (group 3, score 1, Best's Carmine paint, H.E. 450×).

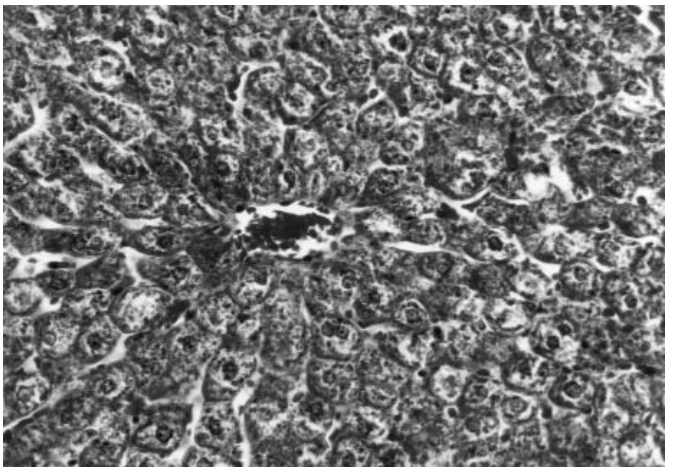

Fig. 4. Glycogen distribution in liver epithelial cells of the nonswimming controls (group 4, score 3, Best's Carmine paint, H.E. 450×).

The plasma glucose levels of the Zn-deficient group were significantly higher than in all the other groups, whereas the plasma glucose levels of the Zn-supplemented group were significantly lower than that of groups 1 and 4. In a study by Khaled et al., the plasma Zn level of 12 football players was determined to be low. In these subjects, hypoglycemia and higher lactate levels were determined *(21).* Because Zn and glucose reciprocally increase the absorption of each other, the higher plasma glucose level would be expected instead of the low Zn level found by Khaled *(6).* The relationship between insulin and Zn should also be taken into account.

Zinc is present in β- and α-cells of the pancreas and plays important roles in the production, storage, and release of insulin *(22).* Low glucose levels in the Zn-supplemented group probably results from Zn-induced increase in insulin production and thereby storage of glucose as glycogen in the liver. An investigation of the effects of Zn on insulin levels during exercise is necessary.

There are reports about a positive correlation between Zn and leptin *(9–11),* but studies about their relationship during exercise are scarce. In our study, the plasma leptin level of the swimming controls was significantly lower than for the Zn-supplemented and the nonswimming control groups, in agreement with reports of decreased leptin levels after exercise *(12,14,23,24)* and in disagreement with studies that have found that there was no change *(25)* or even increased leptin levels following acute exercise *(13).* The leptin level of the Zn-deficient group was the lowest, which may indicate that Zn deficiency is indeed related to decreased leptin levels. Our results indicate that Zn supplementation can be used to prevent decreases in plasma leptin levels during exertion.

The plasma lactate level of the Zn-deficient animals was significantly higher than for all of the other groups. On the other hand, the Zn-supplemented swimming group had significantly lower plasma lactate level than groups 1 and 3. Cordova and Alvarez reported that the muscle Zn of sportsmen decreased as a result of decreased plasma Zn concentration *(6).* Continuous daily exercise may alter Zn metabolism and this, in turn, may lead to fatigue and loss of power *(7).* Because Zn has important roles for the function of various enzymes, severe Zn deficiency may have negative effects on muscle function. Low levels of muscle Zn may result in decreased muscle endurance. It has been reported that Zn supplementation increased muscle power and metabolism during physical activity, but excessive Zn supplementation had negative effects on health *(26,27).* Singh et al. reported that supplementation with Zn and vitamin E has no effect on metabolic responses in women runners. Zinc supplementation, however, was performed by the single administration of Zn, which may not be effective enough *(28).* Other studies that support our finding of increased lactate in the Zn-deficient group include Brun et al., who reported low plasma Zn compared to prospective controls in twenty 12- to 15-yr-old female gymnasts *(29).* They also reported lower serum Zn levels in female than in male gymnasts and a positive correlation between isometric activity force and Zn levels, concluding that low Zn levels could retard pubertal growth and cause dysfunction of muscle performance. These results support our finding of higher plasma lactate levels in Zn-deficient rats. In addition, we found lower plasma lactate in the Zn-supplemented swimming group than in the nonsupplemented swimming rats of group 3. This is of relevance with respect to the relationship between Zn and physical performance. Additional support is found in Khaled's report of low Zn and high lactate levels in professional football players who performed exercise at their maximum capacity on a cycle ergometer, where a clear

correlation between Zn and physical performance was established *(21).* Given this possible relation between Zn and muscle performance and fatigue, future research seems necessary to better understand the relationships between Zn status and physical endurance.

As expected, the plasma Zn level was lowest in the rats fed a low-Zn diet and highest in those that received Zn supplements. Although not statistically significant, the plasma Zn level of the swimming controls was slightly lower than that of the nonswimming controls.

The glycogen content in the liver of the Zn-deficient rats was significantly lower than in the Zn-supplemented and in the nonswimming control groups, and the values for these two groups were significantly higher than for the swimming controls. A search of the literature did not produce a report on the effects of Zn deficiency and supplementation on liver glycogen content. Zn supplementation is thought to help maintain glycogen stores in the liver, probably resulting from Zn-induced insulin release *(30).*

Our results indicate that Zn deficiency has negative effects on physical performance in rats, causing a significant increase of plasma lactate and a decrease in the glycogen stores of liver and of plasma leptin. Because the opposite is true for Zn-supplemented rats, this essential element may have positive effects on physical performance. At least in principle, physiological doses of Zn may be of help to high-endurance athletes.

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