

## Effects of zink deficiency and supplementation on some hematologic parameters of rats performing acute swimming exercise

Abdulkirim Kasim **Baltaci**<sup>1</sup>, Kursat **Ozyurek**<sup>2</sup>, Rasim **Mogulkoc**<sup>1</sup>, Erdal **Kurtoglu**<sup>3</sup>, Esma **Oztekin**<sup>4</sup>, Aylin **Kul**<sup>1</sup>

<sup>1</sup> Department of Physiology,

<sup>3</sup> Department of Hematology,

<sup>4</sup> Department of Biochemistry, Medical School of Selçuk University,

<sup>2</sup> Gymnastics School of Selçuk University, Konya, Turkey

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The aim of the study was to investigate how zinc deficiency and supplementation effect some hematologic parameters of rats performing swimming exercise. Forty adult male Sprague-Dawley rats were divided into 4 groups, zinc deficient swimming group (Group 1, n=10), zinc supplemented swimming group (Group 2, n=10), swimming control group (Group 3, n=10), and control group (Group 4, n=10). Blood samples were taken by decapitation and analyzed for the determination of erythrocyte, hemoglobin level, hematocrit, leukocyte, lymphocyte, platelet count and plasma zinc level at the end of the 4 week experiment.

Erythrocyte count of group 1 was the lowest whereas erythrocyte count in group 3 was significantly lower than that in group 2 and 4 ( $p<0.05$ ). Hemoglobin level of group 1 was significantly lower than that of groups 2 and 4 ( $p<0.05$ ). Hematocrit was significantly lower in both group 1 and group 3 than both groups 2 and 4 ( $p<0.05$ ). Lymphocyte count in group 2 was significantly higher than in all other groups ( $p<0.05$ ). Platelet counts in group 2 was significantly lower than in all other groups ( $p<0.05$ ).

Our findings suggest that zinc deficiency effects the hematologic parameters mentioned negatively whereas zinc supplementation has a positive influence.

**Keywords:** hematological parameters, swimming exercise, zinc and rat

There knowledge in literature regarding the effects of zinc on exercise induced changes in hematological parameters is limited. Available studies concentrate on investigating the effects of exercise on the hematological parameters (5, 13, 17, 32). Hematological parameters are among the limiting factors for the type and intensity of exercise. Exercise in turn effects hematological parameters and is of importance concerning

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Correspondence should be addressed to

Dr. Abdulkirim Kasim Baltaci

Department of Physiology, Meram Medical School of Selcuk University, Konya, Turkey

E-mail: baltaci@selcuk.edu.tr

several pathological situations of blood (5). In addition to important role of zinc in carbohydrate, protein and lipid metabolism (9), it has also key roles in cellular mitosis, growth and maturation and functioning of various of enzymes (15), and it may also play role(s) on physical performance. Compared to the above-mentioned roles of zinc, the effects of zinc on blood parameters during exercise is poorly studied (18). Cordova et al. (8) have found that Zn supplementation increased hematologic parameters in rats. Southern and Baker (30) have reported that Zn induced a depression in hematologic parameters. On the other hand Donmez et al. (10) have found that Zn supplementation did not affect haematological parameters. Considering these data on the effects of Zn on blood parameters no agreement has been reached so far.

Therefore in this study we aimed to investigate the effects of zinc on blood parameters of rats performing swimming exercise.

### Materials and Methods

The protocol of this study was approved by the local Ethics Committee on animal experimentation. A total of 40 male adult Sprague–Dawley rats were obtained from Selduk University Experimental Medicine and Application Center and experiments were performed in the same Center. Animals were randomly divided into 4 groups as follows:

#### *Group 1, zinc deficient swimming group (n=10)*

This group of animals were fed with zinc deficient diet (0.650 ppm/g zinc) for 4 weeks and subjected to 30 minutes of acute swimming exercise at the end of the study period.

#### *Group 2, zinc administered group (n=10)*

This group of animals were subjected to administration of intraperitoneal zinc sulphate (3 mg/kg/day) for 4 weeks in addition to normal diet and were also subjected to 30 minutes of swimming exercise at the end of this period.

#### *Group 3, swimming control group (n=10)*

This group of animals were fed with normal diet and subjected to 30 minutes of acute swimming exercise at the end of this period.

#### *Group 4, normal control group (n=10)*

This group was fed with normal diet and no exercise was performed at the end of feeding period.

Swimming exercise was performed in a temperature controlled water (bath 50 cm in depth and 50 cm in wide) which was made of temperature proof glass and maintained at 37 °C. Rats were not allowed to relax and they were made to swim under our

observation. Swimming exercise was performed as a single 30-minute session. At the end of the study period just before decapitation the animals were placed in a swimming pool for swimming exercise by groups of two animals for each group. Two-ml blood samples were obtained and given into EDTA containing tubes from each animal and erythrocyte hemoglobin, hematocrit, leukocyte, lymphocyte and platelet counts were determined by autoanalyser (CELL-DYN-3500 R, USA).

Plasma zinc levels were determined by spectrophotometry (Shimadzu ASC – 600, Japan) and zinc levels are given as  $\mu\text{g/dl}$ .

#### *Statistical evaluation*

Statistical analysis was performed using SPSS statistical programme. Data are given as means  $\pm$  SD. Analysis of variance was performed and difference between the groups were determined by the least significance difference test. Values at  $p < 0.05$  were considered as significant different.

### **Results**

There was no statistically significant difference between the groups with respect to the pre-experiment mean body weights (Table I). But after four weeks of the experimental period, the mean body weight of the experimental group receiving zinc deficient diet, was significantly lower than all the other groups ( $p < 0.05$ , LSD: 12.39, Table I).

Erythrocyte count was lower in the zinc deficient swimming group compared to the other groups (Table II,  $p < 0.05$ , LSD: 0.89). Erythrocyte count in swimming control group was significantly lower than both in the zinc supplemented swimming group and in the control group ( $p < 0.05$ , LSD: 0.89). No statistically significant difference between the zinc supplemented swimming group and control group regarding erythrocyte count was found. Hemoglobin levels of group 1 was significantly lower than that of the zinc supplemented swimming group and normal control group ( $p < 0.05$ , LSD: 1.97). Hemoglobin value of group 3 was lower than group 4 ( $p < 0.05$ , LSD: 1.97). Hematocrit levels of groups 1 and 3 were significantly lower than groups 2 and 4 ( $p < 0.05$ , LSD: 5.64) but the difference between 2 and 4 was not significant.

No significant difference among the groups with respect to leukocyte counts was found. The lymphocyte count in the zinc supplemented group was higher than in the rest of the other groups ( $p < 0.05$ , LSD: 11.95, Table III). There was not a significant difference between groups 1, 3 and 4 with respect to lymphocyte count could be registered. On the other hand, platelet count in zinc supplemented group was significantly lower than the other groups ( $p < 0.05$ , LSD: 148.2). The difference between groups 1, 3 and 4 with respect to platelet count was not significant. As expected, the plasma levels of zinc was the lowest in group 1 and the highest in group 2 ( $p < 0.05$ , LSD: 12.39). No significant difference between groups 3 and 4 was observed.

Table I

*The mean body weight of experimental groups*

Groups	Pre-experimental body weight (g)	Post-experimental body weight (g)
1 Zinc deficient swimming group, n=10	230.56 ± 16.29	211.67 ± 15.00 <sup>b</sup>
2 Zinc supplemented swimming group, n=10	231.00 ± 17.29	259.50 ± 9.56 <sup>a</sup>
3 Swimming control group, n=10	233.75 ± 19.41	261.88 ± 11.00 <sup>a</sup>
4 Control group, n=10	231.75 ± 14.93	258.75 ± 12.50 <sup>a</sup>

\* Different lettering in the same column are indicating statistical significant difference ( $p < 0.05$ )  
 “Post-experimental body weight; a>b”

Table II

*Erythrocyte, hemoglobin and hematocrit values of experimental groups*

Groups	Erythrocyte (Million/ $\mu$ l)	Hemoglobin (g %)	Hematocrit (%)
1 Zinc deficient swimming group, n=10	4.48 ± 0.94 <sup>c</sup>	9.37 ± 2.01 <sup>c</sup>	22.89 ± 5.86 <sup>b</sup>
2 Zinc supplemented swimming group, n=10	6.62 ± 0.64 <sup>a</sup>	12.52 ± 1.70 <sup>ab</sup>	33.83 ± 3.85 <sup>a</sup>
3 Swimming control group, n=10	5.48 ± 1.13 <sup>b</sup>	10.69 ± 2.45 <sup>bc</sup>	26.78 ± 7.57 <sup>b</sup>
4 Control group, n=10	7.27 ± 0.38 <sup>a</sup>	14.18 ± 0.80 <sup>a</sup>	37.70 ± 1.67 <sup>a</sup>

\* Different lettering in the same column are indicating statistical significant difference ( $p < 0.05$ )  
 Erythrocyte; a>b and c, b>c  
 Hemoglobin; a>bc and c, ab>c  
 Hematocrit; a>b”

Table III

*Leukocyte, lymphocyte, platelet and zinc levels of experimental groups*

Groups	Leukocyte (1000/ $\mu$ l)	Lymphocyte (%)	Platelet (1000/ $\mu$ l)	Zinc ( $\mu$ g/dl)
1 Zinc deficient swimming group, n=10	3.65 ± 1.58	54.13 ± 13.49 <sup>b</sup>	596.22 ± 196.74 <sup>a</sup>	50.78 ± 6.28 <sup>c</sup>
2 Zinc supplemented swimming group, n=10	4.38 ± 1.29	75.14 ± 8.86 <sup>a</sup>	317.60 ± 74.37 <sup>b</sup>	186.80 ± 4.52 <sup>a</sup>
3 Swimming control group, n=10	4.48 ± 1.27	62.08 ± 9.54 <sup>b</sup>	488.00 ± 145.26 <sup>a</sup>	108.00 ± 15.46 <sup>b</sup>
4 Control group, n=10	4.52 ± 1.91	52.65 ± 16.99 <sup>b</sup>	562.50 ± 137.35 <sup>a</sup>	111.75 ± 12.55 <sup>b</sup>

\* Different lettering in the same column are indicating statistical significant difference ( $p < 0.05$ )  
 Lymphocyte; a>b  
 Platelet; a>b  
 Zinc; a>b, b>c”

### Discussion

As presented in Table I, there was no significant difference between the groups with respect to mean body weight before the experiment. But, when comparison was made between the groups with respect to post-experimental mean body weights there was a significant weight loss in zinc deficient group. The observed weight loss in the group fed with zinc deficient diet is in agreement with the studies reporting weight loss after feeding with zinc deficient diet (3, 14). Loss of weight due to loss of appetite is generally accepted as most typical symptom of zinc deficiency (20). Therefore the weight loss observed in the zinc deficient group is consistent with the literature (6, 22).

Erythrocyte counts of group 1 were significantly lower than that of the other groups while this value was higher in groups 2 and 4 compared to values found in groups 1 and 3. There is no agreement between the studies available in literature about the effects of exercise on erythrocyte count. Some authors suggest that increase in erythrocyte count after exercise is due to haemoconcentration (1, 32). On the other hand, there are studies reporting decrease in the number of erythrocyte after exercise (30), or no change at all (13). The effect of zinc deficiency or supplementation on erythrocyte level is poorly studied. Since zinc is involved in functioning of many enzymes (7), its involvement in erythrocyte production and growth is inevitable. The role of zinc in a variety of metabolic processes including cell cleavage and growth (21) or co-existence of some anemia types with zinc deficiency (11, 12, 27) can be regarded as further evidence for this hypothesis. The findings of the lowest erythrocyte count in the zinc deficient group and the highest erythrocyte count in group 2 alongside with higher erythrocyte count in group 2 than even the swimming control group suggests that the above-mentioned findings are due to zinc deficiency or supplementation rather than to swimming exercise.

The significantly lower erythrocyte count after feeding of rats with zinc deficient diet for four weeks is thought to be responsible for insufficient supply of required oxygen during swimming exercise and thereby it may have a negative effect on physical performance. On the other hand, zinc supplementation may have a positive effect on physical performance by increasing erythrocyte count and oxygen binding capacity of blood.

Hemoglobin and hematocrit values of the zinc deficient group were significantly lower than that of the normal control group and zinc supplemented group and the hematocrit level in group 2 was higher than swimming control group. In addition to findings with erythrocytes, the findings of hemoglobin and hematocrit provides further evidence for a suggestive negative effect of zinc deficiency on physical performance while zinc supplementation may have the opposite effect. Similar to our findings, Cordova et al. (8), has demonstrated higher hemoglobin and hematocrit counts in zinc supplemented rats after swimming exercise.

No significant difference was observed between the groups with respect to leukocyte counts. There are several studies reporting increased leukocyte count after exercise (1, 12, 24, 32). Short duration exercise until fatigue has been reported to

increase leukocyte count and this increase was suggested to be due to metabolic changes during exercise rather than to hemoconcentration (26). Our finding of non-significant change in leukocyte count is controversial to the reports of the above-mentioned studies. But, Cordova et al. (8) has also reported lack of significant difference between groups with respect to leukocyte count after swimming exercise in zinc supplemented rats. Lymphocyte count of zinc supplemented group was significantly higher than that of other groups. Several experimental studies have demonstrated that zinc has an increasing effect of lymphocyte count and it also increases the activity of lymphocyte (28, 29). Normal functions of lymphocytes are mainly dependent on their proliferation, differentiation and growth (23). Division of cells, especially at S phase and at pre-mitotic phase are highly sensitive to zinc deficiency (25). Therefore, there is a strong correlation between the level of immune response and tissue and cellular level of zinc. According to the widely accepted current opinion, deficiency of no other elements has more impact than zinc; and zinc deficiency is among the most common causes of immune deficiency in human (23, 28, 29). The finding of higher lymphocyte count in zinc supplemented group is consistent with the literature. But, this increased lymphocyte count is more likely to be result of zinc supplementation than the result of swimming exercise. König et al. (19) has highlighted the importance of zinc intake not only for physical performance but also for the immune system of sportsmen.

Platelet count of zinc supplemented group was significantly lower than the rest of the other groups. Although the platelet count of zinc deficient group was higher than that of groups 3 and 4 this difference was not statistically significant. Several studies have indicated a significant increase in platelet count following exercise (17, 32). Imhof and Koenig (16) reported increase in platelet count and decrease in bleeding and clotting time was determined following exercise. Our finding of low platelet count in zinc supplemented group is a striking finding when compared with studies investigating the effects of exercise on platelet count. To our best knowledge, however, there is no study investigating the relationship between exercise, zinc and platelet count. Beydagi et al. (4) found that platelet count increased, bleeding and clotting times shortened following exercise. Also it was stated that increased platelet count might be the consequence of high lactate level. The low platelet count in zinc supplemented swimming group is might be due to low level of lactate (2). There is a need for further studies investigating the relationship between exercise, platelet count and zinc for clarification of this matter.

The results of this study indicate that zinc deficiency may negatively effect physical performance by causing significant decrease in the number of erythrocytes, hemoglobin and hematocrit levels. On the other hand, zinc supplementation may have a positive effect on the physical performance of rats by causing a significant increase in the number of erythrocytes, hemoglobin and hematocrit levels. One of the striking results of this study is the decrease in platelet count due to zinc supplementation. In conclusion, we think that zinc supplementation in physiological levels may exert a positive effects on sportive performance.

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