RESEARCH ARTICLE

Effects of zinc supplement on hematological parameters following six-week endurance exercise in male rats

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Abstract

Introduction: The present study was aimed to investigate the effects of zinc supplement on hematological parameters following 6 weeks of endurance training in male rats. **Materials and Methods:** A total of 40 adult male Wistar rats (weighing 185 ± 20 g) were randomly divided into four groups: Saline control (receiving saline, without exercise), exercise control, and two intervention groups receiving conventional Zn (1 mg/kg, i.p.) daily (5 days per week) for 6 weeks with and without exercise. 30 min after injection of each exercise session, exercise groups performed the daily exercise protocol. Finally, the hematological parameters were evaluated by complete blood count test.The collected data were analyzed with SPSS using analysis of variance and Tukey *post hoc* tests and the significance level was set at $P \leq 0.05$ for all tests. **Results:** The results showed zinc supplement caused significant increase red blood cells (RBCs), hemoglobin (Hb), and hematocrit (HCT) compared with the control and exercise groups. In addition, zinc supplement with exercise showed significant difference with the exercise alone group. Moreover, exercise significantly decreased the mean RBCs, Hb, and HCT compared with the control. Other hematological parameters (white blood cell, platelet, mean corpuscular volume, mean corpuscular hemoglobin [MCH], and MCH concentration) were not significantly different between the groups. **Conclusion:** Findings of this study showed that supplementation of zinc following aerobic exercise may improve some of the hematological parameters of blood.

Key words: Endurance training, hematological parameters, rat, ZnO

INTRODUCTION

Blood plays a crucial role in sports physiology due to its major role in transports of oxygen, carbon dioxide, and other materials needed for tissues and their products.[1] Blood factors, particularly red blood cells (RBCs) and hemoglobin (Hb), are responsible for transporting the nutrients and oxygen to the active tissues and also carrying waste materials and carbon dioxide to repel from the lungs.[2] Like other organs of the body, blood does not respond equally to any kind of physical activity. There are several factors that body appropriately reacts to like the type of activity, time, intensity, and duration.[3,4] Energy supply for active tissues requires the cooperation of body tissues, especially the blood tissue. Studies on various hematological parameters have demonstrated different findings. In previous research, the effect of physical activity on the amount of RBCs, Hb, hematocrit (HCT), white blood cells (WBCs), and platelets (PLTs) showed reducing, increasing, or not changing these factors. Wu *et al*. observed a significant increase of PLT and WBC following a 24-h

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ultramarathon race; however, they observed no significant change in the amount of RBC, Hb, HCT, mean corpuscular hemoglobin (MCH), MCH concentration (MCHC), and mean corpuscular volume (MCV).[5] Fujitsuka *et al*. reported that the RBCs significantly decreased following 12-week endurance training (ET), whereas the amount of PLT was not changed.[6] Fujitsuka *et al*. represented an increase of WBC after an aerobic exercise session in young and adult athletes while some studies do not confirm the results.[7] In recent years, athletes and nutritionists use different methods such as consumption of dietary supplements to improve athletic performance. Consuming mineral supplements like zinc have also gained great attention among athletes. Zinc is an essential element in a series of biochemical processes, and it is required for the activity of more than 300 enzymes. It has important effects on the metabolism of carbohydrates, protein, and fat. Moreover, zinc plays a key role in the division, growth, and maturation of the cell, enzymes function, and to some extent on physical performance.[8-10]

Some studies have reported that performing exercise can lead to excessive repelling of zinc through sweat and urine which can cause zinc deficiency.[11] Some studies have concluded that zinc supplements can improve performance.[12] On the other hand, few studies have investigated the effects of zinc supplements on hematological parameters of blood in athletes. Cordova *et al*. represented that zinc supplements increased hematological parameters in rats.[13] Southern and Baker reported decrease of hematological parameters, while Donmez *et al*. observed no significant change in hematological parameters following zinc supplements administration.[14]

There are limited and sometimes controversial findings on the effects of zinc supplements on hematological parameters after aerobic activities. Therefore, the present study aims to investigate the effects of zinc supplements on hematological parameters following 6 weeks of ET in male rats.

MATERIALS AND METHODS

A total of 40 male adult Wistar rats weighing 185 ± 25 g were used. They were housed individually in cages with a constant temperature (22°C ± 2°C), humidity (55% ± 4%), and artificial 12:12-h light:dark cycle. They were acclimated for at least 1 week before the experiment and were randomly divided into four groups including:

First group: The control group that received only 0.9% saline and they did no exercise.

Second group: The exercise control group that received no supplements and only did exercise.

Third group: Animals in the third group were subjected to the intraperitoneal injection of 1 mg/kg zinc oxide for 6 weeks (5 days a week) and they did no exercise.

Fourth group: Received intraperitoneal injection of 1 mg/kg zinc oxide supplement for 5 days per week and for 6 weeks. In addition, they performed exercise for 30 min after receiving supplement.

In the second group, all animals became familiar with how to perform physical activity on a rat treadmill (made in Iran). Familiarity program included three sessions of walking at a speed of 5–8 m/min and the slope of zero degree for 5–10 min.[16] Sports-based groups (the second and fourth groups) did physical activities for 6 weeks (5 days per week). The whole training period was divided into three stages including familiarity (1st week), overload (2nd and 3rd week), and maintaining or stabilizing of the work intensity (4th–6th week), and the slope of the treadmill was fixed on zero for all the experiments. Furthermore, from the total activity time, animals were allowed to warm up and cool for 5 min.[17] Time schedule and details of the physical activity stages are presented in Table 1.

1 day following the last training session, the rats that had a full-fledged fasting night (12 h) were anesthetized with ether. Blood samples of the rats were then taken directly from the heart and placed in tubes containing ethylenediaminetetraacetic acid and transferred to the laboratory to measure the hematological parameters (WBC, RBC, Hb, HCT, MCH, MCHC, and MCV).

Statistical analyses were performed with IBM SPSS version 16 using one-way analysis of variance followed by a *post hoc* Bonferroni's test to compare the differences between the different groups. The significance level was set at $P < 0.05$ for all analyses.

RESULTS

The results showed significant differences in the hematological parameters between the experimental groups. According to the results, the amount of RBC, Hb, and HCT was significantly changed between the groups ($P < 0.05$), whereas other parameters were not significantly changed ($P > 0.05$). After the experiment (6 weeks of aerobic training and supplementation), the results suggested that physical activity significantly decreased the mean of RBC, Hb, and HCT ($P < 0.05$). Moreover, injection of zinc supplement led to a significant increase of RBC as compared with the control and the second group ($P < 0.05$); but, the amount of Hb and HCT

Table 1: Time schedule and details of physical activity

Stages	Speed (m*min)	Exercise duration (min)
Familiarity	10	10–15
Overloaded	12–28	15–60
Fixing	28	60

rose significantly in comparison with the second group ($P < 0.05$). However, injection of zinc supplement and physical activity resulted in a significant increase of RBC, Hb, and HCT compared to the second group, but these factors were not significantly changed in comparison with the control and the group only received zinc supplement (third group). There was no significant change in other parameters (WBC, PLT, MCV, MCH, and MCHC) between other experimental groups ($P > 0.05$). The results are presented in Table 2.

DISCUSSION

The present study investigated the effects of zinc supplement following 6 weeks of ET on the hematological parameters (PLT, MCHC, MCH, MCV, HCT, Hb, WBC, and RBC) in male rats.

After a 6-week ET, the number of erythrocytes significantly increased in groups receiving zinc supplement compared to the groups that only performed physical activities. There is no a general agreement on how the number of erythrocytes is affected by physical activities. Some studies showed reduction of erythrocytes number,[18] while some research reported increase of erythrocytes number[19,20] following physical activity. However, some investigations revealed that physical activity cannot change this number.[21,22] The results exhibited decrease of erythrocytes following 6 weeks of ET in male rats which confirmed the results of Fujitsuka *et al*. and Schumacher *et al*. whereas is inconsistent with the results of the study performed by Wu *et al*.[5,6,23] The reduction of RBC in the present study may be due to two factors including: (1) Decrease of RBC production due to the lack of RBC precursors, (2) RBC destruction due to the foot mechanical kick and damage of old cells in small vortex flows and gastrointestinal bleeding; on the other hand, the reduction of HCT may be due to the increase of plasma volume.[24] The decline of the number of Hb may be related to the blood volume alterations. Moreover, the possibility of damage to the RBCs should be considered. In contrast to our results, Donmez *et al*. found no significant changes in the number of erythrocytes as a result of injection of zinc supplements in the chickens.[15] The difference in the results of the study performed by Donmez *et al*. and our results may be due to a large difference between the injected doses. In support of our results, some studies revealed that the amount of hematological parameters decreased due to the lack of zinc[25] and consumption of zinc supplements can improve these parameters.[26]

Baltaci *et al*. reported increase of the number of erythrocytes in rats swimming in the pool while zinc deficiency led to the reduction of these erythrocytes.[27] On the other hand, Kilic *et al*.[28] and Polat[29] demonstrated that zinc supplementation and doing physical activities caused to a significant surge of the number of RBC, Hb, and HCT as compared to the others who only performed exercise and consumed no zinc supplement.

Since zinc is involved in the function of majority of enzymes,[30] consumption of zinc supplement might be associated with the formation and maturation of RBC. In addition, zinc plays a crucial role in the growth, reproduction, and maturation of different cells and many types of anemia occur due to the zinc deficiency[31-33] which can also confirm our results.

An important issue in physical activity is an increasing need for oxygen.[34] The enhancement of erythrocytes number in athletes consuming zinc supplements suggests that zinc may improve the athletes' performance through transferring oxygen to tissues. Similarly, the amount of Hb and HCT in animals consuming zinc supplement was significantly higher than those only doing physical activity. Some studies confirmed the results and they reported increase of Hb and HCT following consumption of zinc supplements in rats swimming in the pool.[13,27]

Table 2: Mean±standard deviation of changes in hematological parameters of different groups

Hematological parameters	No consumption of supplement		Consumption of supplement	
	Control	Exercise	Relax+zinc supplement	Exercise+zinc supplement
RBC*10^6 µL	7.122±0.959	6.128±0.564	7.837±0.444	7.176±0.561
HCT%	38.240±4.403	33.060±0.818	38.731±0.558	37.992±1.052
Hb (g/L)	14.330±0.874	12.650±1.036	14.754±0.355	13.992±0.394
Mean volume of RBC (MCV) (FL)	53.650±1.290	53.010±0.741	50.980±2.113	51.900±1.635
Mean amount of Hb (MCH) (pg)	17.810±1.350	18.020±0.470	16.810±1.311	16.320±0.746
Mean concentration of hemoglobin (MCHC) (g/dl)	33.360±2.153	33.480±2.087	32.910±1.975	31.050±1.300
WBC*10^3	8.612±0.895	11.450±4.885	10.895±4.213	12.780±1.912
PLT*10^3	797.90±61.156	671.00±225.532	752.20±61.156	643.80±197.864

RBC: Red blood cells, HCT: Hematocrit, Hb: Hemoglobin, MCV: Mean corpuscular volume, FL: MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, WBC: White blood cells, PLT: Platelet

In the present study, the amount of MCH, MCV, and MCHC was not significantly changed after injecting zinc supplements in comparison with the animals receiving no zinc. Besides, a significant change in the number of WBCs following performing physical activity was not observed.

Several studies reported increase of the number of WBCs and PLTs after physical activities, whereas some studies revealed a significant decrease or no significant change in the number of WBCs and PLTs following physical activities. Wu *et al*. showed a significant increase of WBC and PLT after the 24-h ultramarathon race[5] while some studies cannot confirm the results.[8]

Moreover, the number of leukocytes and thrombocytes in both groups receiving zinc supplement was not significantly changed compared to the other groups. Similarly, Cordova *et al*.[13] and Baltaci *et al*.[27] also reported that using zinc supplement cannot affect the number of leukocytes after physical activities. In contrast to our results, Kilic *et al*.[28] and Polat[29] found that physical activity can increase the number of leukocytes.

To now, it has been accepted that zinc deficiency is the most common cause of impairment of the immune system. The zinc deficiency in athletes or individuals regularly doing physical activities not only negatively affects their performance, but it can also impair their immune system.[35-37] Kilic *et al*.[28] and Polat[29] concluded that consumption of zinc supplements cannot significantly change the number of PLTs. However, Baltaci *et al*.[27] demonstrated that the PLTs number in rats who consumed zinc supplements and performed physical activity was significantly lower than the other groups. Some factors might be involved in the difference in the results such as the difference in the supplementation dose, the difference in the supplementation duration, the difference in the type of physical activity protocol, and the type of subjects of the experiments. In general, little evidence is available about the effect of consumption of zinc supplements on the hematological parameters. Thus, the exact conclusion in this context requires further studies.

CONCLUSION

The present results showed that zinc supplementation either alone or in combination with physical activity can significantly increase some hematological parameters indicating that zinc supplement may produce positive effects on physical performance of rats.

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