African Journal of Pharmacy and Pharmacology Vol. 5(12), pp. 1436-1440, 29 September, 2011
Available online at http://www.academicjournals.org/AJPP
DOI: 10.5897/AJPP11.062
ISSN 1996-0816

Full Length Research Paper

Effects of zinc supplementation on hematological parameters of high performance athletes

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Accepted 16 August, 2011

The purpose of the present study is to determine the effect of eight weeks zinc supplementation on the erythrocyte and leukocyte counts and other hematological parameters in male kick boxers. Twenty-four subjects were included in the study. They were equally divided into three groups as follows: Group EZ, training and receiving 2.5 mg/kg zinc supplement per day; Group SZ, receiving the zinc supplement but no training and Group E, who exercised but received no supplement. Erythrocyte, platelet and leukocyte counts, hematocrit, hemoglobin and the mean corpuscular volume (MCV) were determined in blood samples taken from each participant at rest and exhaustion. The erythrocyte count of Group EZ was significantly higher than in the E and SZ groups, $p < 0.001$. The number of leukocytes was higher in the two groups that trained. The hemoglobin and hematocrit levels were increased in the EZ group ($p < 0.05$).The platelet number increased with exhaustion in the E and EZ groups ($p < 0.001$). The MCV values were lower in group EZ as compared to the other two groups. The E and EZ subjects had higher neutrophil counts ($p < 0.05$).These results suggest that the combined effects of exercise and zinc supplementation have a positive effect in the hematological parameters of athletes, which may result in better performance and increased endurance.

Key words: Kick boxers, male athletes, exhaustion, zinc-supplementation, blood parameters.

INTRODUCTION

Physical activity has a beneficial effect on the organism. However, exercise may lead to the production of reactive oxygen species imposing a higher oxidative stress on antioxidant defense mechanisms (Muñoz et al., 2010).There is evidence that zinc and magnesium play key roles in cardio-respiratory functions and zinc takes part in life-supporting biochemical processes (Lukaski, 2000, 2001; Micheletti et al., 2001). Loss of zinc in the body increases during exercise, requiring zinc-supplementation especially in women (Clarkson and Haymes, 1994). Some deterioration of blood viscosity and performance occurs due to zinc deficiency (Khaled et al., 1999). Similarly, it was proved that zinc-supplementation corrected antioxidant enzymes in red blood cells and hematology in lithium-treated rats (Malhotra and Dhawan, 2008).

Changes in hematocrit, red blood cell counts and hemoglobin in relation to hemodilution were reported in marathon runners (Duca et al., 2006). There is a report on the effect of high intensity acute exercise on neutrophil infiltration and accumulation in muscle tissues of trained and untrained rats (Morozov et al., 2006).

To determine if zinc supplementation exerts a beneficial effect on blood parameters of trained athletes, the present study was carried out in kick boxers and sedentary subjects at rest and exhaustion.

MATERIALS AND METHODS

Subjects

Twenty-four healthy young males, age range of 12 to 14 years were voluntarily included in the study. All were subject to a full medical examination to rule out any physical or medical condition that would prevent them from participating in the experiments. They were randomly divided into three groups of eight subjects each, as follows:

Group E: Exercise-only group, performing 90 to 120 min kick boxing training until their heartbeat reached of 120 to 140 beats per minute, three times a week for eight weeks with 24 h rest intervals.
Group ZS: Supplemented-sedentary group, subjects that did not exercise while receiving 2.5 mg/kg per day of zinc as zinc picolinate.
Group EZ: Exercising and supplemented group, subjects who trained as those of group E while supplemented with zinc at the

Table 1. Training routines followed by subjects in the exercising groups.

A_1	A_2	A_3
*Reaction time	*Short term anaerobic *endurance	*Coordination between extremities
*First acceleration	*Fast strength	*Maximal speed
*Moderate-term anaerobic endurance	*Speed continuity	*Relative strength
*Maximal strength	*Strength continuity	*Long term anaerobic endurance

Table 2. Blood parameters of participants before and after zinc supplementation and/or exhaustion.

Parameter	Exercise				Zinc-supplemented			
	rbs	ras	ebs	eas	rbs	ras	ebs	eas
Erytrocyte counts ($\times 10^6$)	5.03 ± 0.26^{c}	5.16 ± 0.25^{by}	5.20 ± 0.27^{b}	$5.40 \pm 0.16^{a\ y}$	5.07 ± 0.27^{c}	$5.11 \pm 0.25^{b\ z}$	5.25 ± 0.23^{b}	$5.17 \pm 0.26^{b\ z}$
Leukocyte counts ($\times 10^3$)	6.33 ± 1.30^{d}	6.90 ± 1.23^{c}	8.85 ± 1.57^{by}	$10.47 \pm 1.10^{a\ z}$	6.51 ± 1.16^{d}	$7.47 \pm 1.50^{c\ x}$	$10.17 \pm 2.48^{b\ x}$	$12.51 \pm 2.03^{a\ x}$
Hematocrit (%)	41.73 ± 1.89^{c}	42.76 ± 1.89^{by}	$41.90 \pm 1.44^{b\ z}$	43.61 ± 1.8^{a}	41.28 ± 1.8^{c}	$42.95 \pm 1.18^{b\ y}$	$42.26 \pm 1.79^{b\ y}$	43.10 ± 1.50^{a}
Hemoglobin values (g/dl)	14.12 ± 0.69^{b}	15.07 ± 0.66^{by}	$15.98 \pm 0.41^{c\ x}$	$15.55 \pm 0.63^{a\ x}$	14.00 ± 0.59^{b}	14.00 ± 0.59^{b}	14.00 ± 0.59^{b}	14.00 ± 0.59^{b}
Trombocyte counts ($\times 10^3$)	298.25 ± 51.25^{c}	298.25 ± 51.25^{c}	298.25 ± 51.25^{c}	298.25 ± 51.25^{c}	286.67 ± 67.91^{c}	286.67 ± 67.91^{c}	286.67 ± 67.91^{c}	286.67 ± 67.91^{c}
Mean corpuscular volume (MCV, fL)	81.32 ± 3.79^{a}	81.32 ± 3.79^{a}	81.32 ± 3.79^{a}	81.32 ± 3.79^{a}	81.38 ± 2.73^{a}	81.38 ± 2.73^{a}	81.38 ± 2.73^{a}	81.38 ± 2.73^{a}
Neutrophil values (%)	45.30 ± 9.9^{b}	45.30 ± 9.96^{b}	45.30 ± 9.96^{b}	45.30 ± 9.96^{b}	47.66 ± 8.20^{b}	47.66 ± 8.2^{b}	47.66 ± 8.2^{b}	47.66 ± 8.2^{b}
Lymphocyte values (%)	40.26 ± 8.18^{c}	40.26 ± 8.18^{c}	40.26 ± 8.18^{c}	40.26 ± 8.18^{c}	39.25 ± 7.64^{c}	39.25 ± 7.64^{c}	39.25 ± 7.64^{c}	39.25 ± 7.64^{c}
Monocyte values (%)	9.56 ± 1.2^{a}	9.56 ± 1.2^{a}	9.56 ± 1.2^{a}	9.56 ± 1.2^{a}	9.67 ± 1.13^{a}	9.67 ± 1.13^{a}	9.67 ± 1.13^{a}	9.67 ± 1.13^{a}
Eosinophil values (%)	2.21 ± 0.67^{b}	2.21 ± 0.67^{b}	2.21 ± 0.67^{b}	2.21 ± 0.67^{b}	2.96 ± 1.06^{b}	2.96 ± 1.06^{b}	2.96 ± 1.06^{b}	2.96 ± 1.06^{b}

same dose as group Z.

Training method

After a 10 min warm-up, the subjects underwent a training routine that increased their heartbeat up to 120 and up to 130 beats per minute as measured with a pulse meter during training.

In addition to technical training the subjects also underwent strength, speed, endurance and coordination training under supervision of their advisors. The training program of groups E and EZ are shown in Table 1.

After training, the subjects did 20 min gymnastics to cool down and bring down their heartbeat to 90 pulses per minute or less.

Blood tests

Blood samples were taken from each subject two times. The erythrocyte, hemoglobin, platelet and differentiated leukocyte counts were measured along with the hematocrit, hemoglobin and mean corpuscular volume were measured by standard clinical laboratory procedures by means of a CELL- DYN-3500 R automatic blood analyzer.

Testing of subjects

The tests were applied twice weekly: Rbs: resting before supplementation; Ras: resting after supplementation; Ebs: supplementation before exhaustion; Eas: supplementation after exhaustion. The tests were applied four times during the experimental period. Exhaustion was achieved after a 15min warming until heartbeat was between 90 and 130 beats per minute. Following warm up, the subjects performed short sprints, quick lateral movements and speed training for 20 min. At this point their heartbeat was between 120 and 140 beats per minute. The high-intensity stage followed until exhaustion, characterized by 150 to 179 beats per minute heartbeat.

Statistical analysis

Statistical analysis was carried out with the SPSS v.16.0 software. Variance analysis was used in order to determine differences between and within groups. The Duncan's multiple range test was used in order to determine if there were differences between the study groups. The t-test was used for comparisons of physical tests.

RESULTS

Table 2 shows the blood profile of all participants, before and after zinc supplementation and/or exhaustion. There are no differences in the erythrocyte, leukocyte and platelet counts among the three study groups before supplementation

Table 2. Blood parameters of participants before and after zinc supplementation and/or exhaustion.

Parameter	Exercise + Zinc-supplemented			
	rbs	ras	ebs	eas
Erytrocyte counts (×10^6)	5.07 ± 0.40^{c}	5.42 ± 0.34^{bx}	5.66 ± 0.49^{a}	5.61 ± 0.39^{ax}
Leukocyte counts (×10^3)	6.31 ± 2.75^{c}	6.96 ± 1.73^{d}	10.21 ± 2.49^{bx}	11.20 ± 2.69^{ay}
Hematocrit (%)	41.51 ± 1.63^{c}	42.93 ± 1.76^{bx}	42.96 ± 1.31^{bx}	43.26 ± 1.90^{a}
Hemoglobin values (g/dl)	13.88 ± 1.53^{b}	13.88 ± 1.53^{b}	13.88 ± 1.53^{b}	13.88 ± 1.53^{b}
Trombocyte counts (×10^3)	280.25 ± 90.12^{c}	280.25 ± 90.12^{c}	280.25 ± 90.12^{c}	280.25 ± 90.12^{c}
Mean corpuscular volume (MCV, fL)	76.60 ± 9.36^{b}	76.60 ± 9.36^{b}	76.60 ± 9.36^{b}	76.60 ± 9.36^{b}
Neutrophil values (%)	46.71 ± 6.18^{b}	46.71 ± 6.18^{b}	46.71 ± 61^{b}	46.71 ± 6.18^{b}
Lymphocyte values (%)	40.56 ± 6.03^{d}	40.56 ± 6.03^{d}	40.56 ± 6.03^{d}	40.56 ± 6.03^{d}
Monocyte values (%)	9.38 ± 2.46b	9.38 ± 2.46^{b}	9.38 ± 2.46^{b}	9.38 ± 2.46^{b}
Eosinophil values (%)	2.65 ± 1.53^{c}	2.65 ± 1.53^{c}	2.65 ± 1.53^{c}	2.65 ± 1.53^{c}

^{a,b,c} ($p < 0.001$) Significant for same line. ^{x,y,z} ($p < 0.001$) Significant for different supplemented groups. Rbs: Resting before supplementation, Ras: resting after supplementation, Ebs: exhausting before supplementation, Eas: exhausting after supplementation.

Table 3. Blood parameters of participants before and after zinc supplementation and/or exhaustion.

Zn (ug/dl)	E	ZS	EZ
Rbs	91.20 ± 7.38^{a}	84.09 ± 11.17^{b}	96.31 ± 7.91^{a}
Ebs	93.33 ± 7.78^{a}	86.43 ± 8.43^{b}	96.67 ± 11.89^{a}
Ras	88.69 ± 11.54^{b}	$97.82 \pm 10.17^{a\ x}$	$92.01 \pm 8.05^{b\ y}$
Eas	90.10 ± 12.55^{a}	$87.48 \pm 12.42^{b\ x}$	$81.75 \pm 9.87^{c\ y}$

^{a,b,c}Significant differences in the same row ($p < 0.001$).$^{x,y,\ z}$Significant differences between groups receiving zinc supplements ($p < 0.001$).

at rest or after exhaustion.

After supplementation, the erythrocyte count of group ES was significantly higher than all other groups at rest and after exhaustion, $p < 0.001$. The number of leukocytes increased in the SZ and EZ groups, especially at exhaustion ($p < 0.001$). The platelet number increased after supplementation, especially at exhaustion where it was significantly higher than at rest ($p < 0.001$). The neutrophil, eosinophil, basophil and lymphocyte counts were increased in the SZ and EZ groups ($p < 0.05$), but the number of monocytes slightly decreased in the EZ group at rest and exhaustion.

All groups had higher hematocrit values at exhaustion than at rest. The highest hematocrit values were registered for the EZ group ($p < 0.05$). Similarly, the hemoglobin level was also higher in the EZ group after supplementation ($p < 0.05$). The MCV remains unchanged in all groups throughout the experiment.

Blood serum zinc levels were given in Table 3. As shown in the table, zinc level was reduced after the exercise in groups 1 and 3 ($p < 0.001$). However, zinc supplementation was corrected at these levels.

DISCUSSION

The results show that zinc supplementation positively influences the blood parameters of exercising and sedentary subjects. Previous studies have shown the effects of exercise and trace element supplementation on performance of athletes of different disciplines (Baltaci et al., 2003; Cinar et al., 2010; Lukaski, 2000).

In this work, the effects of zinc supplementation and 8 weeks training on the erythrocyte and leukocyte counts and the distribution of leukocyte subgroups were examined in zinc-supplemented male kick boxers and sedentary controls.

The red-blood cell counts were higher in the two zinc supplemented groups ZS and EZ. When these two groups are compared, the exercising subjects had higher erythrocyte counts than the non-exercising individuals. This is consistent with previous studies conducted on humans and experimental animals (Baltaci et al., 2003; Dönmez et al., 2002; Kilic et al., 2004) where an increase of red blood cells was reported as a result of zinc-supplementation. The fact that this parameter increased with exercise until exhaustion, suggests that is due to liquid loss resulting from intensified exercise.

As expected, the hematocrit and hemoglobin values, both directly related to red-blood cells also increased in the zinc-supplemented groups. The increase in hematocrit level was also higher in subjects that trained to exhaustion, again attributable to liquid loss. This result is consistent with the work of Silva et al. (2006) who reported increased hemoglobin levels after zinc supplementation.

Yet, as mentioned earlier, there are different results about blood parameters obtained in studies involving athletes of various sports branches. Hematological parameters were examined in elite rugby players, where it was found that the hemoglobin and hematocrit levels increased at the beginning of the competitive season but these parameters decreased with increased physical demand towards the end of the season (Banfi et al., 2006). In cyclists, a decrease in hemoglobin levels was reported (Zembron-Lacny et al., 2009). Blood samples taken at rest before, during and after exhaustion were examined along with immune parameter analysis in volleyball players in a 4-month season. In that study, volleyball players had the same erythrocyte, hemoglobin and hematocrit values as the controls (Córdova et al., 2010).

The red blood cells, hemoglobin and hematocrit levels of marathon runners changed due to hemodilution triggered by oxidative stress that occurred during exercise along with increased levels of erythropoietin and reticulocytes (Duca et al., 2006). The reticulocyte and erythropoietin levels were not examined in the present study, but it is possible that zinc-supplementation may have a positive effect against oxidative stress that occurs as a result of hard exercises. In fact, zinc was reported to be as a strong antioxidant in previous studies (Casimiro-Lopes et al., 2009; Khaled et al., 1999; Malhotra and Dhawan, 2008).

The average erythrocyte volume was not significantly influenced by supplementation, but exercise done until exhaustion caused a slight, insignificant increase as compared to rest-levels. This is consistent with another study that zinc-supplementation increased performance by increasing the ability of erythrocyte to change shape and by decreasing the increase in blood viscosity brought about by exercise (Khaled et al., 1999).

The thrombocyte count was not significantly changed by zinc supplementation and exercise, especially in subjects at rest. If the exercise is carried out to exhaustion, no difference in platelet count is seen, consistent with a study conducted with elite rugby players (Banfi et al., 2006).

The total leukocyte count showed a higher increase as a result of supplementation, reaching the highest levels at exhaustion. This is also due to hemoconcentration and because exercise stimulates leukocyte transport to circulating blood. The same happened for leukocyte subgroups such as neutrophils, lymphocytes, eosinophils and basophils. The increase of lymphocyte levels due to zinc-supplementation has been previously reported (Baltaci et al., 2003). Yet, another study demonstrated no change of leukocyte levels, which may be the result of different exercise and supplementation conditions (Banfi et al., 2006). Also contrary to our findings, a decrease in hemoglobin and leukocyte levels was detected in judo athletes (Umeda et al., 2008). We did observe significant increases of lymphocyte levels resulting from supplementation and exercise.

In another study lymphocyte counts were elevated after hard exercise due to the acute phase inflammatory response it stimulated (Córdova et al., 2010). In another study, in spite of finding no changes in erythrocyte and leukocyte counts, zinc supplementation resulted in an increase of lymphocyte percentage in broiler chicks (Dönmez et al., 2002). Zinc supplementation stimulates hemoxigenase1, a stress protein in lymphocytes, monocytes and granulocytes (Fehrenbach et al., 2003). Leukocyte, eosinophil and monocyte values were found to be low in volleyball players and sedentary individuals as compared to long distance runners (Saygin et al., 2006).

In our study, serum zinc levels were reduces after the 8 weeks kick box exercise. Different findings are present in the literature related to issue. Baltaci et al. (1998), stated that zinc levels of child sportsmen were not affected by training. Mogulkoc et al. (1997) reported that training did not affect zinc levels in sportsmen. There is a report in which long-time endurance training significantly decreased resting zinc levels of male and female athletes (Córdova and Alvarez-Mon, 1995).

All the findings in the present study were obtained from adolescents subjects. It is possible that it may be different in various age and sport groups.

The results of this investigation can be taken as evidence that zinc supplementation of kick-box athletes improves blood parameters and may positively influence their performance.

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