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The Effect of Exercise and Zinc Supplement on the Hematological Parameters in Rats

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ABSTRACT

This study evaluates the consequences of a session of intensive short-duration exercise and Zn supplementation on different hematological variables. Forty male Wistar rats were divided into four groups (n = 10): the first nonsupplemented, maintained at rest (R); the second nonsupplemented, undergoing exercise (E); the third supplemented with Zn, kept at rest (ZnR); and the fourth supplemented with Zn, undergoing exercise (ZnE). Zinc supplements (200 ppm) were given in drinking water. The exercise consisted of a single session of swimming until exhaustion. At rest, RBC, Hb, and Hto fell ($p < 0.05$), whereas red cell indices, MCV, and MCH rose ($p < 0.05$) in +ZnR compared with R; MCHC remained unchanged (ZnR vs R). After exercise, RBC, Hb, and Hto increased significantly in E and in ZnE compared with R and ZnR, respectively. In addition, RBC and Hb were lower ($p < 0.01$) in ZnE compared with E; however, MCV and MCH were higher ($p < 0.05$) in the group ZnE vs E. With respect to white blood cells—leukocytes (WBC), limphocytes (LYMPH), and neutrophiles (NEUT)—no significant differences were observed between groups at rest (ZnR vs R). WBC and LYMPH increased signifi-

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cantly in E with respect to the rest situation (E vs R), but this did not happen in supplemented animals (ZnE vs ZnR). Level of pH decreased after exercise both in E and in ZnE, but the fall was lower in the latter. We believe that a single session of swimming until exhaustion leads to an increase in RBC, Hb, and Hto without causing changes in MCV, MCH, and MCHC. On the other hand, Zn supplementation leads to an increase of MCV and MCH, although they remain within normal levels. Furthermore, this supplementation produces lower metabolic acidoses after exercise that leads to leukocyte stability.

Index Entries: Zn; exercise; hematological parameters; rats.

INTRODUCTION

Numerous reports concerning the effect of physical exercise, both as one-off and as a regular training, on hematological parameters have given contradicting results. Most studies reported increases in the hematological variables following single intense exercise (*1–5*). Others, however, have not found any significant postexercise or posttraining changes in the hematological indices concerned, especially in studies on well-trained athletes (*6,7*). Several studies have revealed a drop in the red blood cell count, hemoglobin concentration, and hematocrit values below the initial levels following intense single-session muscular exercises, and following training of variable duration and intensity (*8–10*). Other studies showed that muscular work of varying duration and intensity produces an increase of leukocytes and lymphocytes (*2*).

Some trace elements, such as iron (Fe), copper (Cu), and zinc (Zn), contribute to erythropoiesis: Fe in the formation of hemoglobin; Cu in the erythropoietic function through ferroxidase activity of the ceruloplasmin, which favors the absorption and mobilization of Fe (*11–13*); and Zn in the synthesis of the hemo group by its participation in enzyme porfobilinogeno-syntetase (*14*). Therefore, the deficiency of these trace minerals can lead to a drop in hemoglobin concentration, the number of circulating erythrocytes, and the efficiency of their functions. The efficiency of oxygen supply to working tissues is affected by numerous factors, including the volume of circulating blood and its oxygen-carrying capacity, which is determined by hemoglobin concentration and by the number of circulating erythrocytes.

The administration of supplementary minerals with the intention of improving physical form or endurance level can cause modifications in some hematological parameters. In the case of zinc, these variations seem to be basically owing to:

1. Its relationship with the leukocitary system (*11,14*);
2. Its implication in the hemo group synthesis (*14*); and

3. Its capacity to interact with other essential trace minerals like Cu and Fe (*14–17*).

In this study, an attempt is made to evaluate the repercussions of one session of short duration intensive exercise on different hematological variables and the influence of dietetic Zn supplementation on hematological parameters, before and after the realization of the exercise.

MATERIAL AND METHODS

Forty male Wistar rats were used weighing 250 ± 10 g, which were kept in a room of controlled temperature (21–23°C) and with a light/dark cycle of 12 h/d. The animals were divided into four groups (n = 10): the first nonsupplemented group, maintained at rest (R); the second nonsupplemented group, undergoing exercise (CE); the third Zn-supplemented group, kept at rest (ZnR); and the fourth Zn-supplemented group undergoing exercise (ZnE). The Zn supplement was given in drinking water (*ad libitum*) at a rate of 200 ppm of Zn, in the $ZnCl_2$ form, for 14 d. The exercise was carried out in a bath (50 × 50 × 75 cm) with water kept at 20°C, with a bubbled air system, in order to obtain a regular exercise. This consisted of a single session of swimming until exhaustion, which was reached when the animals remained more than 15 s submerged. At rest and after exercise, under anesthetic with penthobarbital (50 mg/kg), blood was extracted from the abdominal aorta, and the following variables were measured: erythrocytes (RBC), leukocytes (WBC), limphocytes (LYMPH), neutrophiles (NEUT), eosinophiles (EOS), and monocytes (MON), and the red cell indices calculated: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) in a Coulter Counter autoanalyzer (Coulter Electronics Ltd.). Hemoglobin (Hb) was calculated by the cyanohemoglobin colorimetric method, and the hematocrit (Hto) by the micromethod, all measurements being taken twice. Total proteins (TP) were determined by colorimetry by the biuret quantitative method in an autoanalyzer (Hitachi 705). Serum Zn and Fe were measured by atomic absorption spectrophotometry (Perkin Elmer 272), and blood pH was analyzed in a separate ABL-30 (Radiometer). The ▲PV (%) was calculated by following equation:

$$\blacktriangle PV = [100/(100 - Hto_1)] \times [100 \times (Hto_1 - Hto_2)/Hto_2]$$

The results are expressed as $X \pm SD$. Statistical analyses of the results were carried out by analysis of variance for two factors, and only when this was significant ($p < 0.05$) was the Student's t-test used for the comparison of the mean of independent samples, considering significant variations for $p < 0.05$.

RESULTS

In Table 1, the values displayed correspond to hematological parameters RBC, Hb, and Hto and to red cell indices MCV, MCH, and MCHC. At rest, RBC, Hb, and Hto decreased, whereas MCV and MCH increased significantly in supplemented animals (ZnR) with respect to the nonsupplemented group (R); MCHC remained unchanged. After exercise, the nonsupplemented group (E) and the supplemented group (ZnE) showed significant increases in RBC, Hb, and Hto compared with their respective groups at rest (R and ZnR). The red cell indices MCV, MCH, and MCHC also rose slightly, although the increases were not significant. RBC and Hb were significantly lower in supplemented animals (ZnE) than in nonsupplemented ones (E); after exercise, however, MCV and MCH were higher in the former (ZnE).

In Fig. 1 can be seen the data showing the behavior of white series: leukocytes (WBC), limphocytes (LYMPH), and neutrophiles (NEUT). No significant differences were observed in these parameters between the resting groups (ZnR vs R). After exercise, significant increases in WBC and LYMPH were noticed in the nonsupplemented group with respect to the resting group (E vs R). Comparing the exercising groups (ZnE vs E), WBC and LYMPH were lower in the supplemented one than in the nonsupplemented.

Table 2 shows the variations in total proteins (TP), serum Zn and Fe, blood pH, and saturation of oxygen (SaO_2). Comparing the resting groups (ZnR vs R), serum Cu and Zn increased significantly in the Zn-supplemented one. After exercise, serum Zn and TP increased in the nonsupplemented animals (E vs R). In the supplemented group after exercise (ZnE vs ZnR), serum Zn fell; however, if serum Zn is expressed in relation to TP (μg/g protein), this element increase (ZnR = 168 vs ZnE = 172.5). After exercise, pH decreased, but falls were lower in the supplemented (ZnE) than in the nonsupplemented group (E). Plasma volume changes were similar after exercise in both cases (ZnE and E), accounting for (−14%) and (−17%), respectively. Swimming time was similar in the two groups undergoing exercise.

DISCUSSION

The present study demonstrates that the supplementation with Zn at physiological doses provokes a lower metabolic acidoses after maximal exercise that leads to leukocyte stability. After the realization of short-duration intensive exercise, hemoconcentration has been described. Most authors have reported increases in hemoglobin concentration (Hb), hematocrit (Hto), and erythrocyte count (RBC) in peripheral blood after realization of one-off physical effort (*1,3,7,18,19*). In our study, a decrease in plasma volume was observed in the groups that carried out

Table 1
Hematological Variations in the Red Series and in the Indices Calculated (MCV, MCH, and MCHC) in Rats, Brought About by Exercise and by a Supplementation of 200 ppm of Zn for 14 Days

	Nonsupplemented		Zn Supplemented	
	Rest, R	Exercise, E	Rest, ZnR	Exercise, +ZnE
RBC (10^6/mm^3)	8.7 ± 0.7	9.4 ± 0.5*	7.6 ± 0.4[a]	8.5 ± 0.8*,[b]
Hb (g/dl)	14.6 ± 0.8	16.7 ± 0.6*	13.8 ± 0.6[a]	15.9 ± 0.5*,[b]
Hto (%)	42.6 ± 3.2	47.3 ± 2.2*	41.3 ± 2.1[a]	46.4 ± 2.3*
MCV (fl)	48.8 ± 2.2	49.8 ± 1.2	52.7 ± 1.6[a]	53 ± 2.1[b]
MCH (pg)	17.2 ± 0.7	17.7 ± 0.5	18.2 ± 0.4[a]	18.5 ± 0.7[b]
MCHC (%)	35 ± 1.4	35.3 ± 1.1	34.7 ± 1.2	34.8 ± 1.1

A mean ± SD is given. Significant differences ($p < 0.05$) for all comparisons.

*Significant differences between the resting and exercising situations, both in supplemented and nonsupplemented animals.

[a]Significant differences in the comparison between supplemented and nonsupplemented animals at rest (ZnR vs R).

[b]Significant differences in the comparison between supplemented and nonsupplemented animals after exercise (ZnE vs E).

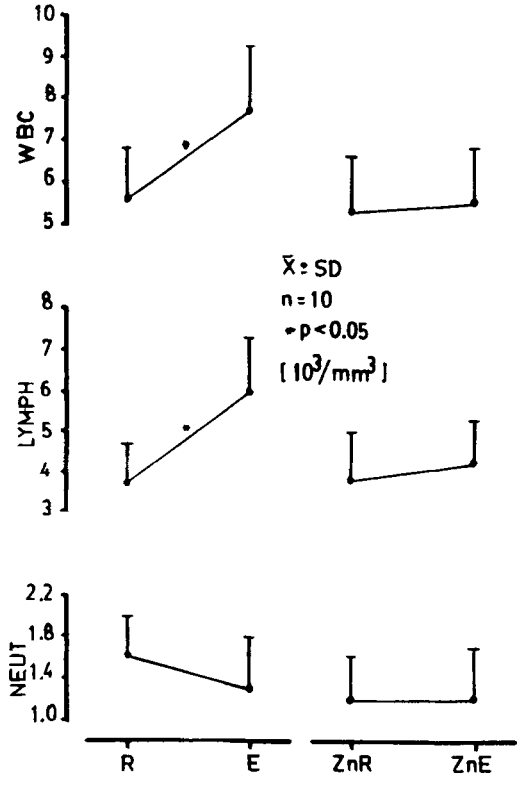

Fig. 1. Variations in white blood cell (WBC, LYMPH, and NEUT) in rats, brought about by exercise and by a supplementation of 200 ppm of Zn for 14 d. Significant differences for $p < 0.05$

Table 2
Variations in Serum Zn, Fe, and TP (Total Proteins), of Blood pH and Saturation of Oxygen (SaO_2) in Rats, Brought About by Exercise and Zn Supplementation (200 ppm)

	Nonsupplemented		Zn Supplemented	
	Rest, R	Exercise, E	Rest, ZnR	Exercise, ZnE
pH	7.39 ± 0.04	7.04 ± 0.08*	7.41 ± 0.03	7.16 ± 0.08*,[b]
SaO_2 (%)	95.3 ± 0.76	99.6 ± 0.12*	94.9 ± 0.98	99.7 ± 0.10*
TP (g/dl)	6.2 ± 0.3	8.0 ± 0.4*	6.0 ± 0.4	6.9 ± 0.7*,[b]
Zn (μg/dl)	99.0 ± 7.9	164.0 ± 9.9*	168.0 ± 10.7[a]	150.0 ± 10.2*,[b]
Fe (μg/dl)	159.0 ± 25.3	312.0 ± 33.3*	167.0 ± 23.5	269.0 ± 28.0*,[b]
S.T. (min)[c]	–	31.0 ± 3.5	–	29.9 ± 7.1

A mean ± SD is given. Significant differences ($p < 0.05$) for all comparisons.

*Significant differences between the resting and exercising situations, both in supplemented and nonsupplemented animals.

[a]Significant differences in the comparison between supplemented and nonsupplemented animals at rest (ZnR vs R).

[b]Significant differences in the comparison between supplemented and nonsupplemented animals after exercise (ZnE vs E).

[c]Swimming time (S.T.) in those animals that carried out exercise.

exercise (E and ZnE). Hemoconcentration is the result of the fluid loss that occurs during exercise, and is caused mainly by elimination of sweat and by water evaporation through the lungs. Two basic circumstances have been suggested as causing the modifications in the intensity of the abovementioned changes: (1) the more intensive the effort, the greater the modifications, and (2) the greater the training level, the less apparent the changes. Dehydration seems to be the principal cause of hematological variations, but there are other phenomena that participate in the changes: (1) the increase in catecholamines during exercise that causes a contraction of blood reservoirs like spleen or liver, helping the liberation of red cells to circulation, and that produces an increase of blood flow probably causing the disappearance of leukocitary margination (*20,21*); (2) the stress generated by exercise and by water temperature that produces an increase in ACTH and cortisol secretion, stimulating the liberation of blood cells from the bone marrow (*22,23*).

As far as red cell indices are concerned, it has been described that short periods of effort up to exhaustion do not seem to modify them (*1,9,24,25*). In this study, MCV and MCH increased in the supplemented groups (ZnR and ZnE) compared with the nonsupplemented (R and E), at rest and after exercise, respectively. We believe that this fact is owing to decreases in RBC after supplementation, since although Hb and Hto decrease too, this fall was proportionately lower than the one in RBC. In this sense, Storey and Greger (*26*) have shown that consumption of great quantities of Zn can affect Fe metabolism, reducing its absorption and retention, which causes hematocrit drop. Furthermore, excessive Zn has negative effects of Cu metabolism affecting indirectly Fe management,

which can develop ferropenic anemia (*11,13,14*). In our study, extra administration of Zn (200 ppm/14 d) had no functional repercussions on Hb, Hto, and Fe, which were found to be within normal values.

With regard to the leukocitary system, after physical exercise and training, a marked increase in white corpuscles had been observed (*2,10*). In addition to all other circumstances that affect red cells, the leukocytosis has been also attributed to: (1) an inflammatory response to local tissue injury, caused by repeated vascular microtraumatism and by high muscle stress/strain (*10*, and 2) the acidosis, which has also been involved in the leukocytosis development and in the platelets mobilization. So, exercises with a lower level of acidosis seem to display lower values of leukocytes (2). In the supplemented group, we did not observe postexercise leukocitosis, and most abovementioned factors cannot explain it. Variation in plasma volume was similar in the exercising groups; the type and duration of exercise were identical; the inflammatory phenomena related to microtraumatism do not seem likely during the swimming period, and if they exist, they will be similar for both groups. Nevertheless, we observed a clear acidosis situation, both in the nonsupplemented and in the supplemented group, acidosis being lower in the latter. This lower acidosis of ZnE could be owing to the Zn participation in carbonic anhidrase (mataloenzyme of Zn), which is involved in the regulation of the acid-base balance (*13*), something that may justify the different behavior of white blood cells in supplemented animals showing a greater leukocyte stability, with no significant tendency toward leukocitosis.

To summarize, we believe that a single session of swimming until exhaustion leads to an increase in RBC, Hb, and Hto without changes in MCV, MCH, and MCHC. Animals that take Zn supplements show a lower metabolic acidoses after maximal exercise that leads to leukocyte stability. Supplementation does not change the maximum exercise level (swimming time was similar in groups E and ZnE), but the lower metabolic acidoses observed could be important in the recuperation period and in the face of long-duration exercise.

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