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MINERAL METABOLISM AND OXIDATIVE STRESS IN BRAZILIAN MILITARY UNDER UNDERGOING STRENUOUS EXERCISE

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ABSTRACT

After strenuous exercise performed by the Brazilian military, there was a moderate increase in oxidative stress confirmed by the increment in TBARS, so the health risk should have been minimal. The basic blood count showed values considered healthy indicating a rigorous homeostatic control of the hematological parameters for this type of exercise. The remaining parameters suffered significant alterations: decrease in sodium, increase in potassium, decrease in chlorine, decrease in magnesium and increase in copper. So there was a good metabolic adaptation to the conditions of physical overload, without representing a risk of mineral misbalance.

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INTRODUCTION

The efficiency of physical training essentially depends on the intensity, volume, periodization and modus of the training stimuli. After each bout of physical exercise a catabolic phase exists at first with decreased tolerance of effort, characterized by reversible biochemical, hormonal, immunologic and other changes. A well-balanced training program therefore needs to include adequate phases of regeneration and regular assessments of the current individual tolerance of stress (Kjaer *et al.,* 2003). By definition, endurance exercise can typically be defined as prolonged steady-state exercise performed for durations between four minutes and four hours. This could therefore encompass middle distance events (e.g. track cycling, rowing, and swimming) through to marathon running and extended stages of road cycling. The exercise of durations beyond four hours, such as 'Ironman triathlons' are classified as ultraendurance (MacLaren and Morton, 2012).

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However, despite its evident beneficial effects, exercise, especially if unusual or exhausting, or training above habitual intensity, may exceed the endogenous antioxidant system's capacity and often results in oxidative stress and injuries. This oxidative overload can produce harmful organic changes in skeletal muscles. Such changes have also been reported in many other organs and body systems responsible for regulating and maintaining homeostasis (Bastos and Silva, 2012). Thus, in a broader sense the concept of injury includes physiological and metabolic reactions to exhausting exercises that are responsible for alterations in blood count, electrolyte content and trace elements concentrations. These damaging effects, with their consequent inflammatory processes, can jeopardize physical performance and lead to overtraining syndrome, besides potentially contributing to an increased future risk of cardiovascular disease (Thompson *et al.,* 2001). It was shown in recent study from Finland that the sustained training load during the last four weeks of basic training led to oxidative stress observable both at rest and after submaximal exercise. The authors concluded that the oxidative stress may be a marker of insufficient recovery leading possibly to non-functional overreaching resulting in stagnation or decrements in performance capacity (Tanskanen *et al.,* 2011).During arduous military training, a combination of stressors could impair host immune defenses. However, in a research carried out with Parachute Regiment recruits in the UK, no differences in immune function and upper respiratory tract infections had been observed as compared with control subjects. It was suggested that the progressive decrease in saliva flow rate during exercises might have indicated an ensuing state of hypohydration (Welsh *et al.,* 2006). In the military, marching is a part of physical training, while "forced march" is a march greater in extent than the distance usually covered and often carried out under difficulties. The intensity, duration, and energy expenditure required to produce these acute exercise effects are not clearly defined.As for the evaluation of oxidative stress markers, they were measured in a case of 35 soldiers (age 19.7 ±0.3 years) at rest and immediately after a 45-min submaximal exercise (Tanskanen *et al.,* 2011). It was concluded that the sustained training load during the last four weeks of basic training led to oxidative stress observable both at rest and after submaximal exercise. To the best of our knowledge, there have been no other reports on this subject so far. Even less is known about the dynamics of hematological and biochemical parameters of the soldiers participating in the forced marches. In this context it is also interesting to evaluate the levels of trace elements copper and zinc with potential benefits for soldiers. Their presence in adequate quantities is known to protect cardiovascular system, enhance wound healing, protein metabolism and many other physiological functions (Stern *et al.,* 2007; McClung *et al.,* 2005). The purpose of this work is to find out if the practice of the forced marches is safe for the health of the Brazilian soldiers, at least in the short term.

MATERIALS AND METHODS

This is a cross-sectional observational analytic study with a quantitative approach. It was approved by the Ethics Committee on Human Research of the Federal University of Mato Grosso do Sul (CAAE: 61369316.6.0000.0021). Written informed consent was obtained from all participants. They were also advised of their right to withdraw from the investigation at any time. One hundred and ten healthy male soldiers of the Brazilian army, aged between 18 and 26 (mean 22) years took part in the research. They belonged to a Combat Engineering Battalion located in the region of Centerwest, Brazil. The research was carried out during September 2016, when daily outdoor temperatures range from 20 to 35ºC, with an average of 26ºC, according to local weather bureau. The conscripts held the march of 24 km (15 miles), armed with full uniform and carrying full combat load of gear (backpack and rifle). The total duration of the march was 7 hours, comprising 5 minutes of rehydration break every 1 hour of march and a snack of 1 hour in the middle of the route (12 km). The plasmatic concentrations of the substances that react with thiobarbituric acid (TBARS) were measured by spectrophotometric method as described elsewhere (Dias *et al.,* 2015). The blood collection was carried out by a trained professional three months before the date marked for the march and three hours after the end of the activity. Serum was separated by centrifugation at 3.000g for 15 min, transferred to demineralized Eppendorf tubes and stored at −18°C for further analytical determinations. The latter were carried out in a Clinical Laboratory of the University School of Medicine. The red blood cell counts, white blood cell counts, Hb concentrations, platelet counts, and minerals were determined by automated analyzers (SE-9000, Sysmex, Kobe and Roche Hitachi, Japan). Blood samples for copper and zinc determinations were collected in polypropylene syringes and immediately transferred to vacuum tubes free of trace elements (BD Vacutainer Systems-Becton, Dickinson & Co). Copper and zinc concentrations were determined using a flame Atomic Absorption Spectometetr Analyst 100 Perkin Elmer. All materials, plastics or glasses were immersed for 24 hours in 5% Extran solution (Merck), rinsed and immersed for at least 24 hours in a 10% solution of ultrapure nitric acid (Merck) for waste decontamination. Then, they were washed with ultrapure water (Milli-Q, Millipore, Bedford, USA) and dried at 40ºC. As for statistical analysis, means, standard error of the mean, minimum and maximum values were calculated. After confirming the parametric character of the data (obtained by Shapiro-Wilks's test), the analyses was carried out using the Student`s test for paired samples. The value p adopted for the calculations was ≤ 0.05. Data were processed using the software BioEstat 5.0.

RESULTS AND DISCUSSION

The soldiers showed a mean age value of 19±0.09 years. Anthropometric measurements were performed for characterized the group (mean±SD): height 1.73±0.06 m; weight 71.56±0.99 kg; body mass index 23.71±0.29 kg/m^2; fat percentage 12.13±0.53%. The data for the oxidative stress and hematologic parameters are summarized in Table 1 (pre- and post-march values). It is known that strenuous exercise leads to an increase in metabolic rate, increases production of reactive oxygen species, and compromises antioxidant defense systems. If the production of free radicals is excessive as observed during strenuous aerobic exercise or if the antioxidant defense mechanisms are impaired, the balance between peroxidants and antioxidants is lost. Thus, there is an apparent paradox between the benefits and disadvantages of strenuous aerobic exercise. It was postulated that, most probably, the results depend on the ability of highly trained soldiers to withstand oxidative stress, provoked by strenuous exercise and is directly related to the plasma antioxidant status (Chevion *et al.,* 2003). In this context, our data seem to suggest that in the participants of this study plasma oxidant status is only moderately altered.
Either anemia or a decrease in the Hb concentrations is often observed in healthy individuals after various intensities of sports and physical exercise. The decrease in red blood counts can also accompany strenuous training program, however, the factors affecting the hematological changes are still not fully elucidated (Fujitsuka *et al.,* 2005). In some cases, iron deficiency could be one of the possible causes responsible for such alterations. However, the biokinetics of iron is slow, and these changes are unlikely to develop in the short term. Another effect of strenuous exercise on hematological changes is an increase in the platelet counts. One of the causes of this increase after exercise is probably due to be a fresh release of platelets from the spleen, bone marrow, and lungs. The magnitude of the increase of coagulation potential, platelet aggregation and fibrinolysis appears to be primarily determined by exercise intensity, but there are conflicting results concerning the effect of exercise on platelet aggregation and activation (Davidson *et al.,* 1987; El-Sayed 1996). In the present investigation we showed that, in accordance to the available data, the platelet counts increased, but to a small extent. The exercise-induced leukocytosis is the main hematological response to strenuous exercise. Its interrelationship with immune function has been widely

covered in the literature. Earlier data on potential impact of physical activity and sport on the immune system are summarized in a brief review (Shephard and Shek, 1994).

Table 1. Oxidative stress and hematological parameters (mean±SD) in Brazilian military (n=110) before and after the march

Parameter	Before	After	*p*-value
TBARS (ng/mL)	593.67±203.08	751.48±401.66	<0.05
Erythrocytes ($10^6/\mu L$)	5.16±0.04	5.17±0.05	0.55
Hemoglobin (g/dL)	15.23±0.15	14.08±0.13	0.02
Platelets ($10^3/\mu L$)	248.98±7.97	267.41±11.72	<0.01
Leukocytes ($10^3/\mu L$)	6.97±0.25	8.95±0.38	<0.01
Neutrophils ($10^3/\mu L$)	2.49±0.20	6.23±0.36	<0.01
Eosinophils ($10^3/\mu L$)	0.27±0.03	0.21±0.01	<0.01
Basophils ($10^3/\mu L$)	0.01±0.004	0.05±0.004	<0.01
Lymphocytes ($10^3/\mu L$)	2.33±0.07	3.90±0.21	<0.01
Monocytes ($10^3/\mu L$)	0.57±0.10	0.76±0.03	<0.01

It was postulated that, first, neutrophils rapidly invade the muscle lesion and promote inflammation by releasing cytokines that attract and activate additional inflammatory cells. Neutrophils may further damage the injured muscle by releasing oxygen free radicals that would damage cell membranes (Kjaer *et al.,* 2003). It was reported in a paper dedicated to the effects of exercise on blood leukocyte count that the leukocytosis persists for 3 hours after exercise, and is due mainly to an increase in the quantity of circulating neutrophils and monocytes, although there may be a small increase in lymphocytes (Natale *et al.,* 2003). The increase in the eosinophil count is mostly assumed as the presence of allergic reaction. However, the strenuous physical exercise may bring about an increase about a normal level which would have adverse effect on the body, indicating additional effects, that is either aggravating allergy or altering immunity status (Setyawan, 2005). In any case, the eosophilia found in this research is within normal values, and hardly reflects such a danger. As for the counting of lymphocytes, there could not been confirmed either a significant decrease or normal values earlier reported in the paper concerning the influence of an arduous Military Training Program on 14 recruits in the United Kingdom (Welsh et al, 2006). On the contrary, we have observed a remarkably significant increase in lymphocytes counts, the difference that may be explained by the larger number of our volunteers (110 *vs* 14).

Table 2. Mineral metabolism parameters (mean±SD) in Brazilian military (n=110) before and after the march

Parameter	Before	After	*p*-value
Sodium (mEq/L)	139.87±0.29	136.05±0.36	<0.01
Potassium (mEq/L)	4.61±0.04	6.16±0.09	<0.01
Chlorine (mEq/L)	107.40±4.15	105.04±4.16	<0.01
Magnesium (mg/dl)	2.34±0.06	2.12±0.01	0.01
iCa (mg/dl)	1.12±0.01	1.08±0.008	0.06
Phosphorus (mEq/L)	4.06±0.06	4.26±0.06	0.19
Copper (mEq/L)	50.68±16.84	60.15±21.28	<0.01
Zinc (mEq/L)	61.79±14.76	61.53±20.24	0.98

As to increase phenomena itself, its origin is unclear. For example, a similar pattern of increase in blood counts for lymphocytes, neutrophils, monocytes, and natural killer cells was also observed after 30 min walk (Nieman *et al.,* 2005). Anyway, it appears that any ethically acceptable form of vigorous exercise provides only a partial model of a clinical inflammatory response. Future research in this area should seek to relate changes in circulating lymphocytes and cytokines to hormonal, symptomatic and enzymatic alterations (Natale *et al.,* 2003). The data on mineral metabolism are summarized in Table 2 (pre- and post-march values). Performing strenuous exercise may result in changes in mineral plasma concentrations, such as an acute exercise effect, or these changes may be kept for a few days. Moreover, at hot temperatures, the loss of minerals can be increased by perspiration. (Keen, 1993).Consequently, sodium loss easily produces considerable hyponatremia, especially in places with a tropical climate (Anastasiou *et al.,* 2009). In fact our data showed statistically significant reduction of plasma sodium after the march, without poseing risk to health (Duh and Cook, 2005) for military personnel who make physical efforts. Potassium, among other functions, is involved in ionic balance by opening and closing alkaline ion channels and creates electrochemical gradients across cell membranes. By this way, nerve impulses and other information are transmitted and cellular function regulated (Crichton, 2008). Rapid and large changes in plasma potassium concentration during and after exercise occur because the sodium-potassium pump has a limited ability to repair potassium into intracellular space (Mebdo and Sejersted, 1990). Our data showed elevated values in plasma potassium concentration of military after the march, which may compromise the proper functioning of the neuromuscular system under these conditions. When the sodium / potassium ratio is established (Table 2), it is verified that not only the exchange of these ions between the extracellular and intracellular space occurred, but a true loss of sodium, probably caused by elimination of sweat. This is the obvious sign of dehydration during exercise (Meyer *et al.,* 2016). Another important observation is that the predicted water replacement may not have occurred sufficiently to avoid this loss during the march performed by the evaluated military personnel.

Thus chlorine, sodium, and potassium are responsible for maintaining water balance and distribution, normal osmotic balance, acid-base balance, and heart rate (Armstrong *et al.,* 2007, Wilmore *et al.,* 2008). Our hypothesis was that the concentration of chlorine should decrease in parallel with the sodium concentration. Actually, the ratio Na_{before}/Na_{after} (1.02) was exactly the same asCl_{before}/Cl_{after} (1.02), confirming that sodium was eliminated as NaCl, without influencing the distribution of potassium between red blood cells and plasma. All this corresponds to normal exercise physiology data (McArdle *et al.,* 2015). Muscle function, electrolyte balance, oxygen consumption and energy production are functions dependent on the presence of magnesium, which under conditions of strenuous exercise is distributed among sites with greater muscular activity. However, most of this element may or may not be removedby sweat (Nielsen and Lukaski, 2006). This may be the true reason for the small decrease in plasma magnesium content in the participants. Bone mineralization and biochemical control of muscle contraction are performed in the presence of calcium. The free ionized calcium (iCa) is the physiologically active form, corresponding to 45-50% of the total. A complex control system involving parathormone is responsible for maintaining constant levels of iCa. The calcium concentrations in human plasma depends, among other factors, on blood pH.It was suggested elsewhere (Motta, 2009) thatat higher pH values a decrease in calcium should be observed. At the same time, strenuous exercise is known to lead to high blood acidosis (Brooks, 2001). Nevertheless the group studied did not show anystatistically significant increase in ionized calcium, a fact that can be explained by the rigorous nature of calcium homeostasis. In this context it is logical that phosphorus concentrations would accompanied calcium dynamics, hence phopshorus content would not be altered. It is well known that copper and zinc plasma concentrations are sensitive to physical exercise. Consequently, copper can undergo considerable changes, depending on the duration, intensity and type of physical exercise performed, and in the case of strenuous exercises, there may be an increase in this mineral (Johnson *et al.,* 1992; Nieman, 2000). As for zinc, the literature indicates that deficiency of this mineral generally occurs among military personnel undergoing intense physical training (McClung *et al.,* 2005). In addition, high muscle activity during exercise increases oxidative stress and, in parallel, the formation of the antioxidant enzyme copper-zinc superoxide dismutase (Cu / Zn-SOD) (Steinbacher and Eckl, 2015). In fact, our study shows that there was an increase in copper, but without the parallel increase of zinc, indicating that this pair was not used to produce a sufficient amount of the Cu/ Zn-SOD enzyme during physical effort. Thus, the statistically significant increase in circulating copper may be attributed to oxidative stress, which, although considered moderate (Table 1), may have been sufficient to cause plasma elevation of that element after the strenuous exercise.

Conclusions

After strenuous exercise performed by the Brazilian military, there was a moderate increase in oxidative stress confirmed by the increment in TBARS, so the health risk should have been minimal. The basic blood count showed values considered healthy indicating a rigorous homeostatic control of the hematological parameters for this type of exercise. The remaining parameters suffered significant alterations: decrease in sodium, increase in potassium, decrease in chlorine, decrease in magnesium and increase in copper. So there was a good metabolic adaptation to the conditions of physical overload, without representing a risk of mineral misbalance.

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