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Serum zinc and blood rheology in sportsmen (football players)

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Abstract. We aimed at investigating relationships between zinc status, blood rheology and blood glucose during exercise. Twenty-one professional football players underwent a triangular maximal exercise test on cycloergometer, with progressively increasing work loads until $VO_{2\,max}$. On the whole these subjects had a low serum zinc because nine of them had a hypozincemia (0.54 ± 0.01 mg/l) which suggested a zinc deficiency. Subjects with low serum zinc were able to perform a lower power output (123 ± 8.71 vs. 166.27 ± 14.84 watts, $p = 0.029$) and exhibited a higher increase in blood lactate during exercise (7.51 ± 0.81 vs. 5.57 ± 0.33 mmol/l, $p = 0.024$) resulting in a lower 2 mmol lactate threshold ($44.7 \pm 3.9\%$ vs. $58.9 \pm 4.8\%$ of maximal power output, $p = 0.04$). They were less able to maintain their plasma glucose and exhibited a tendency towards hypoglycemia ($p = 0.0153$). Hypozincemia was associated with a higher viscometric RBC rigidity index ($p = 0.0009$), and this index was negatively correlated to serum zinc ($r = -0.68$, $p = 0.7 \times 10^{-3}$). Blood viscosity at high shear rate (MT90 viscosimeter) corrected for hematocrit (45%) remained higher during exercise in these hypozincemic subjects ($p = 0.003$). This study suggests that zinc status may influence blood rheology during exercise, either by its direct action on RBC flexibility (demonstrated *in vitro*) or by its effect on lactate accumulation which may in turn modify erythrocyte fexibility.

Keywords: Zinc, football players, exercise, blood lactate, erythrocyte deformability, trace elements, rheology, hypoglycemia

1. Introduction

Zinc is a trace element which plays a role in many aspects of metabolism, since it is associated with more than 200 metalloenzymes [1–3]. In a normal human blood, circulating zinc is contained in erythrocytes (75–88%), plasma (12–20%), and leukocytes (about 3%) [4]. Nutritional insufficiencies in this mineral, causing serious disorders, have been reported in developing countries [5] but seem to exist also in western countries in situations of increased needs like the growth spurt of puberty [6].

There is some evidence for a role for zinc in exercise physiology and sports medicine. Several studies have reported low serum zinc concentrations in athletes [7–9] and adolescent trained gymnasts [10]. Brief exercise sessions acutely induce marked changes in serum zinc and erythrocyte zinc

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distribution [11,12]. On the other hand, zinc seems to play a role in muscle performance [13]. It has insulin-mimetic effects *in vitro* [14] and *in vivo* [15,16], which may influence substrate disposal in muscles. It plays a role in free radical scavenger systems, and muscle lactate-dehydrogenase [17], both systems involved in muscle cellular function.

In the view of the numerous roles of this trace element in physiology and pathology [18,19], some studies have also investigated the influence of zinc on erythrocyte rheology [20–22]. Zinc improves the flexibility of sickle cells, *in vitro* and *in vivo* [21,22]. Further experiments from our laboratory have shown that the deformability of experimentally rigidified erythrocytes is also improved *in vitro* by zinc salts [23,24].

It was thus interesting to investigate the relationships between zinc status and the two classical aspects of blood rheology during muscular activity: the acute exercise-induced blood hyperviscosity [25,26], and the low viscosity associated with fitness in trained athletes [27–29]. Since an influence of nutritional conditions on blood rheology during exercise has been suggested by reports concerning carbohydrates, water intake and polyunsaturated fatty acids [30–32], we hypothesized that zinc could be another nutritional factor influencing blood rheology during exercise.

2. Subjects and methods

Subjects used in this study were 21 professional football players (age 23.5 ± 0.68 yr; weight 75.7 ± 1.35 kg; height was 180.2 ± 1.06 cm; $VO_{2\,max}$ 40.8–80 ml/min/kg) submitted everyday to a physical training program. They were tested together during three days at the end of the sportive season. They underwent a triangular maximal exercise test on cycle ergometer 4000, with progressively increasing work loads until $VO_{2\,max}$ ($VO_{2\,max}$ was directly measured as the maximal value of VO_2). In both protocols pedal speed was kept constant at 60 rpm by the subjects with an rpm-meter. VO_2, heart rate, and blood lactate were monitored during the test. Physical working capacity W_{170} (w/kg) was also calculated, this being the work that the subjects can perform at a heart rate of 170 b/min expressed per kg of body weight [33,34]. Samples were drawn before exercise (T_0), during the test below (T_1) and above (T_2) the 4 mmol/l lactate threshold, and during recovery (T_3). Lactate thresholds at 2 mmol/l and 4 mmol/l were determined as the percentage of $VO_{2\,max}$ at which blood lactate reached this value [35].

Serum zinc was measured by flame atomic absorption spectrophotometry (FAAS; model 2380; Perkin-Elmer). The lower limit of sensitivity of this method is 0.0125 mg/l. Its coefficient of variation (CV) was between 4 and 7%.

Blood samples for hemorheological measurements (7 ml) were drawn with potassium EDTA as the anticoagulant in a vacuum tube Vacutainer. Viscometric measurements were done at very high shear rate (1000 s^{-1}) with a falling ball viscometer (MT 90 Medicatest, F-86280 Saint Benoit) [36, 37]. Accuracy of the measurements was regularly controlled with the Carrimed Rheometer 'CS' (purchased from Rhéo, 91120 Palaiseau, France) [38]. The coefficient of variation of this method ranges between 0.6 and 0.8% [39]. We measured with this device apparent viscosity of whole blood at native hematocrit, plasma viscosity, and blood viscosity at corrected hematocrit (45%) according to the equation of Quemada. Hematocrit was measured with microcentrifuge. Index of erythrocyte rigidity 'k' was calculated according to the equation of Quemada [40].

Lactate was assayed with a kit from DuPont specially adapted to the DuPont de Nemours automatic clinical analyzer. This assay was based on NADH production by rabbit lactate dehydrogenase, the coefficient of variation ranges between 0.7 and 5.6%.

All samples were analyzed for plasma insulin by a radioimmunoassay (kit SB-INSI-5 from the international CIS) and plasma glucose with a Beckman glucose analyzer. The within assay coefficient of variation (CV) for insulin was determined by repetitive measurements of the same sample and was between 8.6% (low values) and 9.7% (high values). The between assay CV for insulin was between 12.5 and 14.4%. The sensitivity (lowest detectable value) was 2 μU/ml.

3. Statistics

Data were analysed by one-way analysis of variance, data are presented as mean $\pm$ the SE of the mean. Correlations were tested by least square fitting for linear relationships. The level of significance was set at $p < 0.05$.

4. Results

4.1. At rest (T_0)

According to serum zinc level at rest, football players were divided into two groups: hypozincemic subjects, *group 1*, (0.54 ± 0.01 mg/l; $n = 9$; age 23.56 ± 1.14 yr; weight 76.5 ± 1.51 kg; height 178.89 ± 1.48 cm) and normozincemic subjects, *group 2*, (0.74 ± 0.03 mg/l; $n = 12$; age 25 ± 0.91 yr; weight 80.96 ± 1.93 kg; height 181.42 ± 1.52 cm). There was no difference in height ($p = 0.2$), weight ($p = 0.1$), and body mass index ($p = 0.3$) between the two groups. Serum zinc was significantly different between the two groups ($p = 3.62 \times 10^{-5}$) (Fig. 1).

Blood viscosity at native hematocrit was significantly lower in hypozincemic subjects when compared to normozincemic subjects (2.62 ± 0.07 vs. 2.92 ± 0.11 mPa·s; $p = 0.042$) (Fig. 2). When this viscosity was corrected for hematocrit (45%) with Quemada's equation, it exhibited only a non significant tendency to be sligthly higher in hypozincemic than in normozincemic subjects (Table 1), as did RBC rigidity index 'k' (Fig. 3). Serum zinc at baseline was positively correlated to hematocrit ($r = 0.6$; $p = 0.004$) (Fig. 4) and negatively to RBC rigidity index 'k' ($r = -0.68$; $p = 0.7 \times 10^{-3}$) (Fig. 5).

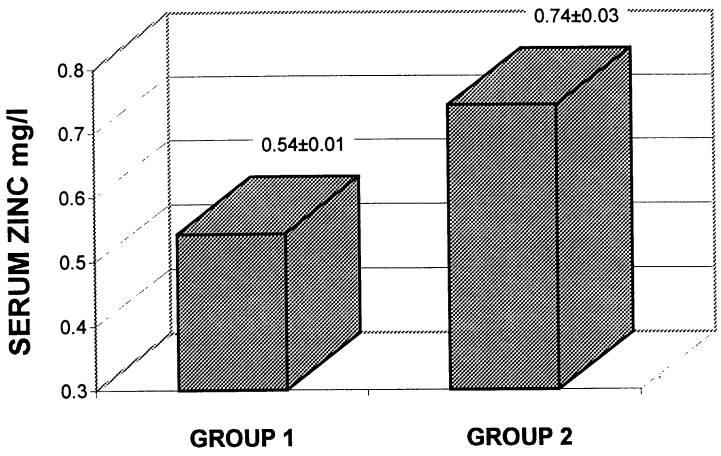

Fig. 1. Serum zinc in the two groups of footbal players at rest, $p = 3.62 \times 10^{-5}$. Group 1 = hypozincemic subjects ($n = 9$); Group 2 = normozincemic subjects ($n = 12$).

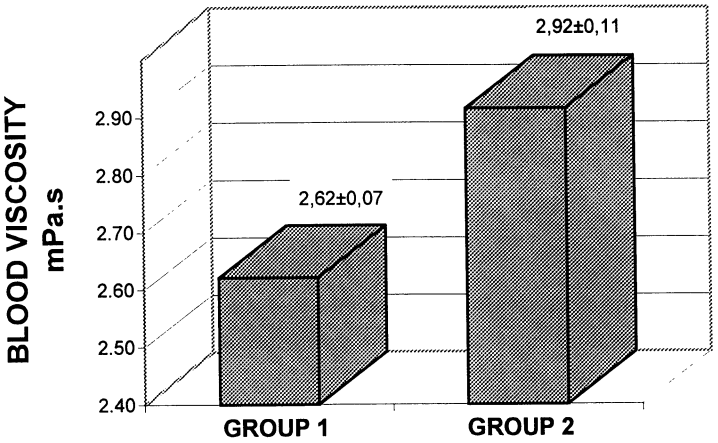

Fig. 2. Blood viscosity at native hematocrit in the two groups of footbal players at rest. Group 1 = hypozincemic subjects ($n = 9$); Group 2 = normozincemic subjects ($n = 12$).

Table 1
Modifications of biological parameters during the exercise test in group 1 (low serum zinc) and group 2 (normal serum zinc). T_0 = at rest; T_1 = during the test below the 4 mmol l^{-1} lactate threshold; T_2 = above this threshold; T_3 = recovery

		At rest	During exercise test		Recovery
	Group	T_0	T_1	T_2	T_3
Hematocrit (%)	1	44.44 ± 0.84	42.56 ± 0.78	42.56 ± 0.80	43.22 ± 0.86
	2	43.33 ± 0.82	44.92 ± 0.87*	44.83 ± 0.52*	44.50 ± 0.63
Insulinemia (UI/ml)	1	12.78 ± 2.86	8.00 ± 1.68**	6.56 ± 1.09	8.78 ± 1.68
	2	8.58 ± 1.37	5.92 ± 0.29**	5.67 ± 0.41	8.58 ± 0.84
Blood viscosity at native hematocrit (mPa·s)	1	2.62 ± 0.07	2.88 ± 0.10	2.99 ± 0.07	2.90 ± 0.12
	2	2.91 ± 0.10*	2.89 ± 0.07	3.01 ± 0.08	3.14 ± 0.09
Blood viscosity corrected for hematocrit (45%) (mPa·s)	1	2.38 ± 0.03	2.42 ± 0.05	2.42 ± 0.04	2.44 ± 0.05
	2	2.33 ± 0.04	2.31 ± 0.04	2.29 ± 0.04*	2.32 ± 0.05

*$p < 0.05$ vs. group 1; **$p < 0.05$ vs. T_0.

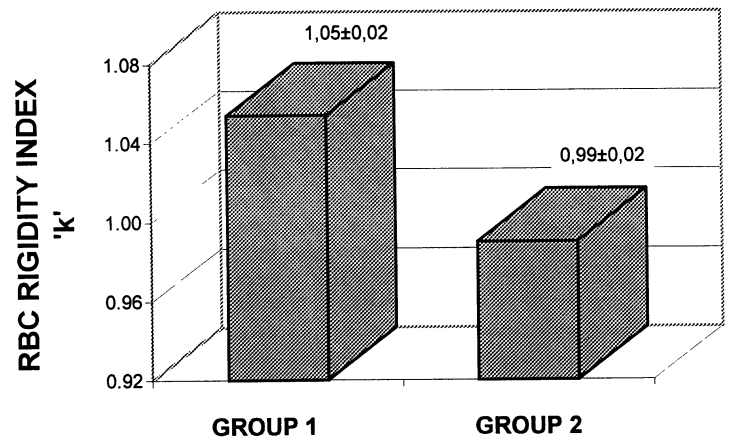

Fig. 3. RBC rigidity index 'k' in the two groups of footbal players at rest. Group 1 = hypozincemic subjects ($n = 9$); Group 2 = normozincemic subjects ($n = 12$).

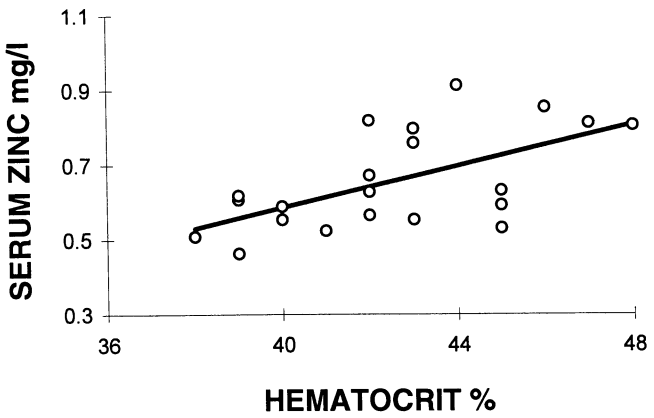

Fig. 4. Correlation between serum zinc and hematocrit at rest, $r = 0.6$, $p = 0.004$.

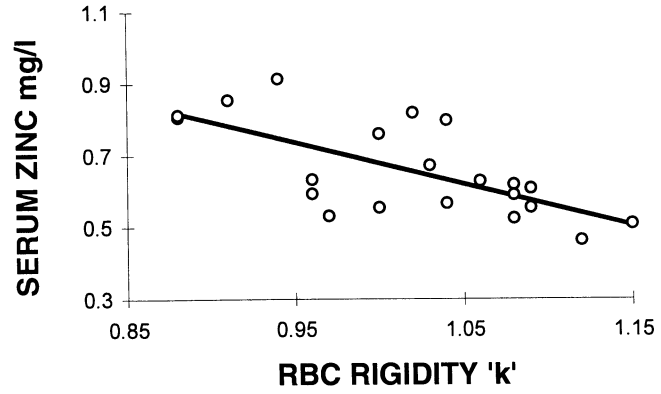

Fig. 5. Correlation between serum zinc and 'k' at rest, $r = -0.68$, $p = 0.7 \times 10^{-3}$.

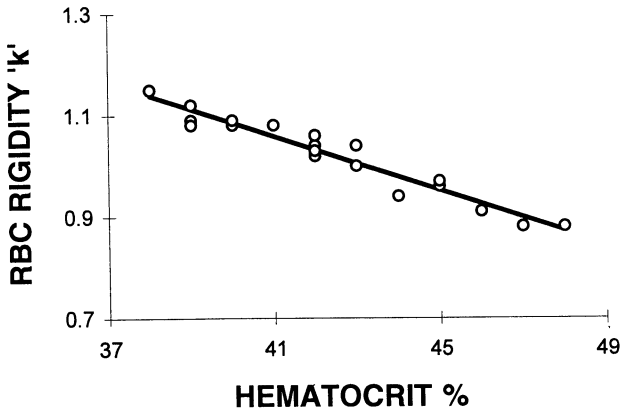

Fig. 6. Correlation between hematocrit and erythrocyte rigidity index 'k', $r = -0.97$, $p < 0.0001$.

4.2. *Responses to exercise* (T_1, T_2)

$VO_{2\,max}$ was found to range between 40.8 and 80 ml/min/kg. W_{170} ranged between 2.37 and 4.17 watt/kg. The lactate peak ranged beteen 3.2 and 11 mmol/l. The 2 mmol/l lactate threshold ranged between 33 and 83% of $VO_{2\,max}$, and the 4 mmol/l lactate threshold ranged between 45 and 100% of $VO_{2\,max}$. Serum zinc remains unchanged during exercise (Fig. 7). Hypozincemic subjects were able to perform a lower output (123 ± 8.7 vs. 166.3 ± 14.8 watt, $p = 0.029$) (Fig. 8) and exhibited a higher increase in blood lactate (7.5 ± 0.8 vs. 5.6 ± 0.3 mmol/l, $p = 0.024$) (Fig. 9), resulting in a lower 2 mmol lactate threshold ($44.7 \pm 3.9\%$ vs. $58.9 \pm 4.8\%$ of maximal power output $p = 0.04$) (Fig. 10).

Glycemia decreased during exercise in hypozincemic subjects while it increased in normozincemic subjects, they were less able to maintain their plasma glucose and exhibited a tendency towards hypoglycemia ($p = 0.0153$) (Fig. 11). Insulinemia decreased significantly during exercise ($p =$

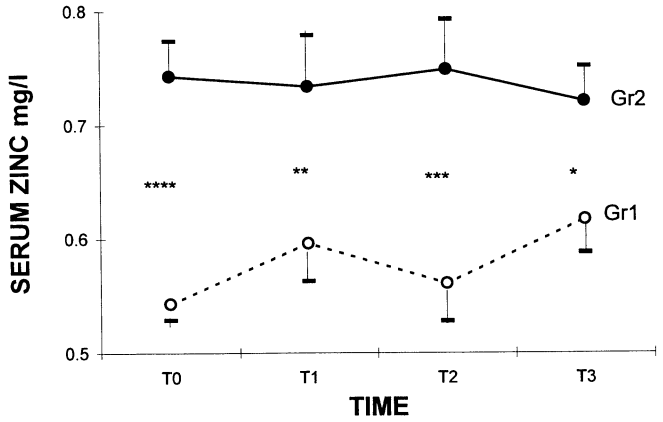

Fig. 7. Serum zinc remains unchanged in the two groups during exercise and remains lower in Group 1 = hypozincemic subjects ($n = 9$) than in Group 2 = normozincemic subjects ($n = 12$).

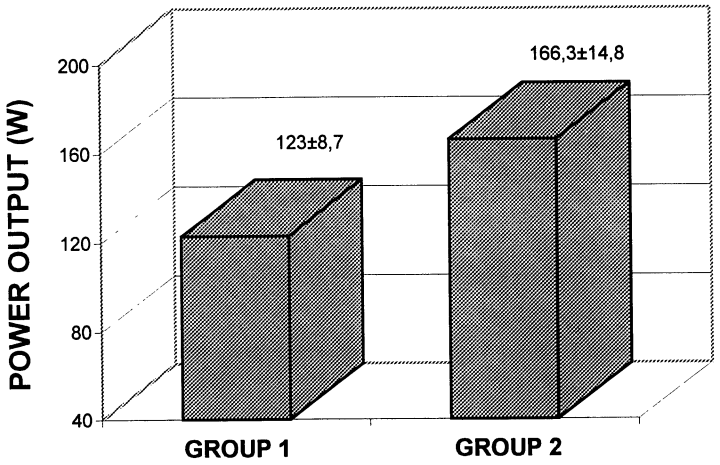

Fig. 8. Hypozincemic subjects had a lower power output than normozincemic subjects, $p = 0.029$. Group 1 = hypozincemic subjects ($n = 9$), Group 2 = normozincemic subjects ($n = 12$).

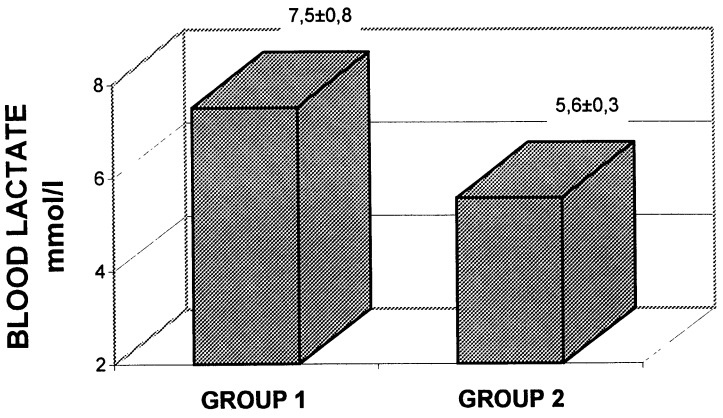

Fig. 9. Hypozincemic subjects had a higher peak blood lactate than normozincemic subjects, $p = 0.024$. Group 1 = hypozincemic subjects ($n = 9$), Group 2 = normozincemic subjects ($n = 12$).

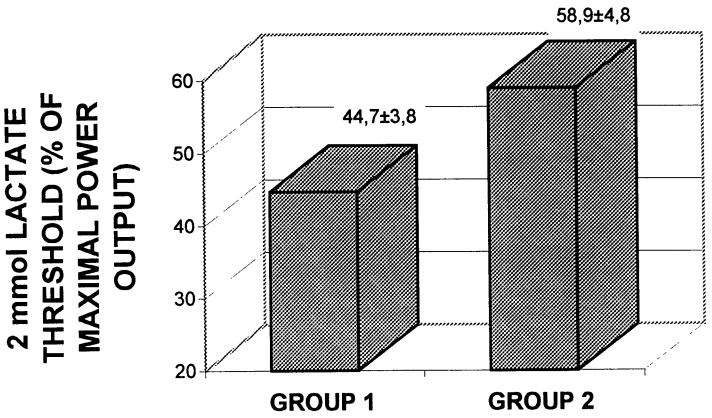

Fig. 10. 2 mmol lactate threshold is lower in hypozincemic subjects, $p = 0.04$. Group 1 = hypozincemic subjects ($n = 9$), Group 2 = normozincemic subjects ($n = 12$).

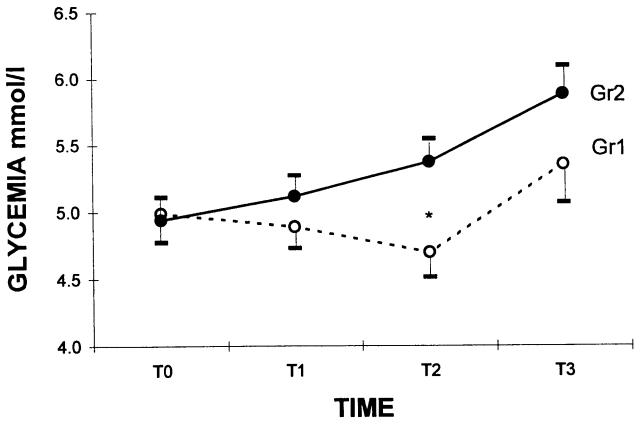

Fig. 11. Glycemia decreased during exercise in hypozincemic subjects, $p = 0.0153$. Group 1 = hypozincemic subjects ($n = 9$), Group 2 = normozincemic subjects ($n = 12$).

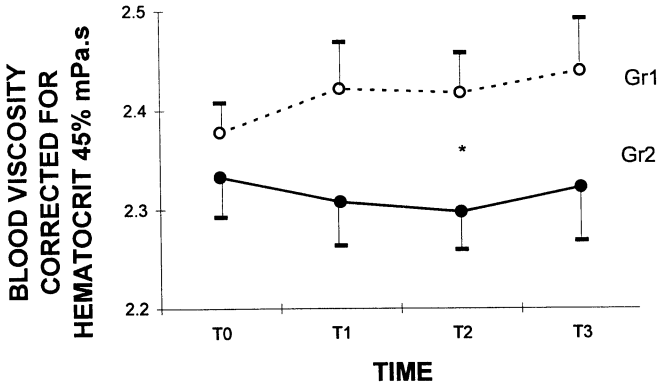

Fig. 12. Blood viscosity corrected for hematocrit (45%) remained higher during exercise in hypozincemic subjects, $p = 0.003$. Group 1 = hypozincemic subjects ($n = 9$), Group 2 = normozincemic subjects ($n = 12$).

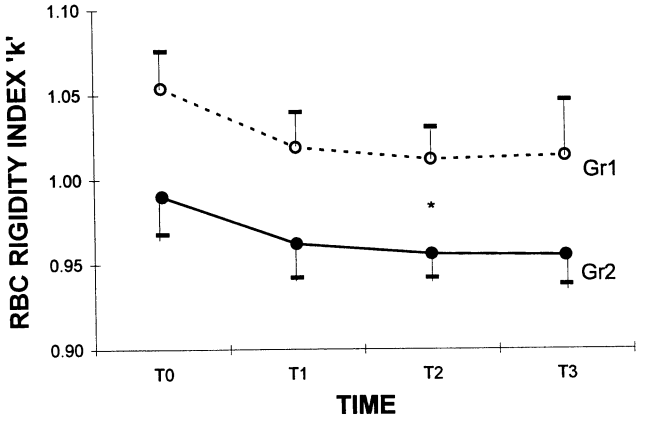

Fig. 13. RBC rigidity index 'k' remained significantly higher during exercise in hypozincemic subjects, $p = 0.0002$. Group 1 = hypozincemic subjects ($n = 9$), Group 2 = normozincemic subjects ($n = 12$).

0.0067) (Table 1). Blood viscosity corrected for hematocrit (45%) remained higher during exercise in these hypozincemic subjects ($p = 0.003$) (Fig. 12). The 'k' RBC rigidity index remained significantly higher during exercise in hypozincemic subjects ($p = 0.0002$) (Fig. 13).

5. Discussion

The aim of this study was to investigate the relationships between zinc status and both blood rheology and glucoregulation during exercise in sportsmen. We observe that footballers with a low serum zinc have a higher blood viscosity, that could be explained by an increased erythrocyte rigidity. A compensatory reduction of hematocrit uncompletely corrects this hyperviscosity.

Hypozincemia is also associated with low blood glucose and a higher increase in blood lactate. This pattern may explain at least in part the lower power output and the lower lactate threshold observed in the group of hypozincemic subject during this exercise protocol.

Our finding of a low serum zinc in this sample of sportsmen is consistent with some previous reports [7,41]. Dressendorfer and Sockolov [7] found a lower serum zinc (0.76 mg/l) in long distance

male runners compared to sedentary controls (0.94 mg/l). Singh [41] reported a lower serum zinc contrasting with a higher concentration of zinc in erythrocytes, in a group of runners compared to controls. Our values are slighthly lower than those reported by these investigators, but in another study we observed in young gymnasts an even lower mean zinc concentration [10] supporting the concept that regular exercise may induce markedly low values of serum zinc, and that this effect can be potentiated by the physiological increase in zinc needs during adolescence.

While serum zinc is not a very sensitive marker of zinc status (i.e., it can be normal in cases of zinc deficiency) such low values of zinc are highly suggestive for a reduction of body's zinc stores [42].

The mechanism for this zinc loss during exercise is uncompletely known. Dressendorfer and Sockolov [7] assume that serum zinc levels are inversely related to the running distance during training sessions. They report mean values of zincemia of 0.81 and 0.67 mg/l for races of respectively 10–19 km and 64–135 km. Clarkson [43] assumes that zinc loss in athletes can be induced by a low zinc containing diet associated with increased losses in urine and sweat. In our opinion, there is no clear evidence of an increase in urinary zinc excretion during exercise, since exercise protocols which markedly increase microalbuminuria in diabetics do not increase urinary zinc [44,45]. Recently Lukaski and coworkers [46–48] stated that neither training nor acute exercise did modify zinc status in adult sportsmen, a conclusion which is not in agreement with our findings. It is clear from our study that in some conditions sportsmen have very low serum zinc values which are highly suggestive for deficiency in this trace element, as we also observed in gymnasts [10]. However, we are unable to conclude whether exercise acutely modifies zincemia or not. Our results show no significant change, but with a β risk (type 2 error) as high as 92%.

Since the zinc status is dependent upon the nutritional status and may in turn affect body size and composition, we investigated the relationships between zincemia and height, weight, and body mass index. Results did not support any correlation between these parameters in this sample.

Our finding of a relationship between zincemia and blood rheology is more original. While some *in vitro* studies [20–24] have demonstrated that this trace element improves erythrocyte deformability in various models of hardened red cells, an *in vivo* action of this cation on this rheologic property has never, to our knowledge, been investigated.

The inverse correlation we find between serum zinc and erythrocyte rigidity (Fig. 5) suggests that low serum zinc is associated with more rigid erythrocytes. The previously reported effect of zinc on the deformability of sickle cells [20–22] concerns a very special model of rigid erythrocytes, but previous experiments of our group on erythrocytes hardened by a 'stress buffer' [23,24] suggest that zinc improves also the deformability of normal red cells submitted to an osmotic damage. The cation Zn^{++} has been hypothesized to protect the red cell again calcium-induced binding of the erythrocyte cytoskeleton to the membrane. In addition, Zn^{++} plays a role in the function of erythrocyte enzymes involved in membrane functions, ion exchanges and protection against free radical damage [1,21,49, 50]. Although further studies will be needed, our results put together with this literature on the *in vitro* effects of zinc (and with preliminary unpublished data from our laboratory) support to some extent the hypothesis of a physiological *in vivo* effect of zinc on red cell deformability.

Blood viscosity (even at a corrected hematocrit 45%) is higher in subjects with low serum zinc. Most of this hyperviscosity seems to be explained by an increase in erythrocyte rigidity index "k". We are unable in this study to detect a significant increase in blood viscosity at corrected hematocrit during exercise, but there is a high value of β risk (82%) and in previous studies from our laboratory this parameter has been shown to increase during similar exercise protocols [51].

A positive correlation between serum zinc and hematocrit (Fig. 4) may be considered surprising. In fact, it seems to be explained by a compensatory decrease in hematocrit resulting from increased

erythrocyte rigidity in subjects with hypozincemia. This explanation is supported by the negative correlation between hematocrit and erythrocyte rigidity (Fig. 6), which seems to reflect the classical mechanism of 'viscoregulation' [52,53], i.e., there is a homeostatic reduction in hematocrit when factors of viscosity increase.

We observe in this protocol the classical increase in blood viscosity during exercise [25,26]. In a previous study we reported that short exercise sessions (15 min) induced a hyperviscosity explained only by an increase in plasma viscosity and hematocrit, while longer sessions (25 min) increased also erythrocyte rigidity proportionally to blood lactate concentrations [26,54]. In this study, there is an increase in blood viscosity at native hematocrit, while neither erythrocyte rigidity nor blood viscosity at corrected hematocrit 45% significantly change. Since the β risks are, respectively, 24 or 82%, it seems reasonable to conclude that this short exercise protocol does not increase erythrocyte rigidity, consistent with our previous reports [26,51,54,55].

A positive correlation between serum zinc and blood viscosity is also found. It may be considered somewhat paradoxical. Actually, partial correlation analysis shows that this correlation does not indicate that zinc increases blood viscosity. It is rather explained by the reduction in hematocrit resulting from high values of erythrocyte rigidity in zinc-deficient subjects.

Finally, we find some relationships among zinc status, blood rheology and fitness. An improvement of blood fluidity has been repeatedly described in trained sportsmen [27–29,51,55]. It seems to result from an increase in extracellular water [56] which can be compared to an 'autohemodilution'. On a theoretical basis, this increase in plasma volume associated with a reduction in blood viscosity may improve heart's work, by increasing preload and reducing postload. However, some other mechanisms could be involved in modifications of blood rheology related to the training status, and thus participate to this relationship between fitness and blood fluidity. There is some preliminary evidence that nutritional status [30–32] modifies these relationships between exercise and rheology. In this context, it was interesting to investigate a possible role for zinc.

In our study, erythrocyte rigidity and blood viscosity (at corrected hematocrit 45%) are higher in hypozincemic sportsmen. Thus, hypozincemia is associated to a tendency to hyperviscosity, together with a lower power output and a higher increase in blood lactate, as shown by the low 2 mmol lactate threshold. In several previous papers of our group, we reported that sportsmen with higher blood viscosity exhibited a stronger accumulation of lactates into blood [29,57,58]. This study is in agreement with those previous reports. In addition, hypozincemic subjects were less able to maintain their plasma glucose and exhibited a tendency towards hypoglycemia which parallels the rise in blood lactate.

Several muscular enzymes (lactate dehydrogenase, pyruvate carboxylase, AMP-deaminase) are dependent upon zinc status [1–3]. A reduction in body's zinc stores may impair aerobic glycolysis and results in both a waste of glucose through anaerobic pathways and an increased lactate production [1–3,13,17]. However, increased blood viscosity resulting from zinc depletion may also, according to our previous findings [29,57,58], explain some of this hyperlactatemia *via* alterations in muscle microcirculation. The experiments of Vicaut's group have provided unequivocal evidence that hyperviscosity (hyperaggregation of red cells) dramatically modifies erythrocyte distribution in muscle microvessels [59].

In conclusion, this study suggests that the zinc status may influence blood rheology during exercise, either by its direct action on RBC flexibility or by an effect related to lactate accumulation which may in turn modify erythrocyte flexibility. We hypothesize that the zinc status influences exercise performance also by hemorheologic effects. A study with zinc supplementation in volunteers will be needed for further elucidating this question.

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