

Effects of zinc supplementation on blood rheology during exercise

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Abstract. We previously reported a higher blood viscosity at corrected hematocrit (45%) (explained by a higher value of erythrocyte rigidity) in football players with low serum zinc (Zn) and thus presumably Zn deficiency; subjects with low serum zinc had also an impairment in performance. This interventional study was undertaken in order to assess the effects of zinc supplementation (compared to placebo) on blood rheology and performance either at rest or during exercise. Ten male healthy volunteers (age: 26 ± 1.3 yr; weight 67.9 ± 2.24 kg; height 177 ± 3 cm) received at random order either zinc (20 mg/day) and placebo, according to a double blind cross-over procedure, during seven days. In each case on the eighth day they performed a 25 min submaximal exercise-test. At rest blood viscosity at corrected hematocrit 45% ($\gamma = 1000 \text{ s}^{-1}$) was lower after Zn (3.56 ± 0.14 vs. 4.13 ± 0.16 mPa.s, $p = 0.009$), explained by a lower RBC rigidity index 'k' according to Quemada's equation (1.65 ± 0.07 vs. 1.84 ± 0.08 , $p = 0.03$). Hematocrit and plasma viscosity were unchanged, but RBC aggregation was decreased (laser retrodiffusion-derived aggregation time 'Ta' 3.52 ± 0.51 vs. 2.75 ± 0.59 , $p = 0.02$). The increase in blood viscosity during exercise is lower after Zn than placebo. Blood viscosity at corrected hematocrit 45% remains unchanged during exercise after Zn, yet it increases after placebo. RBC rigidity index 'k' remains lower during exercise after Zn. The rating of perceived exertion (Borg's scale) at the 20th minute of exercise is lower after zinc (5.6 ± 0.4 vs. 6.6 ± 0.4 , $p = 0.008$). This study confirms that Zn improves erythrocyte deformability, decreases the exercise-induced acute increase in blood viscosity, and improves exercise tolerance. Since Zn deficiencies are not unfrequent in sportsmen, these findings may be potentially relevant to sports nutrition.

Keywords: Zinc supplement, erythrocyte deformability, erythrocyte aggregation, rheology, exercise

1. Introduction

Zinc is a trace element involved in the function of more than 200 metalloenzymes [1–3] and exerts insulinlike and ergogenic effects [4–6]. Although still controversial, there is some evidence for the occurrence of zinc-deficient states in western countries, mostly in situations like puberty or exercise [7–9] where zinc turnover is increased.

In a recent study we observed in elite sportsmen that hypozincemic individuals had both a reduction of performance and an increase in blood viscosity, mainly explained by a higher value of erythrocyte rigidity [10]. However, such a correlational study could not by itself demonstrate that zinc is a factor involved *in vivo* in the regulation of blood rheology in sportsmen, neither it provides any answer on the potential importance of zinc-related rheologic alterations in exercise performance.

Thus, this interventional study was undertaken in order to investigate whether oral zinc supplementation (compared to placebo) influences blood rheology (either at rest or during exercise) and whether these changes modify also exercise performance.

2. Subjects and methods

Ten male healthy controls (age 26 ± 1.3 yr; weight 67.9 ± 2.24 kg; height 177 ± 3 cm; body mass index (BMI) 21.69 ± 0.51 kg/m²) were studied. They received at random order zinc and placebo according to a double blind cross-over procedure, each subject being his own control. Subjects were exercising only at recreational times and were not involved in a training program. They received per os 20 mg daily of zinc gluconate (two capsules of 10 mg), or placebo (two capsules of 10 mg) during seven days in each case. Subjects ingested one capsule 2 h after breakfast and another one 2 h after dinner. The aspect of zinc gluconate and placebo capsules was absolutely identical. Obviously subjects were asked to maintain the same diet during the study. All subjects gave their informed written consent and the study was performed in accordance with the local ethical regulation.

Subjects underwent at the end of each seven days period a 25 min incremental exercise-test with 5 min successive steps (5 min at 50 W, 5 min at 100 W, and 15 min at 150 W). On the first 15 min $\dot{V}O_{2\max}$ was calculated [11] as well as an evaluation of $\dot{V}O_{2\max}$ according to Astrand and Ryhming [12]. During the last 10 min subjects were asked to cycle at 85% of the theoretical maximal heart rate assumed to be $(220 - \text{age})$ [13]. This exercise-test was performed on a cycloergometer (Bodyguard, Jonas Oglaend A.S., N 4301-Sandnes, Norway). Pedal speed was kept constant at 60 rpm during the test. Every 5 min subjects were asked to quote their subjective feeling of exertion on a Borg's category-ratio scale with scores ranging between 0 and 10 [14].

The exercise-test was preceded 2 h before by a standardized breakfast [15] and was followed by a 10 min recovery.

Blood samples were drawn before exercise test (T_0), during the test at 10 (T_{10}) and 25 min (T_{25}) and after 10 min recovery (T_{35}). 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 ($\gamma = 1000 \text{ s}^{-1}$) with a falling ball viscometer (MT 90 Medicatest, F-86280 Saint Benoit) [16,17]. Accuracy of the measurements was regularly controlled with the Carrimed Rheometer 'CS' (purchased from Rhéo, 91120 Palaiseau, France) [18]. The coefficient of variation of this method ranges between 0.6 and 0.8% [19]. 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 [20]. Hematocrit was measured with microcentrifuge. Index of erythrocyte rigidity 'k' was calculated according to the equation of Quemada. The primary aggregation time of red cells ('Ta', i.e., the time necessary for appearance of the first rollers as determined by analysis of the first 5 seconds of stasis) was measured with the erythroaggregometer AFFI-BIO (Licence INSERM, France) [21,22].

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) ranges between 4 and 7%.

Before every exercise-test, isometric measurements of handgrip and adductor strength were performed with specific ergometers designed by J.P. Micallef, INSERM U103 (Biomechanics, Montpellier). These devices are made of a strength gauge with a liquid crystal screen indicating the force in Newtons. Physical

Table 1
Serum zinc and viscosimetric measurements at rest (T_0)

Parameter	Placebo	Zinc	<i>p</i>
Serum zinc (mg/l)	0.74 ± 0.03	0.73 ± 0.05	NS
Blood viscosity at native hematocrit (mPa.s)	3.91 ± 0.16	3.48 ± 0.14	0.05
Blood viscosity corrected for hematocrit (45%) (mPa.s)	4.13 ± 0.16	3.56 ± 0.14	0.009**
RBC rigidity index 'k'	1.84 ± 0.08	1.65 ± 0.07	0.03*
Plasma viscosity (mPa.s)	1.40 ± 0.06	1.38 ± 0.03	NS
Hematocrit (%)	43.50 ± 1.17	44.10 ± 0.50	NS
RBC aggregation time 'Ta' (s)	2.75 ± 0.29	3.52 ± 0.51	0.02*

* $p < 0.05$; ** $p < 0.01$.

working capacity \dot{W}_{170} (W/kg) was calculated, this being the work that the subjects can perform at a heart rate of 170 b/min expressed per kg of body weight [11,23]. The maximal oxygen uptake ($\dot{V}O_{2 \max}$) was also evaluated from the initial submaximal steps according to the classical Astrand and Ryhming procedure [12].

3. Statistics

Values are given as mean \pm standard error on the mean. Comparisons were performed with the Wilcoxon rank sum test for paired data and by two factors analysis of variance when appropriated. Significance level was defined as $p < 0.05$ (first level) and $p < 0.01$ (second level).

4. Results

4.1. At rest (T_0)

As shown in Table 1, serum zinc did not change in zinc-supplemented subjects when compared to placebo. By contrast, apparent blood viscosity at native hematocrit is moderately decreased after zinc. When corrected for hematocrit at 45%, blood viscosity is significantly decreased after zinc (3.56 ± 0.14 vs. 4.13 ± 0.16 , $p = 0.009$) as well as the viscometric index of red blood cell rigidity 'k' given by Quemada's equation (1.65 ± 0.07 vs. 1.84 ± 0.08 , $p = 0.03$). Neither plasma viscosity nor hematocrit did change after zinc. Red cell aggregation time 'Ta' significantly increased after zinc (3.52 ± 0.51 vs. 2.75 ± 0.29 , $p = 0.02$).

4.2. Responses to exercise (T_{10} , T_{25})

Serum zinc was unchanged during exercise, either after zinc or placebo (Fig. 1). Blood viscosity at native hematocrit increased during exercise at T_{25} in zinc-supplemented subjects (4.04 ± 0.24 vs. 3.48 ± 0.16 mPa.s, $p = 0.03$) as well as in subjects who had received placebo (4.52 ± 0.24 vs. 3.91 ± 0.16 mPa.s, $p = 0.02$). However, zinc did not appear to influence this increase (Fig. 2). Plasma viscosity did not appear to be significantly influenced by zinc, but it increased during exercise at T_{10} (1.46 ± 0.03 vs. 1.38 ± 0.03 , $p = 0.037$) and T_{25} (1.47 ± 0.04 vs. 1.38 ± 0.03 , $p = 0.033$) (Fig. 3). Hematocrit increased during exercise: after zinc at T_{10} (46 ± 0.52 vs. 44.1 ± 0.5 , $p = 0.008$) and T_{25} (47.1 ± 0.6 vs. 44.1 ± 0.5 , $p = 0.0006$) and after placebo at T_{10} (46 ± 0.93 vs. 43.5 ± 1.17 , $p = 0.048$) and

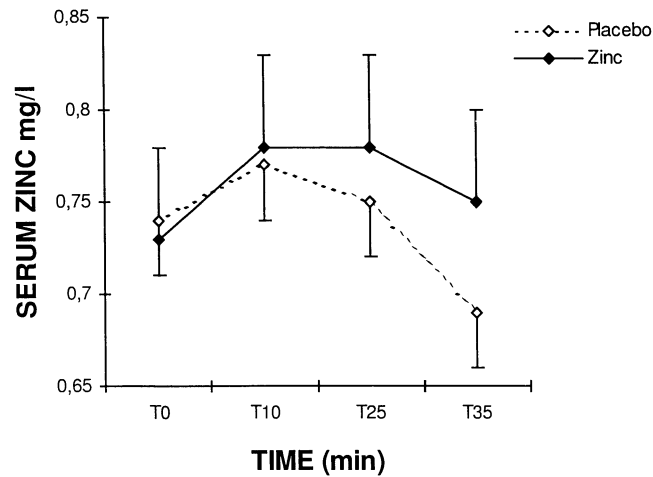


Fig. 1. Serum zinc during exercise after zinc supplementation (compared to placebo) ($n = 10$).

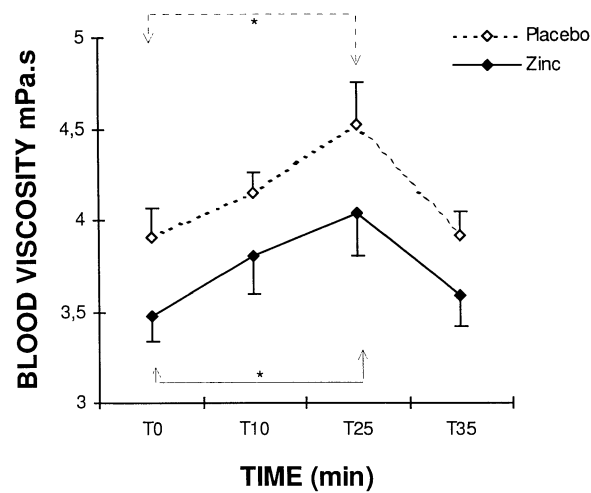


Fig. 2. Blood viscosity at native hematocrit after zinc (compared to placebo) during exercise ($n = 10$, $*p < 0.05$).

T₂₅ (46.6 ± 1.33 vs. 43.5 ± 1.17 , $p = 0.048$). However, there was no difference between the curves obtained in zinc supplemented subjects and placebo-treated subjects (Fig. 4). Corrected blood viscosity at hematocrit 45% did not significantly change during exercise but it remained significantly lower in zinc-supplemented subjects at T₁₀ (3.71 ± 0.2 vs. 4.06 ± 0.13 , $p = 0.024$) and T₂₅ (3.81 ± 0.21 vs. 4.32 ± 0.2 , $p = 0.014$) (Fig. 5). The erythrocyte rigidity index 'k' calculated from Quemada's equation remained unchanged during exercise, but was significantly lower in zinc-supplemented subjects at both T₁₀ (1.63 ± 0.08 vs. 1.80 ± 0.09 , $p = 0.04$) and T₂₅ (1.65 ± 0.07 vs. 1.80 ± 0.06 , $p = 0.005$) (Fig. 6). Red blood cell aggregation time 'Ta' did not appear to be influenced by either zinc or exercise (at T₁₀: 3.17 ± 0.87 vs. 2.60 ± 0.34 , at T₂₅: 2.64 ± 0.5 vs. 2.53 ± 0.36).

Physical parameters: isometric adductor strength (N), handgrip strength (N), W₁₇₀ (W/kg) and VO_{2 max} (ml/min/kg) were not significantly modified after zinc, compared to placebo (Table 2). By contrast, the rating of perceived exertion (as measured with Borg' scale) was lower in zinc-treated subjects (5.6 ± 0.4 vs. 6.6 ± 0.42 , $p = 0.008$) at the 20th minute of exercise (Fig. 7).

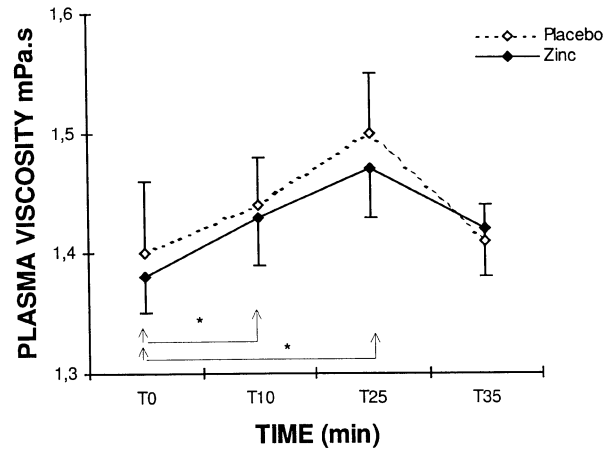


Fig. 3. Plasma viscosity after zinc (compared to placebo) during exercise ($n = 10$, $*p < 0.05$).

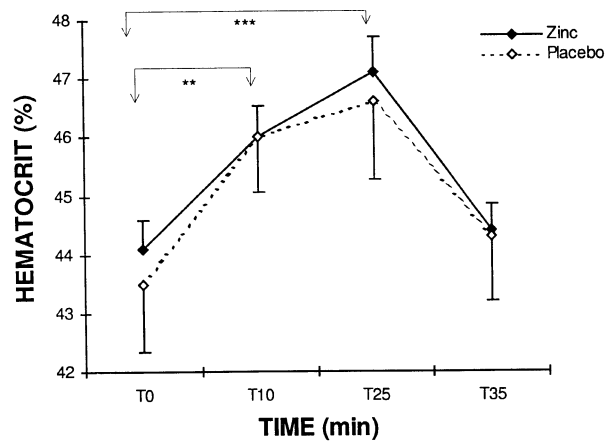


Fig. 4. Hematocrit after zinc (compared to placebo) during exercise ($n = 10$, $**p < 0.01$, $***p < 0.001$).

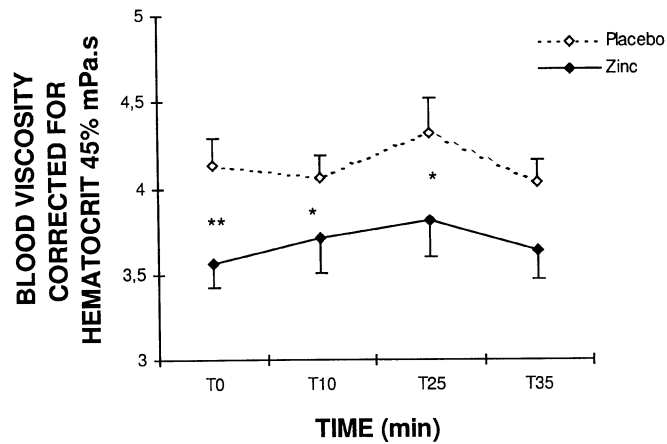


Fig. 5. Blood viscosity corrected for hematocrit (45%) after zinc (compared to placebo) during exercise ($n = 10$, $*p < 0.05$, $**p < 0.01$).

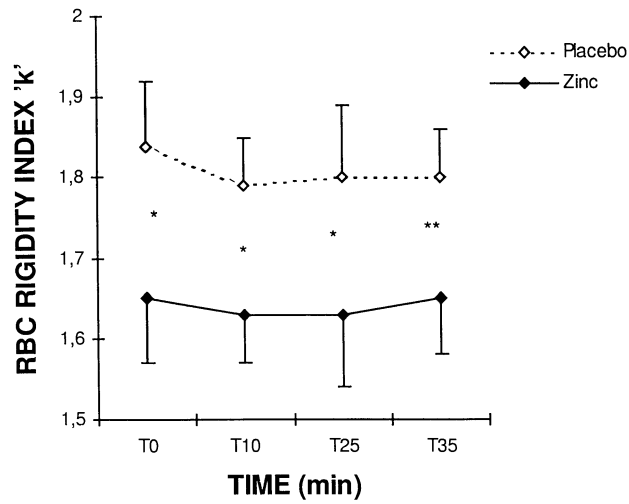


Fig. 6. RBC rigidity index 'k' after zinc (compared to placebo) during exercise ($n = 10$, $*p < 0.05$, $**p < 0.01$).

Table 2

Physical parameters in zinc-supplemented and placebo-supplemented subjects

Parameter	Placebo	Zinc	<i>p</i>
Adductor strength (N)	855.40 ± 55.67	963.40 ± 111.38	0.08
Handgrip strength (N)	576.80 ± 31.75	611.90 ± 37.10	0.12
\dot{W}_{170} (W/kg)	2.30 ± 0.23	2.48 ± 0.31	0.08
$\dot{V}O_2$ max (ml/min/kg)	39.67 ± 4.32	43.71 ± 6.27	0.08

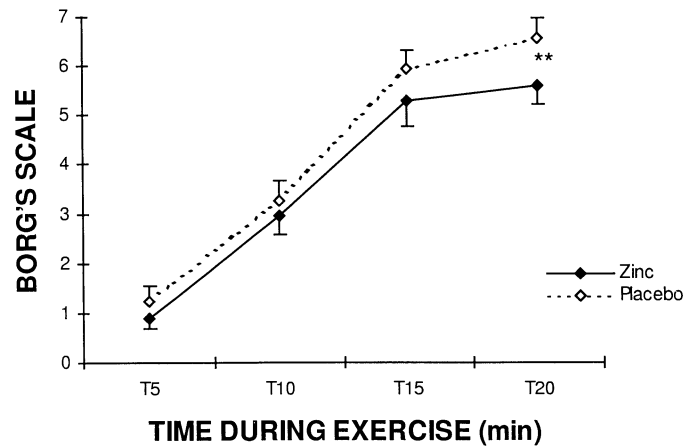


Fig. 7. The rating of perceived exertion (Borg's scale) after zinc (compared to placebo) during exercise ($n = 10$, $**p < 0.01$).

5. Discussion

In a previous study we observed relationships among zinc status, blood rheology, and exercise performance in trained athletes. All the results suggested that zinc had a beneficial effect on both blood rheology and performance [10]. Therefore, this study aimed at determining the effects of a short dura-

tion, low-dose zinc supplementation, on blood rheology, and exercise performance in healthy volunteers matched for age and sex with the sportsmen of the previous study.

Results show that seven day zinc supplementation did not change serum zinc. This is consistent with literature showing that a low dose of zinc (229 $\mu\text{mol/day}$, i.e., recommended intake for young adults) has no effect on zincemia [24]. By contrast, hemorheologic modifications are observed after this zinc supplementation, compared to placebo.

Blood viscosity at native hematocrit is lower after zinc. This is not explained by differences in either plasma viscosity or hematocrit, since both of these parameters are not modified by zinc compared to placebo. By contrast, there is a significant decrease of the erythrocyte rigidity index 'k' after zinc, that could explain a decrease in blood viscosity at corrected hematocrit 45% after zinc intake.

It is interesting to point out that this picture is closely similar to what we previously reported in a cross-sectional study of footballers: when athletes had a low serum zinc at rest, their blood viscosity at corrected hematocrit 45% was higher than in normozincemic subjects, and a higher value of 'k' (that was negatively correlated to serum zinc) was likely to explained this difference [10].

There are a few studies showing that zinc salts improve blood fluidity both *in vitro* and *in vivo*, by improving deformability of hardened red cells in patients suffering from sickle-cell disease [25,26]. Similar results were found in our laboratory with artificially hardened red cells when concentrations of zinc similar to those found *in vivo* were added [27]. Zinc appeared to rapidly improve deformability of red cells hardened by either heating or an hyperosmolar hypercalcic medium [28]. It has been suggested that zinc modifies some properties of cell membranes [1,26,29]. However, most of the effect of zinc could be due to an interaction with intracellular calcium [30], since zinc (as well as magnesium [28]) has been reported to antagonize this other bivalent cation. When important quantities of zinc are contained in the cell membrane, this membrane appears to be stabilized and protected while zinc deficiency has the opposite effect and impairs membrane functions, so that membrane dysfunction is a symptom of zinc deficiency [31].

On the whole, our results together with those of the previous studies reported above support the concept of zinc being a factor physiologically involved in the regulation of erythrocyte deformability.

Our data on erythrocyte aggregation require some comments. The aggregation time 'Ta' significantly increases in zinc-treated subjects, indicating that erythrocyte aggregability is decreased. Therefore, zinc is likely to improve also erythrocyte aggregability. To our knowledge, a role for zinc in the regulation of this physiological property of erythrocyte has not yet been reported. By comparison with red cell deformability, a competition of zinc with calcium may be hypothesized, but there are no experimental data to support this assumption. This aspect requires further investigation.

In this study, serum zinc is unchanged during exercise, whatever the zinc intake during the preceding week. In untrained subjects, an increase in serum zinc during short term exercise has been described, resulting probably from hemoconcentration [32–35]. Such an increase has also been reported in long-duration exercise sessions [36,37], but a decrease has also been reported in such a case [38], presumably explained by stress and acute phase reaction [35]. In fact, zinc status seems to influence the serum zinc response to a given exercise protocol, since Lukaski and coworkers [39] have demonstrated that alimentary zinc restriction in healthy individuals decreases their serum zinc response to exercise while increased zinc intake (33.6 mg/day during 30 days) increases it. Thus, serum zinc response during exercise may be mostly the reflect of body zinc stores [10]. However, in this study, we find no significant difference between subjects who had received seven days zinc and subjects treated with placebo supplementation.

During exercise, increases in blood viscosity, plasma viscosity and hematocrit are classical findings [40,41] that are evidenced here once again. By contrast, 'k' index and blood viscosity at corrected hema-

tocrit (45%), that are both indices of erythrocyte rigidity, remain unchanged during the exercise protocol, although they are lower in zinc supplemented individuals and remain lower throughout the exercise test. This is consistent with our previous cross-sectional study on trained footballers that evidenced quite the same difference between normozincemic and hypozincemic sportsmen [10].

In this study, erythrocyte aggregation time is not influenced by exercise, whatever the zinc intake during the previous week. This is consistent with most studies showing no change of this rheologic property during short exercise bouts [42]. However, we recently reported in trained sportsmen a decrease in erythrocyte disaggregability during the same kind of exercise protocol [43]. The lack of change in aggregability in this study may be a type 2 error resulting from the low number of subjects.

Finally, some comments on ergometric measurements are required. High doses of zinc (135 mg/day during 14 days) have been shown to increase isometric and isokinetic exercise performance in 15 women [44] when compared to placebo. In two previous studies, we reported a decrease in performance when sportsmen become hypozincemic [10]. By contrast, in this experiment, subjects had a normal zinc status and the doses of zinc used for supplementation were 6-fold lower, in order to remain within a physiological range. These two points are likely to explain why neither isometric strength nor aerobic working capacity (\dot{W}_{170} or $\dot{V}O_{2\max}$) are modified by zinc in our study. Presumably, the effects of zinc on performance are obvious in situations of zinc deficiency (impaired performance) or when supraphysiological doses are administered [44]. Actually, a moderate ergogenic effect of zinc even in the situation of this experiment should not be ruled out and might perhaps be observed if the sample of patients were larger, as suggested by the improvement of the subjective feeling of exertion when subjects were given zinc. We think that either a stronger dose of zinc, or a larger number of subjects, or a study in subjects with zinc deficiency would probably detect an ergogenic effect of zinc, consistent with the previous literature.

On the whole, this study clearly demonstrates that zinc intake, at a physiological dose, improves blood rheology in healthy sedentary subjects. This effect is mainly explained by an increase in red cell deformability, and possibly a decrease in red cell aggregability. Whether this effect has a beneficial effect on exercise performance is less clear, although the subjective feeling of exercise tolerance is significantly improved. The next step of this study will be to analyze the consequences of this zinc supplementation on blood glucose and lactate kinetics.

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