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Opposite effects of in vitro lactate on erythrocyte deformability in athletes and untrained subjects

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Abstract. Exercise transiently increases blood viscosity: however data on red cell deformability in this process remain inconsistent, since studies report either impairment (proportional to blood lactate accumulation), a lack of effect, or even in some cases an improvement. To test whether these inconsistencies may be due to physiological differences among populations studied, we compared the effects of in vitro lactate (2 mM, 4 mM and 10 mM) on erythrocyte rigidity in venous blood drawn at rest in 10 untrained vs 10 aerobically-trained subjects. After adjustment of osmolality and pH and incubation at 37°C during 2 minutes, viscometric measurements were performed at 1000 s^{-1} with the MT90 (falling ball) viscometer and Dintenfass's 'Tk' was calculated. While at baseline there was no significant difference in Tk between the two groups, it decreased in the aerobically-trained subjects between 2 and 10 mM lactate concentrations ($p < 0.05$) and increased in the untrained group between 2 and 4 mM ($p < 0.05$). Thus, it seems that endurance training influences erythrocyte response to lactate. Lactate impaired erythrocyte deformability in untrained subjects but it (unexpectedly) improved it in trained subjects. This difference may be due to training-induced adaptations in erythrocyte metabolism, possibly including transmembrane transfer via monocarboxylate transporters.

Keywords: Red blood cell, deformability, endurance, lactate

1. Introduction

Several authors have studied the effect of lactate anion on blood rheology [1–7]. They observed a decrease in erythrocyte deformability when lactate concentration was artificially (lactate anion adjunction in blood samples) or naturally increased (during exercise). Decrease of erythrocyte deformability during exercise has been described in subjects with low physical fitness [7,8]. Lipovac et al. [4] and Reinhart et al. [9] explain that in vitro lactate adjunction or in vivo lactate accumulation in blood leads to an increase in extra-erythrocyte osmolarity. Because of hyperosmolar conditions, red blood cells (RBCs)

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can lose water progressively leading to the increase of internal viscosity and to the decrease of erythrocyte deformability. Hardeman et al. [10] and Connes et al. [11] have reported a paradoxical increase in erythrocyte deformability in endurance athletes after performing a progressive and maximal exercise. Increase of plasma lactate concentration was significant in these endurance athletes between rest and maximal exercise [10,11]. In another study, we observed no change in erythrocyte deformability in trained athletes performing submaximal exercise whereas plasma lactate concentration increased [12]. These discrepancies about the influence of lactate on erythrocyte deformability have never been discussed.

We hypothesised that relationships between erythrocyte deformability and lactate anion could be dependent on the training status of the population. So, we have compared in vitro RBC deformability from untrained and endurance trained subjects in response to different lactate concentrations.

2. Materials and methods

2.1. Subjects

Twenty volunteers participated in the study: 10 untrained subjects practicing physical activity less than three hours a week (SET group) and 10 aerobically trained subjects (ATH group). These subjects were runners, triathletes and cyclists. Major exclusion criteria were tobacco use, muscle or joint diseases, cardiorespiratory diseases, blood diseases and allergies.

2.2. Protocol

Subjects performed an incremental maximal exercise on cyclo-ergometer conducted until maximal oxygen consumption ($VO_{2\,max}$). Blood samples were collected at rest from the cubital vein and hemorheological parameters were assessed. Then, blood samples were mixed with lactate solutions at 3 concentrations (2, 4 and 10 mM) and hemorheological parameters were re-assessed in each group.

2.3. Exercise test

$VO_{2\,max}$ was measured in all athletes and 8 untrained subjects. Each subject performed an incremental maximal exercise. The test began with a warm up of 3 min at 60 watts. Pedalling speed remained constant (above 60 r.p.m.) throughout the test, and the load was increased by step of 30 watts every minute until exhaustion to determine $VO_{2\,max}$. All the ventilatory parameters were measured continuously using a breath-by-breath automated exercise metabolic system (Vmax 229, Sensor Medics, USA). A ten-lead ECG (Hellige, Marquette Medical Systems, Germany) was monitored continuously during testing and was recorded at the end of every minute of the entire test to determine heart rate. Oxygen uptake was considered maximal if at least three of the following criteria were met. (1) An increase in VO_2 lower than 100 ml with the last increase in work rate, (2) a respiratory exchange ratio greater than 1.10, (3) attainment of age predicted maximal heart rate $[210-(0.65\times \text{Age})\pm 10\%]$ and (4) an inability to maintain the required pedalling frequency despite maximum effort and verbal encouragement. Then, subjects had a 5 min recovery period with 2 min pedalling and 3 min at rest.

2.4. Preparation of lactate solutions

Sodium lactate solutions were prepared at three concentrations (20 mM, 40 mM and 100 mM) using N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES) buffer (90 mM NaCl, 50 mM HEPES, pH 7.4, 37°C, osmolality $\approx$ 267 mosmol/kg H_2O) which is an isotonic solution of non-penetrating compounds. Osmolality and pH were adjusted at 310 mosmol/l (with chloride sodium) and 7.4 (with NaOH or HCl) respectively.

2.5. Blood sampling and hemorheological measurements

Blood samples were drawn at rest from the cubital vein of each subject and collected in EDTA tube to assess baseline hemorheological parameters. Measurements were performed in accordance with guidelines of the International Committee of Standardization in Haematology [13]. Each blood sample was then divided in three aliquots of 4.5 ml. 0.5 ml of one of the three sodium lactate solutions was added in the aliquots. Each aliquot received a different concentration. Because of the dilution with the blood sample, final external [La] adjuncted to blood samples were estimated to be 2 mM, 4 mM and 10 mM.

Aliquots were incubated at 37°C during two minutes. Then, viscometric measurements were performed at very high shear rate (1000 s^{-1}) and at 37°C with a falling ball viscometer (MT 90 Medicatest, F-86280 Saint Benoit) [14,15]. Accuracy of the measurements was regularly controlled with the carrimed rheometer "CS" (purchased from Rheo, 91120 Palaiseau, France) [16]. The coefficient of variation of this method ranged between 0.6 and 0.8% [17]. We measured with this device ηb (blood viscosity) and ηp (plasma viscosity). Hct (hematocrit) was measured with microcentrifuge. Index of erythrocyte rigidity "Tk" was calculated according to the equation of Dintenfass [18]:

$$\mathrm{Tk} = \left(\eta\mathrm{r}^{0.4} - 1\right)/\left(\eta\mathrm{r}^{0.4} \cdot \mathrm{Hct}\right),$$

where ηr was relative blood viscosity (ηb/ηp).

2.6. Statistics

We have verified the normality of the distribution of our data using the Kolmogorov-Smirnov' test and then, we have proceded to the statistical treatment of these data. Comparisons between characteristics from the two groups (age, weight, height, $VO_{2\,max}$ and hemorheological parameters at rest) were performed using an unpaired Student t-test. Statistical comparison between ATH and SET for hemorheological data influenced by external [La] adjunction was performed by a two factors analysis of variance (ANOVA) and Scheffe test for multiple comparisons. Values are given as mean $\pm$ standard error of the mean (SEM). Significance level was defined as $p < 0.05$.

3. Results

3.1. Characteristics and resting hemorheological data in two groups

No significant difference was observed between two groups concerning age (24.6 ± 0.9 for SET and 22.3 ± 0.8 for ATH), weight (79.2 ± 2.5 kg for SET and 72.9 ± 1.3 kg for ATH) and height (186 ± 0.2 cm for SET and 181.6 ± 2.1 cm for ATH). There was no statistical difference between untrained subjects and endurance-trained athletes for ηb, ηp, Hct and Tk at rest (Table 1).

Table 1
Resting hemorheological data in two groups

	SET	ATH
Whole blood viscosity (mPa s)	3.12 ± 0.08	3.11 ± 0.05
Plasma viscosity (mPa s)	1.30 ± 0.02	1.29 ± 0.01
Hematocrit (%)	43.5 ± 0.52	43.25 ± 0.55
Tk	0.67 ± 0.01	0.68 ± 0.001

No significance difference between two groups.

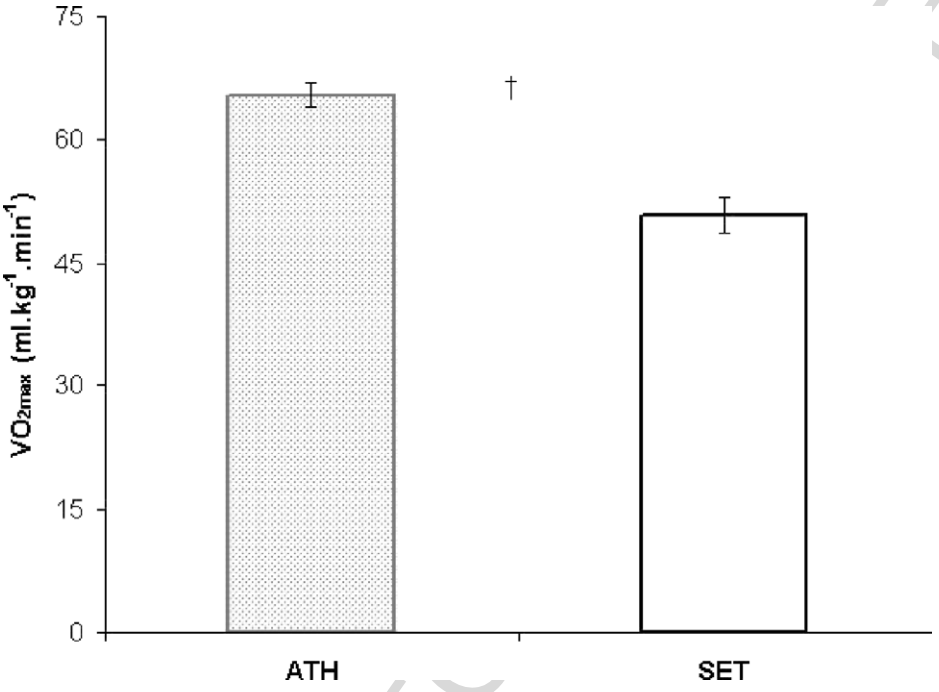

Fig. 1. $VO_{2\,max}$ in each group. $VO_{2\,max}$ was higher in ATH compared to SET ($^{\dagger}p < 0.001$).

3.2. Maximal oxygen consumption

$VO_{2\,max}$ was greater in ATH compared to SET ($p < 0.001$) (Fig. 1).

3.3. Hemorheological data after lactate adjunction in each group

As shown in Fig. 2, ηb increased in SET between 2 and 4 mM of external [La] ($p < 0.05$) and between 2 and 10 mM ($p < 0.05$). No statistical difference was observed between 4 and 10 mM. In ATH, ηb remained constant whichever external [La] (Fig. 2). Plasma viscosity increased in both groups between 2 and 10 mM ($p < 0.01$) and between 4 and 10 mM ($p < 0.05$) (Fig. 3). Hct was identical in both groups for each [La] and remained constant with the increase of [La]. In SET (Fig. 4). Tk increased in SET between 2 and 4 mM ($p < 0.05$) but no significant difference was observed between 2 mM and 10 mM and between 4 mM and 10 mM. Tk decreased between 2 and 10 mM in ATH ($p < 0.05$) but no significant difference was observed between others [La] (Fig. 4). At 2 mM, ATH had a greater Tk than SET ($p < 0.001$). At 4 mM and 10 mM, both had same values.

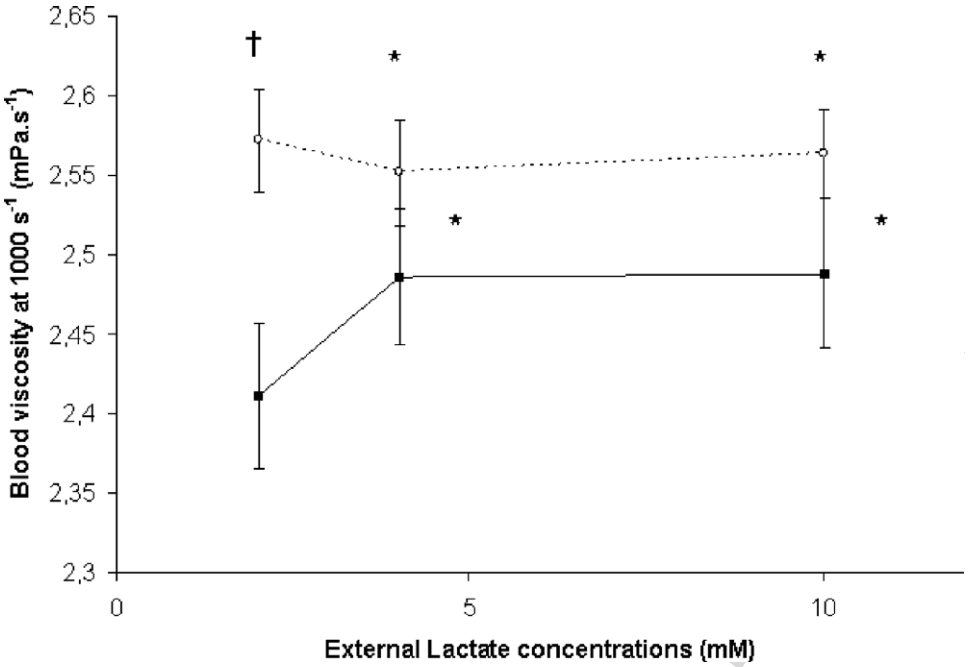

Fig. 2. Comparisons of ηb between ATH (○) and SET (■) at three external lactate concentrations. ηb was higher in ATH at 2 mM ($^{\dagger}p < 0.001$), 4 mM and 10 mM ($^{*}p < 0.05$). ηb increased between 2 mM and 4 mM in SET ($^{*}p < 0.05$) and between 2 mM and 10 mM ($^{*}p < 0.05$).

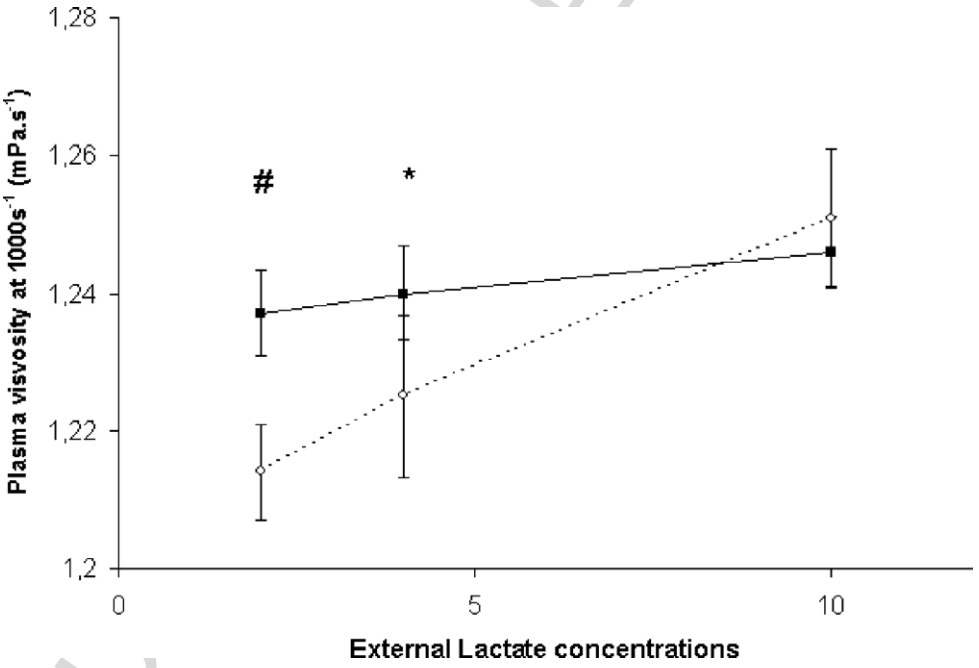

Fig. 3. Comparisons of ηp between ATH (○) and SET (■) at three external lactate concentrations. ηp increased in both groups between 2 and 10 mM ($^{\#}p < 0.01$) and between 4 and 10 mM ($^{*}p < 0.05$).

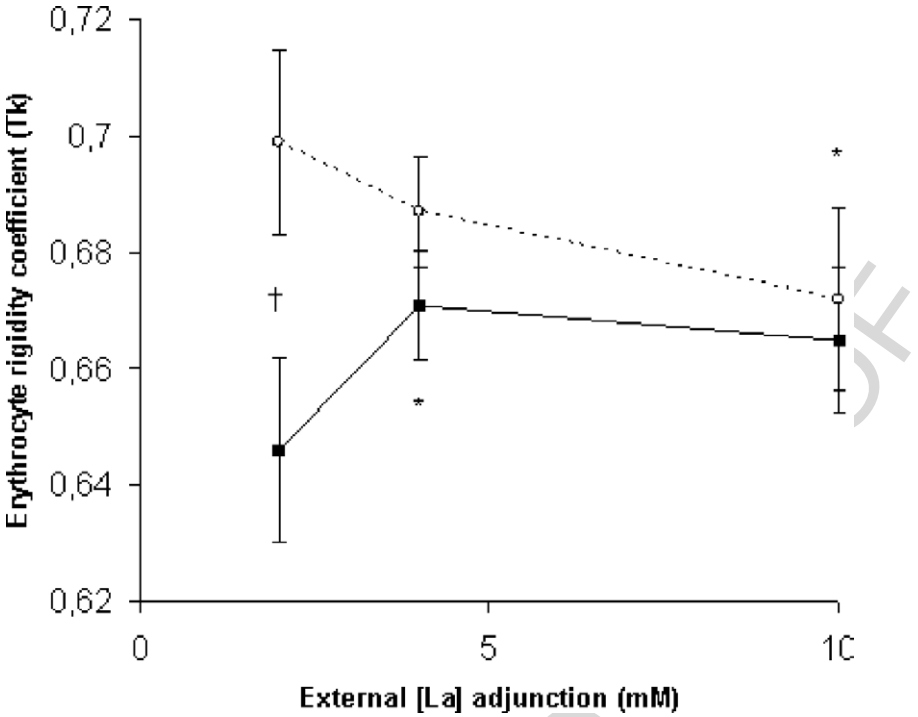

Fig. 4. Comparisons of Tk between ATH (○) and SET (■) at three external lactate concentrations. Tk was higher in ATH at 2 mM ($^{\dagger}p < 0.001$). Tk increased between 2 mM and 4 mM in SET ($^{*}p < 0.05$) and decreased between 2 mM and 10 mM in ATH ($^{*}p < 0.05$).

4. Discussion

Our results showed that Tk increased with lactate adjunction in SET between 2 and 4 mM whereas it decreased in ATH between 2 and 10 mM.

4.1. Methods

Because blood osmolarity is around 310 mosmol/l, we adjusted lactate solutions osmolarities at 310 mosmol/l using NaCl (sodium chloride). The aim of this operation was to avoid osmotic shock when lactate solutions were mixed with blood [19,20]. Influence of pH on hemorheological parameters has also been reported [19]. Then, pH of lactate' solutions were always fixed at 7.4 using NaOH (sodium hydroxide) or HCl (chlorhydric acid). By doing this, we only tested the effect of lactate on blood rheology.

Before hemorheological measurements, each sample containing 4.5 ml of blood and 0.5 ml of one of the three lactate solutions was incubated during 120 seconds at 37°C. The aim of this operation was to facilitate lactate entry into red blood cells, as suggested experimental results [21,22].

4.2. Results

$VO_{2\,max}$ from ATH was higher than $VO_{2\,max}$ from SET. It was not surprising because ATH practised cycle, running or triathlon more than 12 hours a week and SET performed a physical activity less than 3 hours a week. ATH had higher physical aerobic fitness than SET.

At rest, ATH and SET had same hemorheological profile. It could seem surprising because, for example, Schumacher et al. [23] explained that training induces hemodilution. Hct from trained subjects is habitually lower than Hct from sedentary subjects. However, overtraining induces the opposite [24]. In our study, we could explain this lack of difference because SET group was not composed of extreme sedentary subjects: mean $VO_{2\,max}$ of this group was around 50 ml kg^{-1} min^{-1}. Subjects form the SET group had stopped praticing sports since 1 or 2 years before the protocol: thus they were not sedentary but just untrained.

Blood viscosity increased only in SET with external [La]. This increase is essentially linked to the increase of ηp and Tk. On contrary, ηb remained constant in ATH because increase of ηp was compensated by the decrease of Tk. Hct was identical in both groups at each concentration. So, Hct did not influence ηb. The increase of erythrocyte rigidity induced by the increase of [La] has already been described by several authors [4,5,9]. Cellular mechanisms involved in the decrease of erythrocyte deformability induced by lactate could imply an increase in extra cellular osmolarity [4,9]. Because of hyperosmolar conditions in blood, RBCs could lose water and become more rigid. Osmolarities of our different lactate solutions were equilibrated to the same values (i.e., 310 mosmols l^{-1}). So, another cellular mechanism could be involved to explain the increase in Tk in SET between 2 and 4 mM. In ATH, RBC rigidity decreased between 2 and 10 mM. Only two studies have described a paradoxical increase of erythrocyte deformability while lactatemia increased and pH decreased [10,11], but no physiological explanations were found to explain this phenomenon. Some studies have reported that RBCs from aerobically trained subjects have a faster rate of lactate influx than others subjects like sedentary subjects or sprinters [25] that indicates that lactate uptake by RBCs from ATH would have been greater than in SET and would have lead to higher increase of erythrocyte rigidity in ATH. On contrary, Tk decreased in ATH. At 2 mM, Tk was higher in ATH whereas both groups had same values at rest. No experimental data are available to explain this difference. Relationships linking lactate anion and erythrocyte deformability seem to be very complex, but aerobic training status could play an important role in the erythrocyte response to lactate anion.

Improvement of RBC deformability could have some beneficial physiological effects. Betticher et al. [26] measured oxygen diffusion capacity (DLO_2) in isolated rabbit lung preparation with a suspension of chemically modified RBC. They observed an increase of DLO_2 when RBC deformability was improved while DLO_2 decreased with more rigid RBC. During exercise, it is well known that plasma lactate concentration increases. As suggest our results, lactate anion could improve RBC deformability in aerobically trained subjects. This higher RBC deformability could increase O_2 diffusion from alveoli to pulmonary capillaries, resulting in higher blood oxygen content. This could constitute an advantage during exercise because exercising muscle oxygenation could be improved. Moreover, when RBCs are very deformable, they could be hypothesized to carry O_2 up to very small muscle capillaries [27], which could be better for physical performance.

5. Conclusion

Relationships between lactate anion and red blood cell rheology could be linked to the training status and aerobic physical capacity of subjects. Behavior of RBCs in response to lactate anion differs between endurance athletes and non specifically endurance trained subjects. These specific physiological responses could be related to the rate of lactate influx into erythrocytes, which also differs between untrained subjects and endurance trained subjects [25] but there is still a lack of relevant experimental data

available concerning this issue. Improvement of RBC deformability with lactate could be advantageous during exercise by improving hemodynamic profile and gas exchange. Nevertheless, further investigations will be necessary to understand relationships between lactate anion and erythrocyte deformability in these different populations.

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