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Connes, Philippe, Didier Bouix, Guillaume Py, Corinne Caillaud, Pascale Kippelen, Jean-Frédéric Brun, Alain Varray, Christian Prefaut, and Jacques Mercier. Does exercise-induced hypoxemia modify lactate influx into erythrocytes and hemorheological parameters in athletes? *J Appl Physiol* 97: 1053–1058, 2004. First published April 30, 2004; 10.1152/japplphysiol.00993.2003.—This study investigated *1*) red blood cells (RBC) rigidity and *2*) lactate influxes into RBCs in endurance-trained athletes with and without exercise-induced hypoxemia (EIH). Nine EIH and six non-EIH subjects performed a submaximal steady-state exercise on a cycloergometer at 60% of maximal aerobic power for 10 min, followed by 15 min at 85% of maximal aerobic power. At rest and at the end of exercise, arterialized blood was sampled for analysis of arterialized pressure in oxygen, and venous blood was drawn for analysis of plasma lactate concentrations and hemorheological parameters. Lactate influxes into RBCs were measured at three labeled [U-^{14}C]lactate concentrations (1.6, 8.1, and 41 mM) on venous blood sampled at rest. The EIH subjects had higher maximal oxygen uptake than non-EIH ($P < 0.05$). Total lactate influx was significantly higher in RBCs from EIH compared with non-EIH subjects at 8.1 mM (1,498.1 ± 87.8 vs. 1,035.9 ± 114.8 nmol·ml^{-1}·min^{-1}; $P < 0.05$) and 41 mM (2,562.0 ± 145.0 vs. 1,618.1 ± 149.4 nmol·ml^{-1}·min^{-1}; $P < 0.01$). Monocarboxylate transporter-1-mediated lactate influx was also higher in EIH at 8.1 mM ($P < 0.05$) and 41 mM ($P < 0.01$). The drop in arterial oxygen partial pressure was negatively correlated with total lactate influx measured at 8.1 mM ($r = -0.82$, $P < 0.05$) and 41 mM ($r = -0.84$, $P < 0.05$) in the two groups together. Plasma lactate concentrations and hemorheological data were similar in the two groups at rest and at the end of exercise. The results showed higher monocarboxylate transporter-1-mediated lactate influx in the EIH subjects and suggested that EIH could modify lactate influx into erythrocyte. However, higher lactate influx in EIH subjects was not accompanied by an increase in RBC rigidity.

monocarboxylate transporter; endurance; lactate metabolism; hypoxemia; hemorheology

TRANSPORT OF LACTATE ACROSS the erythrocyte membrane proceeds by three distinct pathways (9): *1*) nonionic diffusion of the undissociated acid; *2*) an inorganic anion-exchange system, often referred to as the band 3 system; and *3*) a monocarboxylate-specific carrier mechanism (23). Juel et al. (18) recently showed that monocarboxylate transporter-1 (MCT-1) and band 3 expressions were increased with chronic hypoxia exposure, suggesting that these proteins may be upregulated by hypoxia. The functional significance of the hypoxia-induced changes is likely an increase of lactate and H^+ fluxes from plasma to erythrocyte. During sea-level exercise, some endurance-trained athletes experience arterial hypoxemia [exercise-induced hypoxemia (EIH)] that can be defined as a decrease in both oxygen arterial partial pressure (Pa_{O_2}) and arterial hemoglobin saturation during exercise (8). Miyachi and Katayama (21) have reported repeated episodes of EIH during training sessions when endurance athletes performed very intense exercise, a result suggesting that athletes develop frequent episodes of mild hypoxemia during training. One can thus think that repeated mild hypoxemia stimulus may lead to increased expression or activity of band 3 and MCT-1 proteins on the red blood cell (RBC) membranes. An increase in lactate and H^+ fluxes into RBCs during exercise may also alter the deformability of these cells, because lactate uptake by RBCs increases their rigidity (32). These changes in RBC deformability could, in turn, alter blood rheological properties and then participate to EIH. Several studies have suggested that blood viscosity and its determinants, i.e., RBC rigidity and Hct, could be involved in pulmonary diffusion impairment (6, 35). First, pulmonary diffusing capacity is widely affected by RBC deformability. When deformability is artificially reduced by chemical treatment, diffusion capacity may decrease by 20% (4). Second, high blood viscosity or RBC rigidity causes higher mechanical stress on the blood-gas barrier and may result in membrane disruptions. Aguilaniu et al. (1) showed that 6 wk of a polyunsaturated fatty acid (PUFA) diet led to an upward shift in Pa_{O_2} in master athletes. PUFA regimens are known to improve RBC deformability and to reduce blood viscosity (15); however, hemorheological measurements were not performed in this study (1).

In the present study, we hypothesized that EIH subjects have *1*) a higher capacity for lactate influx in RBCs and *2*) a reduced RBC deformability and increased blood viscosity during exercise. To test this hypothesis, we compared RBC lactate influx characteristics at rest in subjects with and without EIH, and we studied the hemorheological profiles at rest and at the end of a submaximal exercise bout.

MATERIALS AND METHODS

Subjects

Fifteen male endurance-trained subjects (runners, cyclists, and triathletes) were assigned to one of two groups (EIH or non-EIH) by

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assessing the decrease in oxyhemoglobin saturation [oxyhemoglobin saturation measured by pulse oximetry (Sp_{O_2})], during a first exercise test. A minimum drop of 3–4% in Sp_{O_2} for at least the last three steps of an incremental exercise test is required to conclude that the drop is significant (25). ΔSp_{O_2} was calculated as the difference between Sp_{O_2} measured at rest and during the last step of the incremental exercise. Group assignment was also confirmed by the decrease in Pa_{O_2}, i.e., ΔPa_{O_2}, measured during a subsequent submaximal test. Nine subjects with EIH were included in the EIH group (24 ± 1 yr, 73.4 ± 2.2 kg, 180 ± 3 cm, $\Delta Pa_{O_2} = -12.40 \pm 1.17$ Torr, $\Delta Sp_{O_2} = -4.9 \pm 1.1$), and six subjects without EIH were assigned to the non-EIH group (26 ± 2 yr, 72.7 ± 2.2 kg, 182 ± 2 cm, $\Delta Pa_{O_2} = -2.50 \pm 1.50$ Torr, $\Delta Sp_{O_2} = -1.9 \pm 0.4$).

The major exclusion criteria were tobacco use and muscle, joint, and cardiorespiratory diseases.

Experimental Protocol and Procedures

The local ethics committee approved the study, and each subject gave informed, written consent to participate. The subjects performed two exercise tests on a cycle ergometer (Ergoline type) separated by 1 wk. The first one was a progressive and maximal exercise test to determine the maximal cardiorespiratory parameters and ΔSp_{O_2}. The second was a submaximal steady-state exercise test. Arterialized blood was sampled at rest and at the end of exercise for analysis of Pa_{O_2}, and venous blood was sampled at the same times for analysis of plasma lactate concentrations ([Lac]) and hemorheological parameters. Lactate influxes into RBCs were measured on blood sampled at rest.

Exercise tests. The incremental maximal exercise test began with a 3-min warm-up at 60 W. Pedaling speed remained constant (>70 rpm) throughout testing, and the load was increased by 30 W every minute until maximal oxygen uptake ($\dot{V}O_{2\,max}$) was reached. Oxygen uptake ($\dot{V}O_2$) was considered maximal if at least three of the following criteria were met: *1*) a respiratory exchange ratio of >1.10; *2*) attainment of age-predicted maximal heart rate [210 − (0.65 × age) ± 10%]; *3*) an increase in $\dot{V}O_2$ lower than 100 ml with the last increase in work rate; and *4*) an inability to maintain the required pedaling frequency (70 rpm), despite maximum effort and verbal encouragement. A 5-min recovery period was then respected with 2 min of pedaling and 3 min at rest.

One week later, each subject performed a submaximal steady-state exercise based on the maximal intensity determined during the first test. Before exercise, a catheter was inserted into the antecubital vein of the nondominant arm. Venous blood samples were drawn at rest and at the end of exercise (i.e., during the last seconds of the test). After recording of cardiac, ventilatory, and gas exchange data for 5 min at rest, the test began with a 10-min warm-up at 60% of $\dot{V}O_{2\,max}$ followed by 15 min at 85% of $\dot{V}O_{2\,max}$. The recovery period consisted of 5 min of pedaling at low intensity.

$\dot{V}O_2$, CO_2 output, and ventilation were continuously measured at rest and during exercise and recovery by using a breath-by-breath automated exercise metabolic system (Vmax 229, Sensor Medics). A 12-lead electrocardiogram (Hellige, Marquette Medical Systems) was monitored continuously.

Sp_{O_2} and Pa_{O_2}. Before measurements, the ear lobe was systematically cleaned with alcohol and rubbed with a vasodilator cream (Finalgon, Boehringer Ingelheim, Barcelona, Spain). During the first exercise test, Sp_{O_2} was measured by using a noninvasive pulse oximetry method (Satlite Trans, Helsinki, Finland). The ear oximeter has been proven both valid and reliable for measuring significant falls in Sp_{O_2} during exercise (24, 27).

Pa_{O_2} was determined by using the blood-gas method. Blood was collected at the subject's ear and analyzed immediately (IL Meter 1306, Milan, Italy). We considered a Pa_{O_2} decrease of at least 8 Torr as significant for EIH, in agreement with the study performed by Anselme et al. (3).

Hemorheology. Seven milliliters of venous blood were collected in Vacutainer tubes (Becton Dickinson) containing EDTA as the anticoagulant for the hemorheological measurements. Hct was measured by the micromethod after blood microcentrifugation. Measurements of blood viscosity and plasma viscosity were performed with a falling ball viscometer (MT 90 Medicatest, Saint Benoit, France) (12). The coefficient of variation for this method ranged from 0.6 to 0.8% (14). Plasma was obtained via centrifugation at 2,000 *g* and 4°C for 5 min (Jouan, Saint Nazaire, France). The index of RBC rigidity (Tk) was calculated according to the equation of Dintenfass (11):

$$\mu b = \mu p[1 - (Tk \cdot Hct)]^{-2.5}$$

where μb is blood viscosity (mPa/s), μp is plasma viscosity (mPa/s), and Hct is in percent.

Plasma [Lac]. Venous blood (5 ml) was sampled in heparinized tubes (Becton Dickinson). Plasma was obtained via centrifugation at 2,000 *g* and 4°C for 5 min in a refrigerated centrifuge (Jouan). Plasma was then isolated and frozen at −80°C until assay. Plasma [Lac] was determined by using an enzymatic method (Roche Diagnostics kit, Mannheim, Germany).

RBC lactate transport. For lactate influx into RBCs, 5 ml of venous blood were collected at rest in heparin tubes (heparin, 0.2 U/ml), stored in ice, and prepared before lactate influx measurements.

The techniques for RBC preparation and lactate influx measurement were modified from previously published methods (30, 31). The initial Hct (pre-Hct) was determined for all blood samples. One-half (2.5 ml) of each blood sample was transferred to a 50-ml conical tube, depleted of lactate, and washed by using the following procedure. First, the RBCs were isolated by centrifugation at room temperature (25°C, 15 min, 2,000 *g*). Plasma and buffy coat were removed by aspiration. Thirty volumes of chloride buffer [150 mM NaCl and 10 mM sodium tricine, pH 8.0, at 37°C, osmolality ~315 mosmol/kgH_2O, volume (ml) = 30 × 2.5 × pre-Hct] were added to the pellet, which was mixed by inversion and incubated in a water bath for 30 min at 37°C to ensure complete removal of endogenous lactate (10, 30, 31). After incubation, the RBCs were sedimented at room temperature (25°C, 10 min, 2,000 *g*), and the supernatant was removed by aspiration. The cell pellet was then washed twice with chloride buffer and suspended in a volume of HEPES buffer (90 mM NaCl, 50 mM HEPES, pH 7.4, 37°C, osmolality ~267 mosmol/kgH_2O) equivalent to a 30% Hct level (packed cell volume) to obtain the stock cell suspension for influx measurements. This suspension was divided into two tubes, each containing 1 ml. One of the two tubes contained no lactate transport blockers. The second tube contained 1 mM of *p*-chloromercuribenzenesulfonic acid (PCMBS), which is known to inhibit the monocarboxylate-specific carrier at this concentration (30, 31). The tubes were then incubated in a water bath for an additional 30 min at 37°C. Part of the stock cell suspension was used for Hct determination (post-Hct).

All samples for lactate influx measurements were run in triplicate. At time $t = 0$, a 25-μl sample of stock cell suspension was added to a 13 × 100-mm test tube containing 75 μl of HEPES influx buffer at 37°C. The HEPES influx buffer contained L-[U-^{14}C]lactate (sodium salt, specific activity 50 μCi/mmol) at three [Lac] values of 2, 10, and 50 mM. The HEPES influx buffer was adjusted to pH 7.4. Because of dilution with the stock cell solution, the actual [Lac] values were 1.6, 8.1, and 41 mM. The cells were exposed to the influx buffer and mixed at 37°C for 20 s (30, 31). At that time, 5 ml of an ice-cold stop solution [150 mM NaCl, 10 mM sodium-2-(*N*-morpholino)ethanesulfonic acid, pH 6.5] were then added to each test tube to stop influx. The sample was spun for 15 min at 2,000 *g* and 4°C, and the supernatant was removed by aspiration. Control blanks were run in duplicate at the three [Lac] values for both total and MCT-1-mediated lactate influxes, to correct for any residual extracellular radioactivity and for any transmembrane lactate exchange that might have occurred, despite ice-cold stop solution. An additional wash phase was conducted by adding 5 ml of stop solution to each sample. The cells

were spun again, and the supernatant was removed. The RBC pellet was lysed and deproteinized with 0.5 ml of 4.2% perchloric acid followed by centrifugation of the sample for 15 min (2,000 *g*) at 4°C. A 0.4-ml sample was then placed into scintillation vials containing 5 ml of aqueous counting fluid and counted in a liquid scintillation counter (Tri-Carb Liquid Scintillation Analyzer, model 2200).

Total lactate influx into the RBCs was determined at the three [Lac] values (1.6, 8.1, and 41 mM). For this determination, neither the stock cell solution nor the HEPES influx buffer contained any lactate transport blocker. Influx into PCMBS-treated RBCs was measured at the same three concentrations of lactate. Because PCMBS treatment inhibits the monocarboxylate pathways, influx into the PCMBS-treated RBCs was the sum of lactate transport by the band 3 pathway and nonionic diffusion. Therefore, total lactate influx minus influx into the PCMBS-treated cells represents the MCT-1-mediated lactate influx. Control blanks radioactivity was subtracted from the radioactivity measured in samples.

The percentage of contribution from the MCT-1 pathway was calculated by dividing MCT-1-mediated lactate influx by the corresponding total lactate influx. Lactate influx was calculated in nanomoles of lactate per milliliter of cells (erythrocyte) per minute: the 25 μl of stock solution were multiplied by its Hct fraction (0.30) to obtain a packed cell volume. Total lactate influxes into RBCs and MCT-1-mediated lactate influxes were determined.

Statistical Analysis

Values are presented as means ± SE. Subject characteristics and $\dot{V}_{O_2\,max}$ values were compared between groups by using an unpaired Student's *t*-test. Lactate influxes into RBCs were compared with a two-way ANOVA with repeated measures: two groups (EIH subjects and non-EIH subjects) × three [Lac] values (1.6, 8.1, and 41 mM). The relationships between the drop in Pa_{O_2} (ΔPa_{O_2}) and the total lactate influx values and between $\dot{V}_{O_2\,max}$ and the total lactate influx values were evaluated for each subject by using a Pearson correlation. Hemorheological parameters and plasma [Lac] were compared by using a two-way ANOVA: two groups × sample time (at rest or at the end of submaximal exercise). Pairwise contrasts were used when necessary to determine where the significant differences occurred. The level of significance was set at $\alpha = 0.05$.

RESULTS

$\dot{V}_{O_2\,max}$

$\dot{V}_{O_2\,max}$ was significantly higher in the EIH subjects (65.33 ± 2.09 ml·kg^{-1}·min^{-1}) compared with the non-EIH subjects (60.27 ± 0.85 ml·kg^{-1}·min^{-1}) ($P < 0.05$).

Hemorheological Parameters

The values of the hemorheological parameters were identical in the two groups at rest (Table 1). During exercise, blood viscosity, plasma viscosity, and Hct increased significantly in both groups ($P < 0.001$) and reached similar values. Neither a group nor an exercise effect was observed for RBC rigidity.

Table 1. *Hemorheological parameters at rest and at the end of submaximal exercise in EIH and non-EIH subjects*

	EIH Subjects		Non-EIH Subjects	
	Rest	End of exercise	Rest	End of exercise
μb, mPa/s	3.31±0.13	3.83±0.17*	3.11±0.11	3.61±0.15*
μp, mPa/s	1.37±0.01	1.46±0.01*	1.33±0.01	1.44±0.01*
Hct, %	42.1±0.8	45.9±0.9*	42.7±0.4	46.0±0.9*
Tk	0.69±0.02	0.69±0.01	0.68±0.01	0.68±0.01

Values are means ± SE. EIH, exercise-induced hypoxemia; μb, blood viscosity; μp, plasma viscosity; Hct, hematocrit; Tk, red blood cell rigidity coefficient. *Significantly different between rest and the end of exercise ($P < 0.001$).

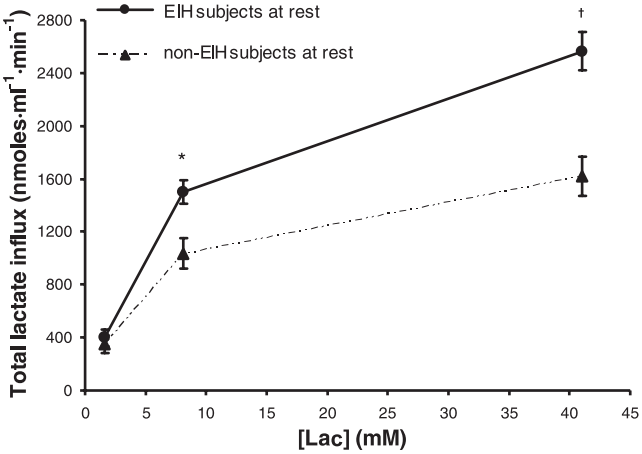

Fig. 1. Total lactate influx into red blood cells (RBCs) from 2 groups at different lactate concentration ([Lac]) values. Values at 8.1 and 41 mM were higher in exercise-induced hypoxemia (EIH) subjects. Values are means ± SE. *$P < 0.05$; †$P < 0.001$.

Plasma [Lac]

Plasma [Lac] values were similar for both groups at rest: 2.15 ± 0.11 and 2.32 ± 0.19 mM for EIH and non-EIH subjects, respectively. At the end of submaximal exercise, the plasma [Lac] was also identical in the two groups: 4.88 ± 1.12 and 5.32 ± 1.34 mM for EIH and non-EIH subjects, respectively.

Lactate Influx into RBCs

Total lactate influxes into RBCs are illustrated in Fig. 1. At 1.6 mM [Lac], no difference between groups was observed. Total lactate influx was greater in the EIH subjects at 8.1 mM ($P < 0.05$) and 41 mM [Lac] ($P < 0.001$) compared with the other group. MCT-1-mediated lactate influx was higher in the EIH subjects at 8.1 mM ($P < 0.05$) and 41 mM [Lac] ($P < 0.001$; Fig. 2).

The fractional contribution of MCT-1 to total lactate influx decreased with external [Lac] (Table 2).

Relationships between ΔPa_{O_2} and total lactate influx values. We found no relationship between ΔPa_{O_2} and total lactate influx measured at 1.6 mM (Fig. 3*A*). Negative correlations were found between ΔPa_{O_2} and total lactate influx measured at 8.1 mM ($r = -0.82$, $P < 0.05$, Fig. 3*B*) and between ΔPa_{O_2} and total lactate influx at 41 mM ($r = -0.84$, $P < 0.05$, Fig. 3*C*).

Relationships between $\dot{V}_{O_2\,max}$ and total lactate influx values. We found no significant correlation between $\dot{V}_{O_2\,max}$ and any of the total lactate influx values.

DISCUSSION

This study mainly showed that, in the resting condition, total and MCT-1-mediated lactate influxes into RBCs were higher in the EIH subjects at 8.1 and 41 mM [Lac]. In addition, there was a strong correlation between ΔPa_{O_2} and total lactate influx. The hemorheological profile was the same in the two groups at rest

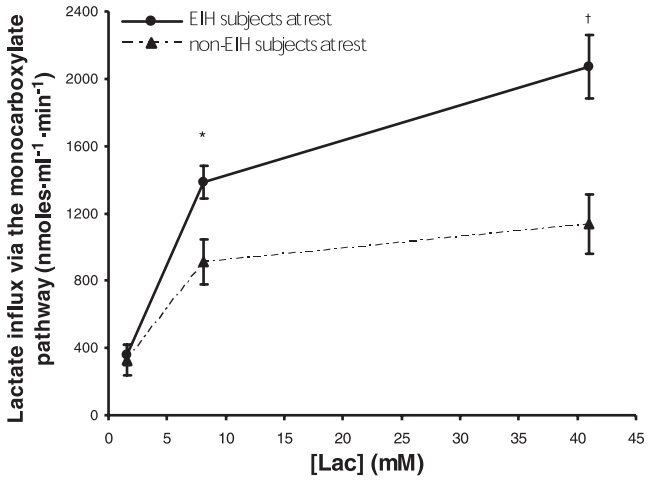

Fig. 2. Lactate influx via the monocarboxylate pathway in RBCs from EIH and non-EIH subjects. Values are means ± SE. Monocarboxylate transporter-1 (MCT-1)-mediated lactate influx was higher in EIH subjects at 8.1 mM (*$P < 0.05$) and 41 mM (†$P < 0.001$).

and at the end of exercise. These results suggest that EIH can modify lactate fluxes into RBC without any significant change in cell deformability.

Lactate Influx

We have observed higher total lactate influx into the RBCs from EIH subjects at moderate (8.1 mM) and high [Lac] (41 mM) than in the other group. We are mindful that the use of another [Lac], as done by Skelton et al. (30), closer to the physiological range would have reinforced our results. From Figs. 1 and 2, we can reasonably think that the use of a [Lac] around 16 mM would have given similar results, i.e., higher total or MCT-1-mediated lactate influx in EIH subjects. The results obtained at the tested concentrations showed in EIH subjects an increased influx via MCT-1, the major pathway of lactate influx into RBCs (30). This could be related to the greater aerobic physical fitness in EIH subject. Indeed, Skelton et al. (31) showed that the lactate influx via MCT-1 was faster in RBC obtained from animals with high-oxidative capacity (dogs and horses) than from animals with low-oxidative capacity (goats and cattle). However, in the present study, we did not find any significant relationship between $\dot{V}_{O_2\,max}$ and total lactate influx. Lactate uptake by RBCs may, nevertheless, play a role in enhancing physical performance. The accumulation of lactate and hydrogen ions in muscle during exercise may inhibit muscular function and cause fatigue (13, 16). High-RBC uptake of these ions could help in establishing a gradient between plasma and interstitial fluid (9, 28). Transport of these metabolites away from the exercising muscles into the blood would reduce the potential for muscle acidosis and delay the onset of fatigue.

Recently, Juel et al. (18) found that band 3 and MCT-1 expressions on RBC membranes from humans were increased after 2 and 8 wk of hypoxia exposure. RBC MCT-1 and band 3 contents increased dramatically (+330 and +150%, respec-

Table 2. *Fractional contribution (%) of the monocarboxylate transporter-1 pathway to total lactate influx at three lactate concentrations*

	1.6 mM	8.1 mM	41 mM
EIH subjects, %	89.7±0.6	92.5±1.4	80.8±4.6
Non-EIH subjects, %	93.8±3.7	88.0±0.8	70.2±3.2

Values are means ± SE. No significant difference was observed between groups at any concentration.

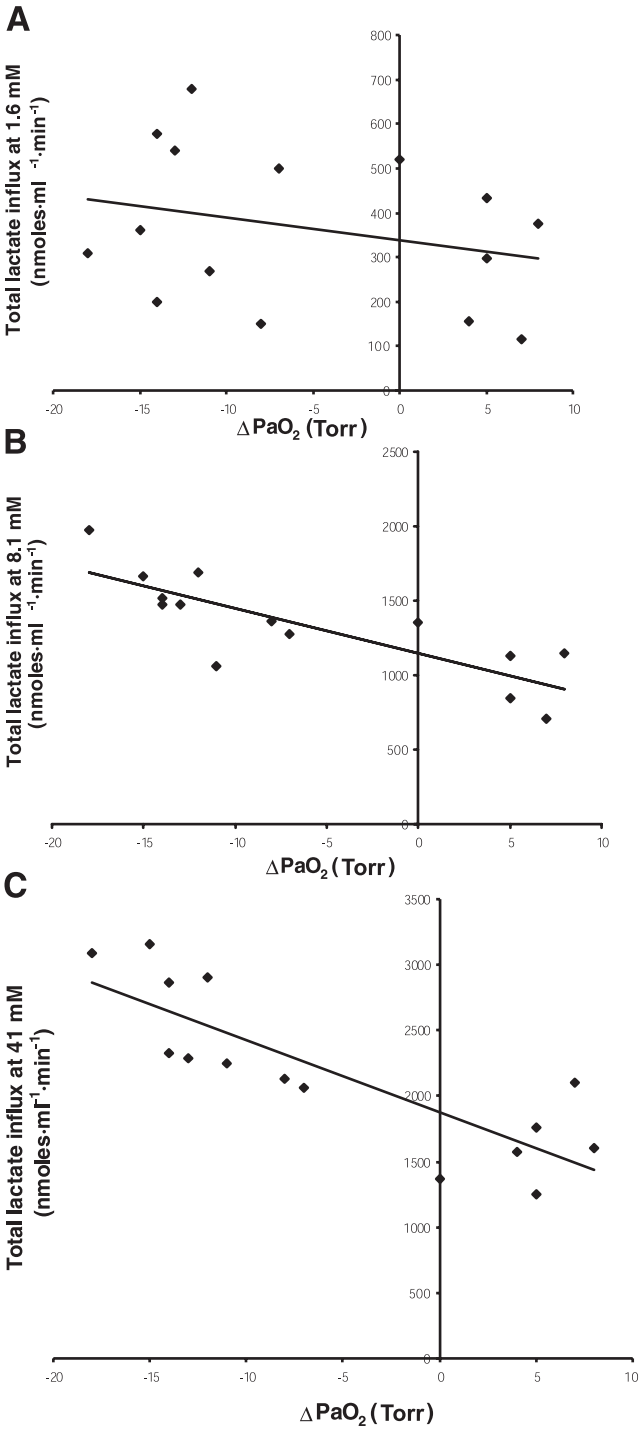

Fig. 3. Relationships between change in oxygen arterial partial pressure (ΔPa_{O_2}) and total lactate influx at 1.6 mM (*A*; $n = 15$), 8.1 mM (*B*; $n = 14$), and 41 mM (*C*; $n = 15$). Significant relationships were found between ΔPa_{O_2} and lactate influx at 8.1 mM ($r = -0.82$, $P < 0.05$) and between ΔPa_{O_2} and lactate influx at 41 mM ($r = -0.84$, $P < 0.05$).

tively) after 2 wk. These data suggest that the proteins involved in RBC lactate transport can be upregulated by hypoxia. In our study, the subjects exhibiting hypoxemia during exercise had the higher RBC lactate transport activity. These subjects trained regularly, sometimes twice a day, and it is possible that they experienced repeated hypoxemic events that had led to changes in RBC lactate transport activity. We found significant relationships between the drop in Pa_{O_2} and the total lactate uptake values measured at 8.1 and 41 mM, i.e., the two [Lac] values at which differences in total lactate uptake were noted between EIH and non-EIH. Although this finding indicates no causality, this correlation strengthens the argument that the enhanced lactate influx is more related to EIH than to $\dot{V}_{O_2\,max}$. Overall, these results suggest that hypoxemia (induced by either exercise or environmental condition) could be a strong stimulus for lactate transport into RBCs by acting on either the expression of the proteins involved or their activity. The underlying physiological mechanism is still unknown but may be related to erythropoietin (Epo) secretion. In a recent study (7), our laboratory showed that 4 wk of treatment with recombinant human Epo led to a significant increase in RBC lactate influx in human subjects. A potential role for Epo in MCT-1 expression might also explain why Juel et al. (18) found an increase in MCT-1 expression on RBC membrane but did not find such a change in muscle samples. Indeed, to our knowledge, there is no Epo receptor in mature human muscular cells.

Hemorheological Measurements

Blood viscosity was assessed by using a falling ball viscometer, the MT 90 Medicatest. This method was shown to be valid for the assessment of both plasma and blood viscosities in humans (12, 14). Moreover, Doffin et al. (12) specifically demonstrated that the MT 90 viscometer measures with a shear rate of 1,000 s^{-1}. Based on the Dintenfass equation, it was thus possible to calculate Tk in our study because this Tk becomes more specific as the shear rate rises (11). Blood viscosity depends on several parameters, including plasma viscosity, Hct, and RBC rigidity (11, 26). In our study, the hemorheological parameters involved in blood viscosity regulation had increased to the same value in both groups at the end of exercise, and thus blood viscosity also showed the same increase. This increase in plasma viscosity during exercise could be related to a rise in plasma protein concentration. For example, Vandewalle et al. (34) observed higher plasma concentrations of α_1-globulins, α_2-globulins, β-globulins, and γ-globulins at the end of a moderate exercise.

Several factors could be responsible for the change in Hct during exercise, including fluid shift, water loss, and RBCs released from the spleen (17, 19, 22, 33), but we did not determine the relative contribution of each factor in this study.

RBC rigidity did not change with exercise in either group. Usually, RBC deformability decreases with exercise (5, 29). The increased plasma [Lac] and decreased pH that occur during exercise lead to water loss by RBCs (osmotic process), which, in turn, results in greater rigidity (20, 32). In our study, however, the rise in plasma [Lac] during exercise was slight in all subjects, which can explain the lack of RBC rigidity change. The subjects exhibiting EIH had higher RBC lactate transport activity at 8.1 and 41 mM [Lac], but, in vivo, the plasma [Lac] reached lower values during exercise ($<$6 mM at the end of exercise). These results could also explain the lack of RBC rigidity difference between the two groups at the end of exercise.

EIH

We investigated blood rheology to clarify its relationship with EIH. Because the measurements were performed at a high-shear rate, we were able to hypothesize about the effects of blood rheological properties in small vessels like the capillaries, even though the blood was drawn from the antecubital vein. At a high-shear rate, blood viscosity depends primarily on erythrocyte rigidity and then on plasma viscosity and Hct, whereas, at a low-shear rate, blood viscosity depends mostly on aggregation properties and Hct.

Previous studies have suggested a potential role of RBC rigidity in pulmonary hemodynamics and pulmonary diffusion limitation. Weiss et al. (35) perfused pony lungs with pentoxifylline-treated RBCs, which are known to be very deformable. They observed a 27% decrease in filtration pressure, resulting in a 10% decrease in pulmonary arterial pressure. In master athletes, Aguilaniu et al. (1, 2) observed a decrease in EIH severity after a PUFA diet. Because a PUFA diet is known to improve RBC deformability, a potential contribution of blood parameters to EIH genesis cannot be excluded. In our study, it seems that hemorheology did not contribute to EIH during the submaximal exercise performed at 85% $\dot{V}_{O_2\,max}$, because no difference between groups was noted for any of the hemorheological parameters. Nevertheless, blood viscosity may contribute to EIH during very intense exercise, i.e., $>$90% $\dot{V}_{O_2\,max}$, because we observed a greater increase in blood viscosity in the endurance-trained subjects with EIH compared with those without EIH during a progressive and maximal exercise (6).

In conclusion, higher total lactate transport activity and greater MCT-1-mediated lactate influx were found in the RBCs from the EIH subjects, but erythrocyte deformability and blood viscosity were similar in the two groups of endurance-trained subjects. We suggest that the higher lactate influx into RBCs may be more closely related to hypoxemia than to aerobic capacity. Further studies are needed to identify the underlying physiological process.

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