Exercise Effects on Erythrocyte Deformability in Exercise-induced Arterial Hypoxemia

Authors
R. Alis[1,5], F. Sanchis-Gomar[1,2,3], D. Ferioli[4], A. La. Torre[4], J. R. Blesa[1,5], M. Romagnoli[1,6]

Affiliations
Affiliation addresses are listed at the end of the article

Key words
- exercise
- exercise-induced arterial hypoxemia
- erythrocyte deformability

Abstract

Exercise-induced arterial hypoxemia (EIAH) is often found in endurance-trained subjects at high exercise intensity. The role of erythrocyte deformability (ED) in EIAH has been scarcely explored. We aimed to explore the role of erythrocyte properties and lactate accumulation in the response of ED in EIAH. ED was determined in 10 sedentary and in 16 trained subjects, both before and after a maximal incremental test, and after recovery, along with mean corpuscular volume (MCV) and red blood cell lactate concentrations. EIAH was found in 6 trained subjects ($\Delta SaO_2 = -8.25 \pm 4.03\,\%$). Sedentary and non-EIAH trained subjects showed reduced ED after exercise, while no effect on ED was found in EIAH trained subjects. After exercise, lactate concentrations rose and MCV increased equally in all groups. ED is strongly driven by cell volume, but the different ED response to exercise in EIAH shows that other cellular mechanisms may be implicated. Interactions between membrane and cytoskeleton, which have been found to be O_2-regulated, play a role in ED. The drop in SaO_2 in EIAH subjects can improve ED response to exercise. This can be an adaptive mechanism that enhances muscular and pulmonary perfusion, and allows the achievement of high exercise intensity in EIAH despite lower O_2 arterial transport.

Introduction

During incremental exercise, a drop in arterial O_2 saturation (SaO_2) is frequently observed in male endurance-trained subjects [19]. This effect, named exercise-induced arterial hipoxemia (EIAH), is believed to reflect an insufficient alveolar gas exchange, probably caused by mismatch in alveolar ventilation to perfusion ratio and diffusion limitations [21]. A rheological parameter, blood viscosity is a function of plasma viscosity, red blood cell (RBC) aggregation, hematocrit and erythrocyte deformability (ED), with ED being the main determinant in microcirculation [4]. Several published observations suggest a role of blood rheological properties in EIAH development: i) blood viscosity rise induces pulmonary vascular resistance increment [20]; ii) an artificially induced decrease in ED diminishes lung O_2 diffusion capacity in animal models [6]; iii) administering polyunsaturated fatty acids to EIAH subjects, which improves ED, reduces EIAH severity during incremental exercise [1,2].

However, the blood rheology response to exercise in EAIH has been scarcely explored. It have been shown that during incremental exercise, blood viscosity increased substantially more in EIAH than in non-EIAH subjects, which may be due to different cellular properties [11]. In fact, increased ED [11], decreased ED [13] or absence of effects on ED [8] have been reported in EIAH, despite most of the published studies having shown that ED decreases with exercise [10].

It has been proposed that lactate accumulation may play a pivotal role in affecting ED during exercise [10], since in vitro, it exerts opposite effects on ED in trained and non-trained subjects [9]. Interestingly in highly trained subjects, those showing EIAH display greater lactate influx activity, mediated by monocarboxylate transporter-1 (MCT-1) [8].

The results for ED in EIAH are controversial and can be attributed to methodological issues such as exercise modality [13] or use of indirect ED measurement [31]. However, the role of ED in EIAH appearance warrants further research.

In the present study, we aimed to ascertain by ektacytometry techniques whether ED response to exercise differs between EIAH and non-EIAH subjects. We also aimed to explore whether RBC

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Correspondence
Rafael Alis
Research Universitary Institute Dr. Viña Giner, Molecular and Mitochondrial Medicine, Catholic University of Valencia San Vicente Mártir
C/Quevedo 2
Valencia 46001
Spain
Tel.: +34/963/637 412
Fax: +34/963/637 412
rafael.alis@ucv.es

lactate uptake during exercise plays a role in ED between EIAH and non-EIAH subjects. For this purpose, trained and non-trained subjects performed a running incremental test until exhaustion, with SaO_2 being monitored. ED, plasma and RBC lactate concentrations were measured before and after exercise, and after a 30-min recovery period.

Materials and Methods

Subjects

Thirty healthy male subjects were enrolled in the study. 20 were physically active (trained group, triathletes and endurance athletes who pursued physical activity weekly > 12 h) and 10 subjects were physically inactive (sedentary group, weekly physical activity < 1 h). Exclusion criteria were hematological, infectious or inflammatory diseases, history of heart disease, hormonal impairments (i. e., hypo- or hyperthyroidism) or presence of cardiovascular risk factors (obesity (BMI > 30 kg/m^2), current tobacco use (> 1 cigarette/day), hypertension (systolic blood pressure > 140 mmHg, diastolic blood pressure > 90 mmHg), hyperlipidemia (total cholesterol > 220 mg/dL and/or triglycerides > 175 mg/dL) or fasting glucose (> 126 mg/dL)). 4 subjects (all belonging to the trained group) were excluded since they did not complete the incremental test or it was considered not maximal. Therefore, 26 healthy male (10 sedentary and 16 trained) subjects were ultimately included in the study (**Table 1**).

All of the participants were informed of the purpose, protocol and procedures before agreeing to participate in the study, which was approved by the Ethical Committee of the Catholic University of Valencia. This work complies with the principles of the Declaration of Helsinki and was performed in accordance with the Spanish laws on research in humans. This study meets the ethical standards of the International Journal of Sports Medicine [14].

Experimental protocol

All participants reported to the laboratory in fasting conditions between 8 and 9 a.m. Subjects performed a maximal incremental test on a motorized treadmill until exhaustion. During the warm-up period (5 min) subjects selected the running speed (between 8 and 12 km/h) according to their preferences and habitual training pace. This speed remained fixed during the test, while the slope was increased by 1 % every minute. The test ended when the subject was unable to keep running despite verbal encouragement. Ventilatory parameters were recorded during exercise through a respiratory valve and a face mask (Hans Rudolph, Inc., Kansas City, MO, USA) using a gas analyzer (MetaLyzer 3B-R2, Cortex GmbH, Germany). The test was considered maximal when at least 2 of the following conditions were fulfilled: respiratory quotient > 1.1 and maximal heart rate > maximal heart rate predicted as 220-age.

During the incremental test, SaO_2 was non-invasively recorded every 15 s by pulse-oximetry using a Cardell 9500 HD Multiparameter Monitor. Presence of EIAH was considered when SaO_2 decreased by at least 4 % ($\Delta SaO_2 \leq -4\%$) from the baseline values in the last 2 min of the incremental test [21].

Blood sampling

Blood samples were taken from the antecubital vein before, just after exercise and after the 30-min recovery period. Blood was collected in standard Vacutainer® tubes containing K_3EDTA for lactate, hematological and hemorheological measurements. Tubes were kept in ice until performing determinations within 2-h after extraction.

Laboratory methods

Erythrocyte deformability was determined by ektacytometry techniques in a Rheodyn shear stress diffractometer (Myrenne GmbH, Germany) at 60 Pa [23,25]. In short, 30 µl of blood were suspended in 2 ml of a dextran solution (24 % dextran 40 000; pH = 7.4, 300–310 mOsm/L and 24 mPa·s viscosity). The blood-dextran suspension was placed between a rotating optical glass disc and a stationary disc. A defined shear force of 0.3–60 Pa was exerted on this suspension. Red blood cells were aligned with the fluid shear forces in parallel to the direction of flow and deformed to ellipsoids. A helium-neon laser beam passed through the red blood cell suspension and a diffraction pattern appeared. The diffraction pattern is circular with red blood cells at rest, but becomes elliptical when cells are deformed by shear. The light intensities of the diffraction pattern were measured along 2 orthogonal axes equidistant from the center of the image, and the erythrocyte elongation index (EEI) was calculated from the width (W) and length (L) of the ellipsoid:

Table 1 Subjects characteristics and incremental test data comparison between the sedentary subjects and trained subjects who did not present exercise-induced arterial hypoxemia (EIAH) and those who did.

	Sedentary (n = 10)	Trained non-EIAH (n = 10)	Trained EIAH (n = 6)	ANOVA p-value
age (years)	30.40 ± 6.40	35.40 ± 7.47	30.50 ± 6.83	0.228
weight (kg)	77.34 ± 5.98	70.77 ± 5.16 *	64.98 ± 3.29 * * *	< 0.001
BMI (kg/m^2)	24.39 ± 2.05	22.83 ± 1.67	21.87 ± 1.32 *	0.028
VO_2max (ml/min/kg)	44.61 ± 4.75	56.53 ± 4.80 * * *	54.82 ± 2.91 * * *	< 0.001
test duration (s)	747 ± 82	779 ± 95	676 ± 152	0.190
test running speed (km/h)	8.80 ± 0.79	10.90 ± 0.66 * * *	12.17 ± 2.38 *	< 0.001
anaerobic threshold (%VO_2max)	84.10 ± 6.73	92.74 ± 5.19 *	85.61 ± 5.67 #	0.009
RER_{max}	1.30 ± 0.11	1.19 ± 0.07 *	1.27 ± 0.04 #	0.020
maximal heart rate (bpm)	187.10 ± 9.76	175.40 ± 7.85 *	183.67 ± 10.71	0.030
post-exercise $[La^-]_{wb}$ (mM)	9.76 ± 2.63	8.91 ± 1.99	8.20 ± 2.08	0.412
post-exercise $[La^-]_{plasma}$ (mM)	11.12 ± 3.11	10.24 ± 2.37	9.92 ± 2.25	0.681
post-exercise $[La^-]_{RBC}$ (mM)	8.38 ± 2.96	7.52 ± 1.70	6.99 ± 2.15	0.547

RER_{max}: maximal respiratory exchange ratio. $[La^-]_{wb}$: whole blood lactate concentration. $[La^-]_{plasma}$: plasma lactate concentration. $[La^-]_{RBC}$: intra-erythrocyte lactate concentration
Data as mean ± standard deviation
* p < 0.05, * * p < 0.01, * * * p < 0.001 vs. the sedentary group
# p < 0.05 vs. non-EIAH trained group

EEI = 100·(L–W)/(L+W). We determined EEI at 60 Pa (EEI60) in duplicate in all of the samples. The inter-assay variation coefficient of the technique was 0.98 %.

Hematocrit (Htc), hemoglobin, RBC and reticulocyte counts, and cellular indices – i.e., mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) – were determined in a Sysmex XE-2100L (Sysmex, Kobe, Japan).

The lactate concentrations in whole blood and plasma were determined just after drawing with a Biosen C-line autoanalyzer (EKF-diagnostic GmbH, Germany). After blood was drawn, two 120 µl pre-cooled capillary tubes were quickly filled with anticoagulated blood and centrifuged for 30 s at 21 000 g and 4 °C (Sorvall Legend Micro 21R Microcentrifuge, Thermo Scientific, Germany). Afterwards, plasma was separated from the pelleted RBCs and used for the lactate measurements. Then 20 µl of either whole blood or plasma were pipetted into the pre-made analyzer tubes, gently agitated and inserted into the previously calibrated analyzer. All of the measures were taken in duplicate. Intra and inter-assay variation coefficientes were 0.92 % and 2.39 % for the whole blood lactate measures, and 0.73 % and 1.21 % for the plasma lactate measures. Intra-erythrocyte lactate concentrations were estimated using the following equation [15]:

$$[La^-]_{RBC} = [(100 \cdot [La^-]_{WB}) - ((100 - Hct) \cdot [La^-]_{Plasma})] \cdot Htc^{-1}$$

where $[La^-]_{RBC}$ is the lactate concentration inside the erythrocyte, $\cdot [La^-]_{WB}$ is the whole blood lactate concentration and $[La^-]_{Plasma}$ is the plasma lactate concentration.

Statistical analysis

All of the data variables were analyzed for normality by the Shapiro-Wilk test. Whenever required, data were log-transformed to improve normality for statistical testing. The statistical analyses were performed by grouping subjects according to their training status and presence of EIAH (sedentary, trained non-EIAH and trained EIAH). Incremental test data, subjects' characteristics and the hematological parameters were analyzed in a one-way ANOVA for independent measures. Homogeneity of variance was assessed by Levene's test. When this assumption was violated, Welch's correction was adopted. Exercise effects on ED, erythrocyte indices and La^- concentrations, were analyzed using a one-way ANOVA for repeated measures (sampling time: pre-exercise, post-exercise and 30-min recovery). Post hoc comparisons were made with Bonferroni's correction. Pearson's correlation coefficients were calculated in order to explore the association between continuous variables. The statistical analysis was performed using SPSS, version 21 (IBM Corporation, Armonk, NY, USA). The results were considered statistically significant at $p < 0.05$. Data were expressed as mean ± standard deviation (SD).

Results

Subjects characteristics

In the last 2 min of the incremental test, 6 trained subjects showed arterial oxygen saturation at least 4% lower than the baseline levels ($\Delta SaO_2 = -8.25 \pm 4.03$ %). For the data analysis, trained subjects were therefore divided into those not showing EIAH and those showing EIAH. Sedentary subjects were heavier, their selected running pace was lower, and their VO_2max was lower than in both trained groups (**Table 1**). Interestingly, trained EIAH subjects showed a higher maximal respiratory exchange ratio (RER_{max}), and their anaerobic threshold was at a lower VO_2max percentage than for the non-EIAH trained subjects (**Table 1**). The mean EEI60 values were no different among the 3 groups ($F(2,25) = 0.225$; $p = 0.800$). Moreover, no differences were found for the hematological parameters (**Suppl. Table 1**).

Exercise effects on ED

We analyzed the effects of exercise on ED, along with MCV and MCHC, since these erythrocyte indices are major determinants of EEI60. Exercise lowered ED accompanied by cell swelling and, accordingly, decreased MCHC in the sedentary and trained non-EIAH subjects (**Fig. 1**). However, in the trained subjects with EIAH, identical exercise-induced effects were observed on MCV and MCHC, but no effects were noted on EEI60 (**Fig. 1**).

Correlation analysis was applied to further investigate the association between the exercise effects on ED and the presence of EIAH and cell swelling (**Fig. 2**). A significant inverse bivariate correlation was found between the exercise-induced change in EEI60 and the arterial O_2 desaturation at the final 2 min of the incremental test ($r = -0.626$, $p < 0.001$, $n = 26$). Interestingly, exercise-induced MCV increment was found to be correlated with the exercise effects on ED only when EIAH subjects were removed from analysis ($r = -0.503$, $p = 0.024$, $n = 20$) (**Suppl. Table 2**).

Exercise effects on plasma and intra-erythrocyte lactate concentrations

We aimed to test the hypothesis that lactate intake plays a role in the ED response to intense exercise in trained-EIAH subjects. Therefore, we measured plasma and whole blood lactate concentrations, and calculated the inside-to-outside RBC lactate ratio before and after exercise, and also after the 30-min recovery period. As expected, the lactate concentrations and the inside-to-outside RBC lactate ratio rose considerably after exercise (**Fig. 3**) in all of the groups. However, no significant differences between groups were found in any lactate-related variable explored ($p \geq 0.346$).

Discussion

To accomplish their biological function RBCs must undergo major deformations in micro and macro circulation. ED is driven by the interplay of 3 parameters: membrane-cytoskeleton flexibility, surface-to-volume ratio and cytoplasmic viscosity. We demonstrate that intense exercise induces an increment in MCV, and a subsequent decrease in MCHC, irrespectively of training status and presence of EIAH. These effects should have a divergent impact on ED. As a result of cell swelling, the surface-to-volume ratio decreased, impairing ED, while cytoplasmic viscosity (directly related to hemoglobin concentration) also dropped, thus improving ED. However, within normal ranges, the effect of MCHC on ED is limited [12]. Therefore, ED should decrease as a result of these factors interaction. Indeed, the EEI60 data showed that exercise decreased ED in the sedentary and non-EIAH trained subjects (**Fig. 1**), but this effect was not observed in the trained EIAH subjects. Moreover, correlation data shows that EIAH appearance distorts the relationship between the exercise-induced cell swelling and ED impairing (**Fig. 2**) – a finding that has been previously reported [22].

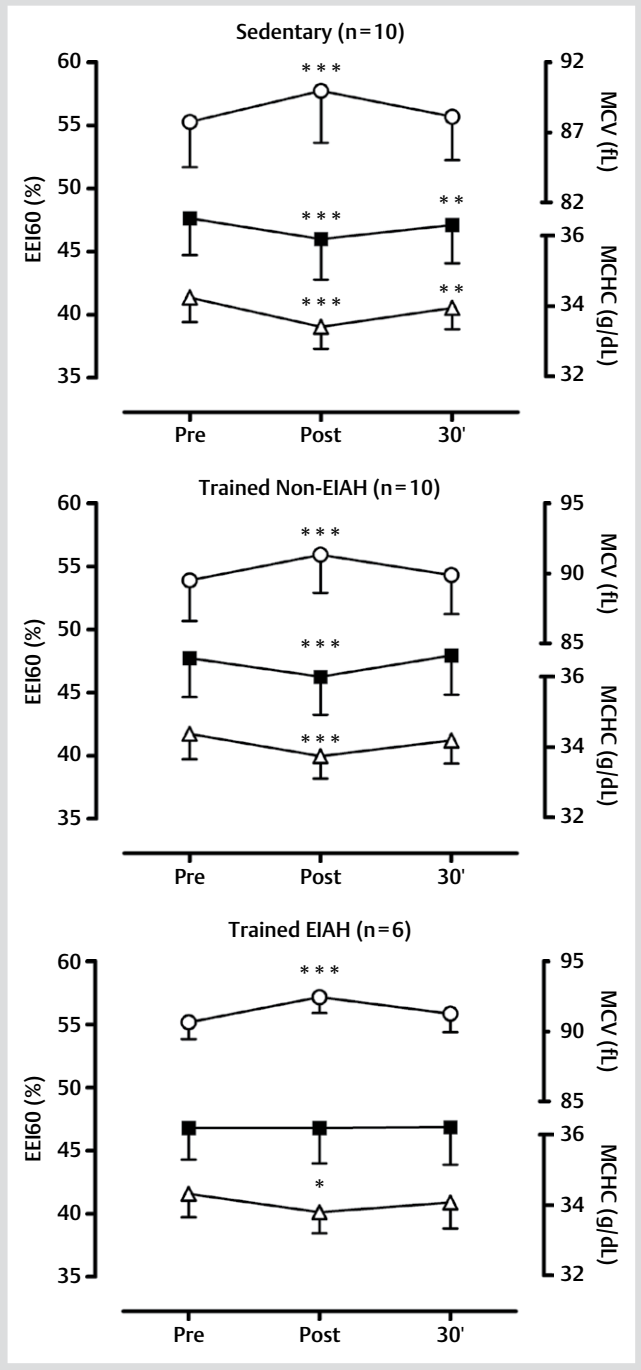

Fig. 1 Erythrocyte deformability (EEI60, ■), erythrocyte mean corpuscular volume (MCV, ○) and erythrocyte mean corpuscular hemoglobin concentration (MCHC, Δ) evolution during exercise and 30-min recovery, in the sedentary, the trained subjects without exercise-induced arterial hypoxemia (EIAH) and the trained subjects with EIAH. Symbols represent the mean values and error bars denote standard deviation. * * $p<0.01$, * * * $p<0.001$ vs. pre-exercise.

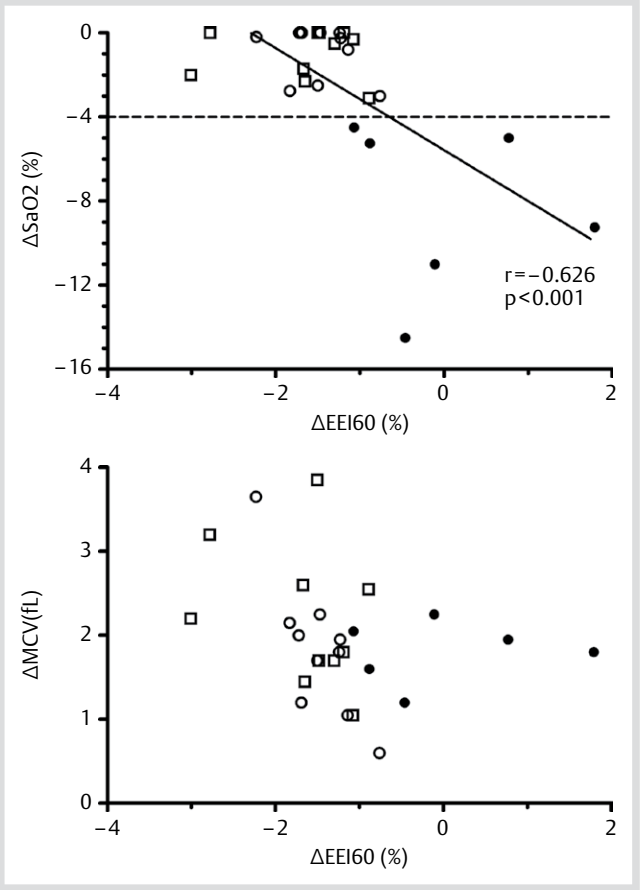

Fig. 2 Correlation plot between the increment of erythrocyte elongation index at 60 Pa ($\Delta EEI60 = EEI60_{post\text{-}exercise} - EEI60_{pre\text{-}exercise}$) and the mean arterial desaturation at the 2 last minutes of the incremental test (up) and the increment in mean corpuscular volume (MCV, down); in the sedentary (□), the trained non-EAIH (○) and the trained EIAH (●) subjects.

To the best of our knowledge, this is the first work to determine ED in EIAH directly by ektacytometry. Previous work that studied exercise effects on ED in EIAH have reported increased [11], decreased [13] or no changes [8] in ED. However, indirect ED measures (i.e., Tk calculations), which are currently outdated [5,31], were employed [8,11,13].

It has been previously shown that lactate uptake during exercise causes RBC swelling and decreased cell density [26], which is in agreement with the results of the present study. The trained EIAH subjects showed the same exercise effects on lactate concentrations (**Fig. 3**) and MCV increment (**Fig. 1**) as the sedentary and the trained non-EIAH subjects. Therefore, the greater MCT-1 lactate transport activity that has been reported in these subjects [8] does not seem to have any effect on the erythrocyte cellular properties linked to ED.

Several studies have reported divergent results regarding the effect of exercise on MCV [3,28–30]. The exercise type (running vs. cycling), methodological issues, and more importantly exercise intensity, are important factors to take into account when MCV values are compared. However, none of the aforementioned studies reported data about exercise intensity or blood lactate levels, which are crucial for understanding the effects of exercise on MCV.

Our data reveal that RBC membrane-cytoskeleton properties in the trained-EIAH subjects must be affected distinctly. The erythrocyte bilayer membrane is connected to the cytoskeleton through interactions between the cytoplasmic domain of integral membrane proteins and the spectrin network. These linkages stabilizes the bilayer membrane and are organized into 2 structures: one around the ankyrin protein that links band 3 (HCO_3^-/Cl^- exchanger, SLC4A1) and β-spectrin [18], and the other around protein 4.1R that forms a junctional complex with actin and β-spectrin, and is linked to several membrane proteins, which are probably organized in a band 3-centred metabolon

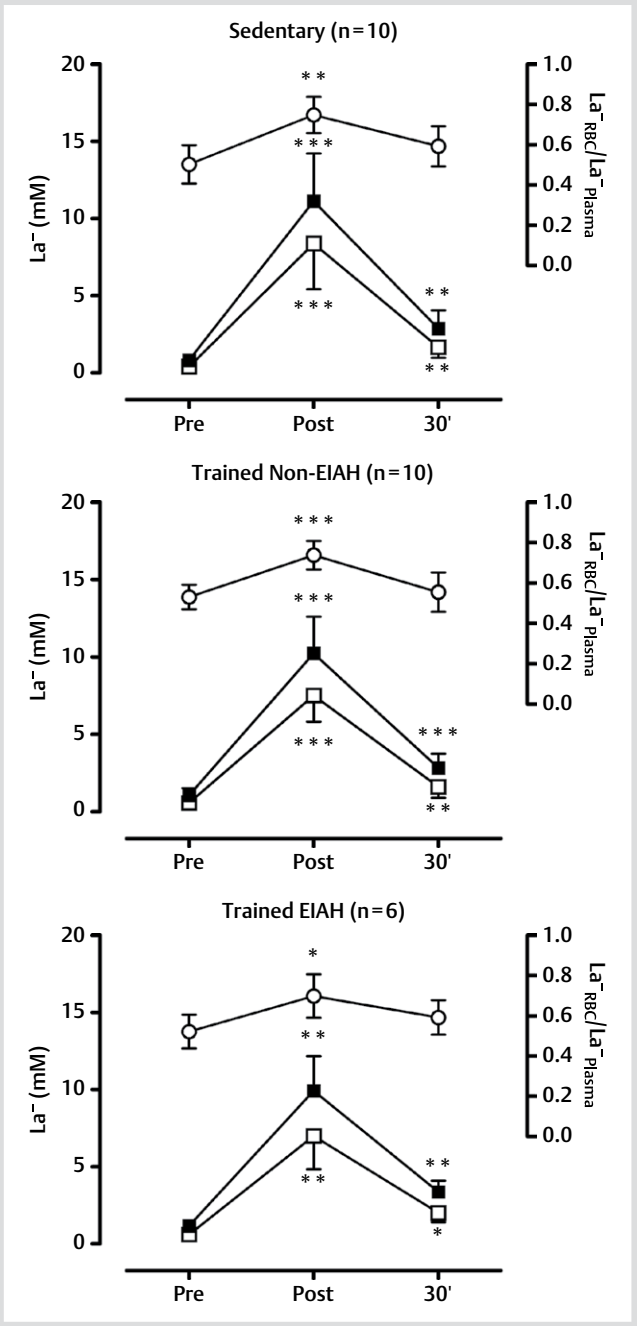

Fig. 3 Effect of exercise and the 30-min recovery period on lactate concentrations in plasma (■), inside erythrocyte (□) and on the inside-to-outside erythrocyte lactate ratio (○) in the sedentary, the trained subjects without exercise-induced arterial hypoxemia (EIAH) and the trained subjects with EIAH. Symbols represent the mean values and error bars denote standard deviation. * $p<0.05$, * * $p<0.01$, * * * $p<0.001$ vs. pre-exercise.

[24]. Weakening these interactions leads to decreased membrane stability and enhanced ED [16,17]. Interestingly, it has been recently shown that deoxyhemoglobin binds to the cytoplasmic domain of band 3 inhibiting the ankyrin-band 3 interactions [27]. Therefore, the lowered O_2 saturation during exercise in the trained-EIAH subjects can be the factor that explains the lack of exercise-induced effects on ED, if compared to sedentary and non-EIAH trained subjects, and despite having the same impact on lactate concentration, MCV and MCHC.

It has been previously hypothesized that ED can play a role in the appearance of EIAH [7]. Yet according to our results and current knowledge on RBC membrane physiology, it rather seems that the appearance of EIAH should have an effect on ED. In line with this, drops in SaO_2 should improve ED at high exercise intensity. This would prove to be an adaptive mechanism for improving pulmonary and muscular perfusion, and thus allowing stabilization of SaO_2 and muscular metabolism. It could, therefore, explain why high exercise intensity can be achieved despite impaired O_2 arterial transport in the EIAH subjects. In fact, no differences in maximal O_2 consumption or higher lactate concentrations were found when comparing trained EIAH and non-EIAH, although in the former, the anaerobic threshold was found at a lower VO_2max percentage and the maximal RER was higher.

In conclusion, ED is not impaired by intense exercise when SaO_2 considerably drops. This may be related to a newly discovered O_2-driven cellular mechanism that regulates membrane-cytoskeleton interactions [27], which may constitute an adaptive mechanism in exercise.

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Affiliations

[1] Research Universitary Institute Dr. Viña Giner, Molecular and Mitochondrial Medicine, Catholic University of Valencia San Vicente Mártir, Valencia, Spain
[2] Department of Physiology, University of Valencia, Valencia, Spain
[3] Fundación Investigación Hospital Clínico Universitario/INCLIVA, Valencia, Spain
[4] Department of Biomedical Sciences for Health, Università degli Studi di Milano, Milano, Italy
[5] Faculty of Medicine, Catholic University of Valencia "San Vicente Mártir", Valencia, Spain
[6] Department of Physical Education and Sports, University of Valencia, Valencia, Spain

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