Introduction

In a normoxic environment, recombinant human erythropoietin (rhEpo) has long been known for its ergogenic properties. Evidence of this is supported by numerous reports showing that prolonged rhEpo treatment induces a consistent increase in maximal oxygen uptake (VO_{2max}) [1–4]. Furthermore, it has been shown that endurance performance is dramatically augmented after rhEpo treatment [5]. The ergogenic effect of rhEpo is primarily meditated by hematological changes, since rhEpo stimulates - as endogenous renal Epo does - the proliferation of erythroid progenitor cells that ultimately augments red blood cell production, total hemoglobin mass and arterial oxygen content [6,7], both factors being key determinants of oxygen transport capacity and aerobic performance [8].

On the other hand, the ergogenic effect of rhEpo in a hypoxic environment is much less documented. While exercise capacity is consistently increased after rhEpo in normoxia, this is not the case with exercise in hypoxia, since neither rhEpo injections [9] nor autologous blood transfusion [10] were found to improve VO_{2max} at altitudes above 4000 m, in spite of enhanced arterial O$_2$ content (CaO$_2$). Likewise, with altitude acclimatization, CaO$_2$ and systemic oxygen delivery are restored close to sea-level values, however VO$_{2max}$ measured at high altitude is only barely ameliorated [11,12]. The main mechanism of this dissociation appears to be of cardiovascular origin, involving a reduction in peak muscle blood flow with altitude acclimatization [11,13] caused by a reduction in peak cardiac output and a redistribution of blood flow toward non-exercising tissues in response to higher CaO$_2$ [12]. Alternatively, it has been argued that in hypoxia VO$_{2max}$ is not as tightly dependent on CaO$_2$ as in normoxic conditions, but is rather limited by the pressure gradient driving diffusion from the capillaries to the muscle mitochondria, due to the reduced arterial oxygen pressure (PaO$_2$) [14,15]. Nevertheless, since previous studies examining the influence of CaO$_2$ on the cardiovascular response to exercise have been carried out after prolonged exposure to hypoxia, where accelerated erythropoiesis and hypoxia coexist, it has not been possible to separate putative effects linked to altitude acclimatization from those elicited by the increase in CaO$_2$.

Abstract

Treatment with recombinant human erythropoietin (rhEpo) induces a rise in blood oxygen-carrying capacity (CaO$_2$) that unequivocally enhances maximal oxygen uptake (VO$_{2max}$) during exercise in normoxia, but not when exercise is carried out in severe acute hypoxia. This implies that there should be a threshold altitude at which VO$_{2max}$ is less dependent on CaO$_2$. To ascertain which are the mechanisms explaining the interactions between hypoxia, CaO$_2$ and VO$_{2max}$ we measured systemic and leg O$_2$ transport and utilization during incremental exercise to exhaustion in normoxia and with different degrees of acute hypoxia in eight rhEpo-treated subjects. Following prolonged rhEpo treatment, the gain in systemic VO$_{2max}$ observed in normoxia (6–7%) persisted during mild hypoxia (8% at inspired O$_2$ fraction (FIO$_2$) of 0.173) and was even larger during moderate hypoxia (14–17% at FIO$_2$ = 0.153–0.134). When hypoxia was further augmented to FIO$_2$ = 0.115, there was no rhEpo-induced enhancement of systemic VO$_{2max}$ or peak leg VO$_2$. The mechanism highlighted by our data is that besides its strong influence on CaO$_2$, rhEpo was found to enhance leg VO$_{2max}$ in normoxia through a preferential redistribution of cardiac output toward the exercising legs, whereas this advantageous effect disappeared during severe hypoxia, leaving augmented CaO$_2$ alone insufficient for improving peak leg O$_2$ delivery and VO$_2$. Finally, that VO$_{2max}$ was largely dependent on CaO$_2$ during moderate hypoxia but became abruptly CaO$_2$-independent by slightly increasing the severity of hypoxia could be an indirect evidence of the appearance of central fatigue.
The fact that recombinant Epo boosts maximal oxygen transport at sea level but not at high altitude implies the existence of an “altitude threshold” beyond which the ergogenic effect of rhEpo on VO₂max vanishes. Determining this threshold may give further insights into the minimal level of hypoxia required to initiate some mechanisms countering the advantageous hematological adaptations conferred by rhEpo.

The first objective of this study was to determine how does the rhEpo-induced rise in VO₂max vary with the severity of hypoxia. From the results obtained in response to this first question, our second objective was to gain insights into the mechanisms involved in the interactions between hypoxia, CaO₂ and VO₂max.

Methods

Subjects

Eight Caucasian healthy male volunteers participated in the study. Their characteristics (mean±SD) were age: 27±7 yr, height: 180±4 cm, body mass: 83±7 kg, and maximal oxygen uptake: 4.1±0.2 L/min⁻¹. The subjects were active in recreational sport activities such as jogging 1–2 times per week. During the entire study period, the subjects were asked not to deviate from their normal lifestyle, and this was well accepted by all. Written informed consent to participate in the study was obtained from all subjects. This study was carried out according to the Declaration of Helsinki and was approved by the Ethical Committee of Copenhagen and Frederiksberg Counties, Denmark. All the study took place at sea level in Copenhagen, Denmark.

Treatment with Recombinant Human erythropoietin (rhEpo)

After baseline measurements, treatment with rhEpo (NeoR-ecormon, Roche, Mannheim, Germany) was started and lasted for fifteen weeks. On each occasion, 5000 IU was injected as follows. First two weeks: one injection every second day; third week: three injections on three consecutive days; week four to fifteen: one injection every week. All injections occurred between 08:00 and 10:00 a.m., and were preceded by 30 min of supine rest. Two weeks prior to rhEpo treatment all subjects received 100 mg iron.day⁻¹ orally, and this was maintained throughout the entire study period. The present rhEpo treatment is an exploratory model, which has been developed by the Copenhagen Muscle Research Centre both for physiological studies and also for doping testing purposes. Our rhEpo treatment, which aimed to mimic the subject was sitting at rest on the ergometer, was used to calibrate the thoracic impedance device. Heart rate and stroke volume were continuously measured during the test. Cardiac output was averaged over 15-s intervals.

Validity of cardiac impedance for cardiac output measurement. In order to evaluate the validity of our cardiac impedance method for cardiac output measurement during the non-invasive study, cardiac output during incremental exercise in the invasive study was measured simultaneously by the cardiac impedance technique and the cardio-green dye dilution method. The agreement between the two techniques was assessed by the method of Bland and Altman [19]. The Bland-Altman plot (Fig. 2), obtained from 55 simultaneous measurements, shows that the mean bias was –0.41 L/min⁻¹ with 95% confidence interval ranging between –4.83 and 4.01 L/min⁻¹.

Invasive experiments. In order to gain insights into the control of maximal oxygen transport in hypoxia following prolonged rhEpo treatment, seven of the eight subjects (subjects #1–4 and #6–8) underwent an invasive experiment on two separate occasions, i.e. before and after fourteen weeks of rhEpo treatment. On each occasion, the subjects performed an incremental cycling exercise until exhaustion in two conditions, i.e. in normoxia and in normobaric hypoxia equivalent to the altitudes of 1500 m (FIO₂ = 0.174), 2500 m (FIO₂ = 0.153), 3500 m (FIO₂ = 0.134) and 4500 m (FIO₂ = 0.115).
transducer positioned at the height of the fourth parasternal intercostal space (T100209A, Baxter, Unterschleissheim, Germany) and was also used to sample arterial blood. A similar catheter was inserted in the same femoral artery 1–2 cm below the previous catheter and advanced 5–10 cm in the proximal direction for cardiac output measurement. Another 20 gauge catheter (Arrow, Ref. ES-14150, Reading, PA, USA) was inserted in the right femoral vein, 2–3 cm below the inguinal ligament and advanced 8 cm in the distal direction, beyond the merger with the saphenous vein for femoral venous blood sampling. This catheter was also connected to a blood pressure transducer positioned at the height of the fourth intercostal parasternal space (T100209A, Baxter, Unterschleissheim, Germany) to measure femoral vein pressure. In the same femoral vein, a venous catheter with side holes (Radiopack TFE, Cook, Bjaerverskov, Denmark) was inserted and advanced ~5 cm proximal to the inguinal ligament for the injection of ice-cold physiological saline solution. A thin polyethylene-coated thermistor (model 94-030-2.5F T.D. Probe, Edwards Edslab, Baxter, Irvine, CA, USA) was inserted through the venous catheter for blood flow measurements by the constant infusion thermodilution technique [21]. All these catheters were connected to three-way stopcocks and, along with the thermistor, sutured to the skin, under local anesthesia to minimize the risk of movement during exercise. An additional venous catheter was inserted into an antecubital vein to inject indocyanine green (ICG, Akorn Inc, IL) for measuring cardiac output by the dye-dilution method, as previously reported [22,23]. A three-lead electrocardiogram (ECG) was displayed on a monitor during catheterization and the rest of the experimental procedures (Dialogue 2000, Danica, Copenhagen, Denmark). The ECG, blood pressure and the temperatures registered by the thermistor, as well as the infusate temperatures were recorded simultaneously with the data acquisition system (MacLab 16/s ADInstruments, Sydney, Australia). Cardiac output was measured continuously during exercise by the cardiac impedance technique. Respiratory variables were measured continuously, as explained below.

**Experimental protocol**

**Incremental exercise.** The experimental protocol consisted of an incremental exercise bout during which the subjects cycled with a cadence close to 80 revolutions per minute on an electrically-braked ergometer (Excalibur, Lode, Groningen, The Netherlands), first at
100 watts (W) during 15 min, then by increasing workload by 40 W every 90 seconds (s) until the subject could not sustain the pedaling pace. The maximal workload (Wmax) was calculated with the formula [24]: Wmax = Wcomp + 40 × (t/90), where Wcomp is the last workload completed, t is the number of seconds that the final, not-completed workload was sustained and 40 (watts) is the workload increment. During the invasive experiment, a blood sample was obtained simultaneously from the femoral vein and artery, followed by the measurement of leg blood flow, and cardiac output at the 6th and 12th minutes at 100 W, then the last 45 s at each workload and at maximal exercise, as close as possible to exhaustion. In this specific study, only the measurements at the 12th minute at 100 W and maximal exercise are reported. Also during normoxic trials the values at 260 W were given as an estimate of the corresponding peak maximal exercise are reported. Also during normoxic trials the highest 15-second measurement of V\dot{O}_2 was taken as representative workload completed, and 40 (watts) is the workload difference between leg blood flow and the pressure difference between the femoral artery and the femoral vein. All pressures were referred to blood pH, CO₂, O₂ tensions, lactate, and potassium (ABL700 Series, Radiometer, Copenhagen, Denmark). Capillary hemocrit values obtained with the gas analyzer (ABL 700) were tested against the micro-centrifugation method during a separate procedure. A satisfactory agreement was found between the two methods (results not shown). Blood gases were corrected for measured femoral vein blood temperature (femoral venous and arterial blood gases). Plasma norepinephrine concentration was measured by ELISA (R&D Systems, Minneapolis, MN, USA). Blood O₂ content (mL^{-1}) in femoral artery and vein (CaO₂ and C_aO₂, respectively) was computed from the saturation and [Hb], i.e. (1.34×[Hb])×SO₂+0.003×PO₂. Systemic oxygen delivery was computed as the product of cardiac output and CaO₂, while leg O₂ delivery was obtained multiplying leg blood flow by CaO₂. Leg VO₂ was calculated using the direct Fick method, i.e. as the product of leg blood flow and arterial-venous difference in oxygen content. The systemic a-v O₂ difference was calculated using the measured whole body VO₂ and cardiac output (indirect Fick method). Leg lactate and potassium releases were calculated as the product of leg blood flow and the venous-arterial difference of lactate and potassium, respectively.

Blood samples and calculations. Blood was sampled anaerobically in heparinized syringes (or in capillary tubes (Radiometer) for the non-invasive experiment, according to the manufacturer’s instructions) and immediately analyzed for hemoglobin concentration ([Hb]), hematocrit, oxygen saturation, blood pH, CO₂ and O₂ tensions, lactate, and potassium (ABL700 Series, Radiometer, Copenhagen, Denmark). Capillary hemocrit values obtained with the gas analyzer (ABL 700) were tested against the micro-centrifugation method during a separate procedure. A satisfactory agreement was found between the two methods (results not shown). Blood gases were corrected for measured femoral vein blood temperature (femoral venous and arterial blood gases). Plasma norepinephrine concentration was measured by ELISA (R&D Systems, Minneapolis, MN, USA). Blood O₂ content (mL^{-1}) in femoral artery and vein (CaO₂ and C_aO₂, respectively) was computed from the saturation and [Hb], i.e. (1.34×[Hb])×SO₂+0.003×PO₂. Systemic oxygen delivery was computed as the product of cardiac output and CaO₂, while leg O₂ delivery was obtained multiplying leg blood flow by CaO₂. Leg VO₂ was calculated using the direct Fick method, i.e. as the product of leg blood flow and arterial-venous difference in oxygen content. The systemic a-v O₂ difference was calculated using the measured whole body VO₂ and cardiac output (indirect Fick method). Leg lactate and potassium releases were calculated as the product of leg blood flow and the venous-arterial difference of lactate and potassium, respectively.

Red blood cell volume. Red blood cell volume was determined by a carbon monoxide rebreathing method twice before rhEpo treatment, and after 5 and 13 weeks of rhEpo treatment. This dataset has been reported elsewhere [6].

Statistical analysis
The Kolmogorov-Smirnov’s test was applied to examine the normality in the distribution of data. Due to the small number of participants in our study (n = 7), for each parameter, all the data that were obtained at a given power output (i.e. at 100 W or at peak exercise) were pooled before data distribution was examined (n = 28). The Bartlett’s test was used to evaluate the uniformity of variance between conditions. If variance was not found to be homogeneous, ANOVA was replaced by the Welch’s test. Once normality and variance homogeneity were verified, the differences in the measured variables among experimental conditions and exercise levels were analyzed using two-way ANOVA with repeated measures, with rhEpo and power output as within-subjects factors. Two separated ANOVA analyses were performed, one for testing the effect of rhEpo in normoxia and the other one for testing the effect of rhEpo in hypoxia. Another two-way ANOVA (rhEpo × hypoxia) was applied to the measured variables 1) at 100 W and 2) at peak exercise. When F was significant in the ANOVA, planned pair-wise specific comparisons were carried out using Student’s paired t test adjusted for multiple comparisons with the Newman-Keul’s procedure. Statistics were done with the Statview software, version 5.0. (SAS Institute, Cary, NC). The values are reported as arithmetic means±SE. Differences were considered as significant for P<0.05.

Results
rhEpo increases red blood cell volume and maximal oxygen uptake in normoxia.

The resting hematological changes observed among our rhEpo-treated subjects have been reported elsewhere [6]. Briefly, rhEpo was found to increase red blood cell volume by 9.4% after 5 weeks and by 8.1% after 13 weeks. This was associated with an increase
rhEpo increases maximal oxygen uptake during acute moderate hypoxia (1500–3500 m)

Our non-invasive study reveals that after 5 weeks of rhEpo treatment the improvement in pulmonary VO\(_2\)max observed in normoxia, of 5.8%, did not decline with increasing levels of inspired hypoxia up to 3500 m (Fig. 3A and 3B). Our results instead suggest that the stimulating effect of rhEpo on maximal oxygen transport was even more pronounced during moderate hypoxia, as the gain in VO\(_2\)max reached 14.0% at 2500 m (P = 0.09 vs normoxia) and 17.5% at 3500 m (P < 0.05 vs normoxia) (Fig. 3B). Although the absence of any leg oxygen transport measurement in this part of the study did not allow us to unravel the underlying mechanisms, our results at least indicate that this unexpectedly large gain in VO\(_2\)max during moderate hypoxia was related neither to a higher maximal cardiac output nor to a larger rise in CaO\(_2\) following rhEpo in this condition (Table 1).

rhEpo does not increase maximal oxygen uptake during acute severe hypoxia (4500 m)

Our results demonstrate that the potential for rhEpo to improve maximal oxygen transport, evidenced both in normoxia and during moderate hypoxia up to 3500 m, vanished during a more severe hypoxic challenge equivalent to 4500 m. This finding appears to be independent of the duration of rhEpo treatment, as our data showed the same insignificant change in pulmonary VO\(_2\)max, of 1.9% after 5 weeks (Fig. 3A and 3B) and of 2.0% after 14 weeks of treatment (Fig. 3A). The demonstration that active muscles failed to utilize more oxygen after rhEpo in severe hypoxia of 4500 m comes from the observation of an unchanged peak leg VO\(_2\) in this condition (Fig. 4B). Taken together, our data thus suggest that the minimal level of inspired hypoxia required to abolish the ergogenic properties of rhEpo is above 3500 m and below 4500 m.

Why does rhEpo fail to improve maximal oxygen uptake during acute severe hypoxia?

The fact that VO\(_2\)max did not increase during hypoxia (4500 m) following rhEpo treatment in spite of elevated CaO\(_2\) levels raises the question of the mechanisms involved. The results here below, obtained from our invasive study, provide some insights into these mechanisms.

**Pulmonary gas exchange.** Peak pulmonary ventilation slightly increased after rhEpo in hypoxia (Table 3), without any concomitant improvement in alveolar PO\(_2\) (Table 2). Alveolar-to-arterial O\(_2\) difference reached its highest value during maximal exercise in hypoxia, regardless of rhEpo treatment. By contrast, in normoxia, peak alveolar-to-arterial O\(_2\) difference tended to decrease (P = 0.06) after rhEpo (Table 2). Finally, SaO\(_2\) was not altered by rhEpo treatment, either during normoxia or during hypoxia (Table 2).

**Systemic oxygen transport.** In normoxia, neither maximal heart rate nor maximal stroke volume was affected by rhEpo and this was also true in hypoxia (Fig. 5A and 5B). As a consequence, maximal cardiac output was found similar in all conditions (Fig. 6A). Whatever the oxygenation condition, CaO\(_2\) was increased after rhEpo (Table 2) resulting in a higher maximal systemic O\(_2\) delivery both in normoxia (P = 0.06) and in hypoxia (P < 0.05) (Fig. 6B). Systemic arterio-venous O\(_2\) difference, although not wider following rhEpo, increased systematically as power output rose, except during hypoxic exercise (Fig. 6C). Similarly, systemic fractional O\(_2\) extraction failed to increase along with power output in hypoxia after rhEpo, so that the calculated value during hypoxic exercise was 16% lower than the corresponding value obtained before rhEpo treatment (Fig. 6D).

**Leg oxygen delivery.** Peak leg blood flow was not altered, either by rhEpo or by hypoxia (Fig. 6E). Peak leg O\(_2\) delivery increased after rhEpo in normoxia only (Fig. 6F). Because CaO\(_2\) was augmented after rhEpo without any change in C\(_{\text{av}}\)O\(_2\) (Table 2), leg arterio-venous O\(_2\) difference rose after rhEpo independently of oxygenation (Fig. 6G). Finally, leg fractional O\(_2\) extraction was not altered by rhEpo (Fig. 6H).

**Distribution of cardiac output.** Although neither maximal cardiac output nor leg blood flow were significantly altered by rhEpo, Fig. 7 reveals that the distribution of maximal cardiac output was altered, as the fraction of peak cardiac output directed to the exercising legs was 17% higher after rhEpo in normoxia.

---

**Recombinant Epo and Hypoxia**

**Figure 3. The effect of rhEpo on VO\(_2\)max depends on the O\(_2\) inspiration fraction.** This figure refers to the non-invasive study. Panel A shows the individual VO\(_2\)max values measured before (−) and after (+) a 5-week treatment with recombinant human erythropoietin (rhEpo), during normoxia and acute hypoxia equivalent to altitudes ranging between 1500 m and 4500 m. Panel B indicates the changes in VO\(_2\)max induced by rhEpo, for each altitude condition. Values are means ± SE in seven subjects. *P < 0.05 after vs before rhEpo. †P < 0.05 hypoxia (3500 m) vs normoxia (0 m).

doi:10.1371/journal.pone.0002996.g003
Figure 4. rhEpo increases peak pulmonary and leg V\(\dot{O}_2\) in normoxia but not in severe acute hypoxia. This figure refers to the invasive study. Pulmonary V\(\dot{O}_2\) (panel A) and leg V\(\dot{O}_2\) (panel B) are plotted against power output in the four experimental conditions, i.e. before and after a 14-week treatment with recombinant human erythropoietin (rhEpo) during normoxia and acute hypoxia equivalent to an altitude of 4500 m. Values are means±SE in seven subjects. *\(P<0.05\) after vs before rhEpo. $\P<0.05$ hypoxia vs normoxia.

doi:10.1371/journal.pone.0002996.g004

Conversely, such a change in blood flow distribution did not occur after rhEpo in hypoxia.

**Blood pressure and vascular conductance.** Mean arterial blood pressure during maximal exercise was found to be higher following rhEpo in normoxia, but not in hypoxia (Fig. 8A). The same applied for systolic and diastolic blood pressures (results not shown). Systemic vascular conductance during peak exercise decreased after rhEpo in normoxia, while this parameter was not

| Table 1. Maximal exercise variables during the non-invasive experiment. |
|---------------------------------------------------------------|
| **Maximal exercise**                                       |
|                                                            |
| **Normoxia** | **Hypoxia** |
| 0 m          | 1500 m      | 2500 m      | 3500 m      | 4500 m      |
| Power output, W |
| PRE          | 362±13      | 349±14\(^*$\) | 333±12\(^*$\) | 309±11\(^*$\) | 286±12\(^*$\) |
| POST         | 369±13      | 360±14      | 341±16\(^*$\) | 314±14\(^*$\) | 287±12\(^*$\) |
| Cardiac output, l.min\(^{-1}\) |
| PRE          | 27.0±1.2    | 26.5±1.2    | 26.4±0.8     | 26.2±1.0     | 25.8±1.1     |
| POST         | 28.3±1.1    | 28.0±1.2    | 28.4±1.3     | 27.7±1.3     | 26.8±1.1\(^*$\) |
| Hematocrit\(^*,\) \%, |
| PRE          | 46.9±1.4    | 47.7±1.5    | 48.2±1.4     | 47.4±1.4     | 48.1±1.5     |
| POST         | 50.2±1.7\(^*$\) | 51.9±1.5\(^*$\) | 51.4±1.4\(^*$\) | 52.3±1.5\(^*$\) | 50.8±1.2\(^*$\) |
| [Hb]\(^*,\) g.dl\(^{-1}\) |
| PRE          | 15.3±0.4    | 15.3±0.5    | 15.8±0.5     | 15.6±0.5     | 15.3±0.5     |
| POST         | 17.0±0.4\(^*$\) | 17.1±0.5\(^*$\) | 16.8±0.5\(^*$\) | 16.9±0.5\(^*$\) | 16.8±0.4\(^*$\) |
| SaO\(_2\)\(^*,\) % |
| PRE          | 97.7±0.3    | 90.2±2.5    | 85.2±0.8\(^*$\) | 77.4±2.5\(^*$\) | 70.6±1.3\(^*$\) |
| POST         | 96.8±0.7    | 91.0±1.9    | 85.4±1.6\(^*$\) | 79.2±1.1\(^*$\) | 68.3±1.7\(^*$\) |
| CaO\(_2\)\(^*,\) mL.l\(^{-1}\) |
| PRE          | 197.5±6.0   | 186.7±10.2  | 180.1±6.8\(^*$\) | 161.7±10.4\(^*$\) | 147.1±7.1\(^*$\) |
| POST         | 220.3±7.0\(^*$\) | 206.6±10.5\(^*$\) | 190.6±6.3\(^*$\) | 179.5±8.0\(^*$\) | 153.2±6.4\(^*$\) |
| Lactate\(^*,\) mmol.l\(^{-1}\) |
| PRE          | 13.2±0.8    | 12.6±0.9    | 12.5±0.5     | 13.3±0.9     | 12.4±0.6     |
| POST         | 12.6±0.4    | 11.8±1.1    | 11.6±0.7     | 12.9±1.3     | 11.8±0.9     |
| RER          | 1.14±0.02   | 1.24±0.02\(^*$\) | 1.24±0.02\(^*$\) | 1.24±0.02\(^*$\) | 1.28±0.01\(^*$\) |
| POST         | 1.11±0.03   | 1.18±0.02\(^*$\) | 1.20±0.02\(^*$\) | 1.18±0.03\(^*$\) | 1.33±0.04\(^*$\) |
| Ventilation, l.min\(^{-1}\) |
| PRE          | 157±10      | 156±12      | 156±10      | 157±10      | 147±9        |
| POST         | 154±8       | 162±9       | 160±12      | 160±10      | 149±8        |

Values are means±SE (n = 7). *\(\) reflects arterialized samples from capillary blood; $\) reflects arm venous samples. RER, respiratory exchange ratio. The subjects were evaluated before (PRE) and after a 5-week treatment with recombinant human Epo (POST), in normoxia and in acute normobaric hypoxia equivalent to altitudes ranging from 1500 m to 4500 m. $\P<0.05$ POST vs PRE. $\P<0.05$ hypoxia vs normoxia.

doi:10.1371/journal.pone.0002996.t001
Enhancing effect of rhEpo treatment during exercise in normoxia and hypoxia. During exercise in hypoxia CaO₂ and systemic oxygen delivery associated with higher CaO₂ and systemic oxygen delivery. This finding implies that there is a threshold altitude between 3500 and 4500 m, at which altitude there is no benefit for VO₂max from rhEpo treatment. 

This finding implies that there is a threshold oxygenation between 3500 and 4500 m, at which altitude there is no benefit for VO₂max from rhEpo treatment.

**Table 2. Blood oxygenation during the invasive experiment.**

|                       | Submaximal exercise | Maximal exercise |
|-----------------------|---------------------|------------------|
|                       | Normoxia            | Hypoxia (4500 m) | Normoxia            | Hypoxia (4500 m) |
| Power output, W       | PRE                 | POST             | PRE                 | POST             |
|                       | 100                 | 100              | 260                 | 260              |
|                       | 337±13              | 279±11           |
|                       | 364±13              | 281±10           |
| PAO₂, mmHg            | PRE                 | POST             | PRE                 | POST             |
|                       | 106.6±0.8           | 50.3±1.6                                                                             |
|                       | 107.7±1.3           |
|                       | 112.0±0.8           | 56.9±1.6                                                                             |
|                       | 111.6±0.8           | 56.2±0.7                                                                      |
|                       | 100.1±1.2           | 40.4±1.0                                                                      |
|                       | 103.1±2.4           | 41.0±1.1                                                                      |
|                       | 4.3±0.8             | 13.3±1.0                                                                             |
|                       | 8.5±1.4             |
|                       | 11.9±1.4           | 16.5±1.8                                                                      |
|                       | 1.7±1.6             | 11.2±1.8                                                                             |
|                       | 7.8±2.4             |
|                       | 17.4±0.8           | 10.8±1.5                                                                      |
|                       | 19.4±0.8           | 11.8±1.6                                                                      |
|                       | 98.4±0.1           | 71.0±2.3                                                                             |
|                       | 97.5±0.2           |
|                       | 96.9±0.4           | 71.1±1.4                                                                      |
|                       | 98.1±0.1           | 70.0±1.6                                                                             |
|                       | 97.0±0.2           |
|                       | 96.6±0.5           | 70.0±1.4                                                                      |
|                       | 28.9±1.8           | 15.3±1.9                                                                             |
|                       | 17.9±2.4           |
|                       | 11.0±3.1           | 7.5±2.2                                                                      |
|                       | 27.8±1.7           | 15.2±2.5                                                                             |
|                       | 18.9±2.0           |
|                       | 11.3±1.8           | 7.1±1.8                                                                      |
|                       | 189.7±5.3          | 139.7±5.9                                                                             |
|                       | 189.7±4.2          |
|                       | 191.7±4.0          | 138.1±5.2                                                                      |
|                       | 209.9±5.3          | 151.7±6.5                                                                             |
|                       | 209.5±5.7          |
|                       | 210.5±5.2          | 153.9±6.1                                                                      |
|                       | 55.6±4.2           | 30.5±4.1                                                                             |
|                       | 37.5±5.2           |
|                       | 290±5.3           | 17.4±4.6                                                                      |
|                       | 59.5±4.7           | 32.2±6.0                                                                             |
|                       | 42.1±5.0           |
|                       | 333±4.6           | 19.4±4.3                                                                      |
|                       | 14.1±0.4           | 14.6±0.4                                                                             |
|                       | 14.3±0.3           |
|                       | 14.7±0.4           | 15.0±0.3                                                                      |
|                       | 15.6±0.4           | 15.9±0.5                                                                             |
|                       | 15.9±0.4           |
|                       | 163±0.3           | 16.2±0.5                                                                      |
|                       | 14.2±0.4           | 14.5±0.5                                                                             |
|                       | 14.4±0.4           |
|                       | 14.8±0.4           | 14.8±0.3                                                                      |
|                       | 15.8±0.4           | 15.9±0.6                                                                             |
|                       | 15.9±0.4           |
|                       | 16.2±0.5           | 15.8±0.5                                                                           |
|                       | 43.3±1.3           | 44.6±1.4                                                                             |
|                       | 43.9±1.0           |
|                       | 449±1.1           | 45.8±0.9                                                                      |
|                       | 47.8±1.2           | 48.5±1.5                                                                             |
|                       | 47.5±1.0           |
|                       | 49.8±0.8           | 49.5±1.6                                                                      |
|                       | 43.5±1.3           | 44.7±1.4                                                                             |
|                       | 43.5±1.0           |
|                       | 453±1.3           | 45.4±0.9                                                                      |
|                       | 48.2±1.2           | 49.0±1.8                                                                             |
|                       | 48.6±1.4           |
|                       | 48.5±1.5           | 49.4±1.5                                                                      |

Values are means±SE (n = 7). A, alveolar; a, arterial; f, femoral vein; A-aDO₂, alveolar-arterial O₂ difference. The subjects were evaluated before (PRE) and after a 14-week treatment with recombinant human Epo (POST), each time in normoxia and in acute normobaric hypoxia equivalent to 4500 m of altitude. *P<0.05 POST vs PRE.

Acid-base balance, lactate and norepinephrine concentrations in blood. Acid-base balance and blood lactate concentrations are presented in Tables 1 and 3. The levels of arm venous lactate (Table 1), as well as the levels of arterial and femoral venous lactate (Table 3) and potassium (result not shown) measured during peak exercise were similar in all the experimental conditions. Furthermore, rhEpo did not induce any change in leg lactate release (result not shown), regardless of oxygenation. Finally, leg potassium release during peak exercise was found to be similar in all the conditions (result not shown).

Of note is that rhEpo treatment was associated with a large increase in arterial plasma norepinephrine during maximal exercise, reaching 124% in normoxia and 286% in hypoxia compared to the values observed before rhEpo treatment (Fig. 8D).

**Discussion**

This study demonstrates that the well-known VO₂max-enhancing effect of rhEpo treatment during exercise in normoxia is also present during mild to moderate hypoxia up to 3500 m, but at 4500 m there is no benefit for VO₂max from rhEpo treatment. This finding implies that there is a threshold altitude between 3500 and 4500 m, from which little or no benefit should be expected from an increased hemoglobin concentration. The main mechanism responsible for this phenomenon resides on the distribution of cardiac output between active muscles and the rest of the body. During exercise in normoxia, the administration of rhEpo is associated with higher CaO₂ and systemic oxygen delivery combined with a higher fraction of the cardiac output directed to the legs, leading to higher peak leg O₂ delivery and VO₂. During exercise in hypoxia CaO₂ and systemic oxygen delivery were also higher after rhEpo treatment, however the fraction of the cardiac output directed to the legs was not increased concurrently, leaving the increased CaO₂ per se insufficient to improve leg maximal oxygen delivery.

Because our study addresses the question of the interactions between hypoxia, CaO₂ and VO₂max, a prerequisite for data interpretation is that augmented CaO₂ is the main, if not unique factor by which rhEpo might alter VO₂max. In support of this hypothesis, we separately demonstrated in our rhEpo-treated subjects that if the rise in CaO₂ associated with rhEpo...
treatment was acutely reversed by isovolemic hemodilution, the gain in VO₂max conferred by rhEpo was concurrently abolished [25].

rhEpo improves systemic maximal oxygen uptake during moderate hypoxia

Following rhEpo treatment VO₂max was consistently improved during moderate hypoxia, but not during severe hypoxia. To our knowledge, only one research group has previously evaluated the effect of an acute rise in CaO₂ and red blood cell volume on VO₂max during moderate acute hypoxia. Their studies showed that after autologous blood re-infusion VO₂max was increased by 9.3% at 2278 m [26] and by 13% at 3566 m [27]. It has also recently been reported that at moderate altitude of 2340 m, the 15% increase in hemoglobin concentration induced by three weeks of acclimatization in athletes resulted in a progressive rise in CaO₂ that was associated with a 8.9% increase in VO₂max from acute to chronic exposure to 2340 m [28]. Although hemoglobin concentration rose only by 11% after five weeks in our rhEpo-treated subjects, these previous data are nonetheless in line with the present finding showing that increasing CaO₂ is beneficial for aerobic performance in moderate hypoxia.

A surprising observation was that the improvement in VO₂max was even larger during moderate hypoxia (~15%) than during normoxia (~6%) following rhEpo. The observation of a similar VO₂max response at two consecutive levels of simulated altitudes (2500 m and 3500 m) further supports the plausibility of our observation. Our results indicate that in normoxia, the improvement in VO₂max was smaller than the concomitant rise in O₂ delivery (~18%), while during moderate hypoxia (F(IO₂) = 0.134–0.153), the improvement in VO₂max matched the increase in O₂ delivery (~15%). Whatever the mechanism underlying this tighter coupling might be, we suggest that O₂ utilization responds more “efficiently” to higher O₂ supply when the O₂ transport system must cope with moderate arterial hypoxemia.

In severe hypoxia VO₂max is not increased by rhEpo treatment: potential mechanisms involved

The invasive and non-invasive experiments performed in this study show that rhEpo treatment had no positive effect on VO₂max during severe acute hypoxia. This confirms recent data from our laboratory, showing no increase in VO₂max at 4100 m following prolonged rhEpo treatment [9], as well as earlier evidence demonstrating no augmentation in VO₂max at 4300 m.

Table 3. Acid-base balance, lactate, respiratory exchange ratio and ventilation during the invasive experiment.

|                      | Submaximal exercise | Maximal exercise |
|----------------------|---------------------|------------------|
|                      | Normoxia (4500 m)   | Hypoxia (4500 m) |
| Power output, W      |                     |                  |
| PRE                  | 100                 | 100              |
| POST                 | 100                 | 260              |
| pHₐₕ                 |                     |                  |
| PRE                  | 7.401 ± 0.005       | 7.352 ± 0.007    |
| POST                 | 7.394 ± 0.004       | 7.323 ± 0.012    |
| pHₐₕ                 |                     |                  |
| PRE                  | 7.314 ± 0.004       | 7.218 ± 0.015    |
| POST                 | 7.297 ± 0.005       | 7.179 ± 0.015    |
| PaCO₂, mmHg          |                     |                  |
| PRE                  | 37.3 ± 0.9          | 39.8 ± 1.4       |
| POST                 | 52.6 ± 0.8          | 51.9 ± 0.8       |
| PₐₜCO₂, mmHg         |                     |                  |
| PRE                  | 55.5 ± 1.5          | 66.7 ± 2.7       |
| POST                 | 62.1 ± 1.2          | 78.7 ± 2.8       |
| Arterial HCO₃⁻, mmoL⁻¹ |                  |                  |
| PRE                  | 23.5 ± 0.3          | 213 ± 0.5        |
| POST                 | 23.9 ± 0.3          | 20.8 ± 0.5       |
| Femoral vein HCO₃⁻, mmoL⁻¹ |                |                  |
| PRE                  | 23.4 ± 0.3          | 20.5 ± 0.6       |
| POST                 | 23.9 ± 0.3          | 20.2 ± 0.4       |
| Lactateₑₑ, mmoL⁻¹     |                     |                  |
| PRE                  | 1.0 ± 0.1           | 3.7 ± 0.4        |
| POST                 | 1.2 ± 0.1           | 5.1 ± 0.6        |
| Lactateₑₑ, mmoL⁻¹     |                     |                  |
| PRE                  | 1.0 ± 0.1           | 4.4 ± 0.5        |
| POST                 | 1.2 ± 0.1           | 5.9 ± 0.6        |
| RER                  |                     |                  |
| PRE                  | 0.87 ± 0.01         | 0.99 ± 0.02      |
| POST                 | 0.91 ± 0.01         | 1.04 ± 0.02      |
| Ventilation, l.min⁻¹ |                     |                  |
| PRE                  | 44 ± 2              | 93 ± 5           |
| POST                 | 50 ± 1              | 106 ± 5         |
| Vₑₑ / VO₂             |                     |                  |
| PRE                  | 24 ± 1              | 28 ± 2           |
| POST                 | 25 ± 1              | 29 ± 2           |
| Vₑₑ / VCO₂            |                     |                  |
| PRE                  | 28 ± 1              | 27 ± 1           |
| POST                 | 28 ± 1              | 27 ± 1           |
Although in both situations (severe acute hypoxia after rhEpo or chronic hypoxia) \( \dot{V}O_2 \) max appears to be independent of the acute severe hypoxia, in spite of elevated CaO2. Such cardiac output and leg blood flow were found to be preserved in exercising tissues [12]. By contrast, following rhEpo maximal acclimatization [11,13], not only because cardiac output is lower, peak leg blood flow is also decreased with rhEpo failed to increase \( \dot{V}O_2 \) max in severe hypoxia, thus raising the question of the mechanism(s) abolishing the potential while increasing glycolytic capacity [30].

One major difference between the present data and altitude-acclimatization studies relies on the responses of cardiac output and leg blood flow. With chronic exposure to hypoxia, where \( \dot{V}O_2 \) max is classically found barely changed with altitude acclimatization (>4000 m), in spite of a large improvement in CaO2 [11,12,29]. Although in both situations (severe acute hypoxia after rhEpo or chronic hypoxia) \( \dot{V}O_2 \) max appears to be independent of the enhancement of CaO2, the underlying mechanisms are different. Indeed, prolonged rhEpo treatment stimulates erythropoiesis without increasing hypoxia-inducible factor (HIF-1) levels, while during prolonged hypoxia HIF-1 accumulation triggers Epo gene activation therefore promoting erythropoiesis but also targets many other genes involved in various physiological responses, which may also have a negative effect on \( \dot{V}O_2 \) max. In this regard it has been shown that transgenic mice lacking prolyl hydroxylases which otherwise always have HIF-1 downregulate aerobic potential while increasing glycolytic capacity [30].

One major difference between the present data and altitude-acclimatization studies relies on the responses of cardiac output and leg blood flow. With chronic exposure to hypoxia, where \( \dot{V}O_2 \) max is classically found barely changed with altitude acclimatization (>4000 m), maximal cardiac output is blunted [31] and this is thought to be a major cause of the failure to recover \( \dot{V}O_2 \) max after acclimatization [12]. Peak leg blood flow is also decreased with acclimatization [11,13], not only because cardiac output is lower, but also because blood flow is preferentially directed toward non-exercising tissues [12]. By contrast, following rhEpo maximal cardiac output and leg blood flow were found to be preserved in acute severe hypoxia, in spite of elevated CaO2. Such cardiac output response was in line with other data showing no change in maximal cardiac output during acute hypoxia of similar severity (4000–4500 m) without CaO2 manipulation [32,33]. Even so, rhEpo failed to increase \( \dot{V}O_2 \) max in severe hypoxia, thus raising the question of the mechanism(s) abolishing the potential advantage conferred by high O2 transport capacity.

**Blood flow redistribution.** One possible mechanism, supported by our experimental data, is related to blood flow regulation. In normoxia, rhEpo treatment allowed for a higher fraction of maximal cardiac output to be directed toward exercising muscles, resulting in higher peak leg O2 delivery and leg VO2. Although this blood flow priority given to the active tissue implied a flow limitation into the other vascular beds, it is suggested that oxygenation in non-leg tissues - due to higher CaO2 - was nevertheless sufficient to allow for vital organs such as heart, respiratory muscles or brain to ensure their homeostasis. Since at maximal exercise there is a competition between tissues for a limited amount of O2, a strong activation of the sympathetic system is required to avoid hypotension [34] and maintain an efficient match between O2 demand and blood flow distribution [13]. The higher norepinephrine concentrations in plasma during normoxic exercise after rhEpo compared to the corresponding value before rhEpo is compatible with increased sympathetic activation [35] helping to direct a greater fraction of the cardiac output to the legs.

However, the distribution of blood flow during exercise in severe acute hypoxia was not altered by rhEpo, leaving peak leg O2 delivery and leg VO2 unchanged. It is worth noting that at that time, systemic fractional O2 extraction was reduced, unlike peak O2 delivery, therefore suggesting a limitation in O2 extraction into non-leg tissues. Although we could not establish any causal relationship between systemic O2 extraction and blood flow redistribution, we speculate that the lack of blood flow redistribution toward exercising muscles following rhEpo in hypoxia could be related to the low O2 extraction observed in non-leg tissues. Had blood flow decreased to non-leg tissue areas also in hypoxia, where O2 extraction was already diminished in spite of arterial hypoxemia, it is possible that O2 delivery/utilization in some organs (i.e. brain, heart or respiratory muscles) and hence local O2 homeostasis would have been compromised. Beyond the causes of this lower systemic O2 extraction, which remain unclear, our data showed an increase in mixed venous O2 content during hypoxic peak exercise, from 22 ml\( \cdot \)l\(^{-1} \) before rhEpo to 45 ml\( \cdot \)l\(^{-1} \) after rhEpo, suggesting higher central venous oxygenation levels. Pulmonary gas exchange was not altered by the increase in hemoglobin concentration elicited by rhEpo treatment, and hence, alveolar-to-arterial O2 difference and arterial O2 saturation were

---

**Figure 5. Heart rate and stroke volume after rhEpo in normoxia and in severe acute hypoxia.** This figure refers to the invasive study. Heart rate (panel A) and stroke volume (panel B) are plotted against power output in the four experimental conditions, i.e. before and after a 14-week treatment with recombinant human erythropoieitin (rhEpo) during normoxia and acute hypoxia equivalent to an altitude of 4500 m. Values are means±SE in seven subjects. doi:10.1371/journal.pone.0002996.g005
similar to those observed before rhEpo. In agreement with this finding isovolemic hemodilution at altitude did not alter pulmonary gas exchange in altitude-acclimatized subjects [36].

Data on blood pressure and vascular conductance can also shed light on leg versus systemic cardiovascular events that occurred during maximal exercise following rhEpo. Indeed, we found that blood pressure during normoxic maximal exercise increased after rhEpo. Since such response was abolished after isovolemic hemodilution [25], increased viscosity due to high hematocrit was a likely candidate of this higher blood pressure, although a direct rhEpo vasoconstrictor effect cannot be ruled out [37]. In association with higher blood pressure, we found that plasma
norepinephrine concentration at peak exercise reached higher levels following rhEpo, independently of oxygenation. Although we cannot deduce any causal link between circulating norepinephrine and blood pressure from the present data, one possibility is that the high levels of plasma norepinephrine reflected an overall sympathetically-mediated vasoconstriction [35] which could be the cause of elevated blood pressure. If so, the fact that blood pressure did not increase similarly during hypoxic exercise in spite of elevated norepinephrine could be related to hypoxia itself, which is known to attenuate sympathetic vasoconstriction [38]. Nevertheless, the reason why norepinephrine was higher following rhEpo remains unclear. A direct effect of rhEpo on norepinephrine can be speculated, but has not been verified so far.

In summary, our data indicate that the reason why rhEpo does not boost VO\textsubscript{2}\text{max} in severe acute hypoxia are: 1) a redistribution of blood flow causing a lower peak leg blood flow following rhEpo (see Fig. 6E and 7), such that O\textsubscript{2} delivery to the legs in severe hypoxia did not benefit from the increase in CaO\textsubscript{2} brought up by rhEpo treatment, and 2) a lower systemic O\textsubscript{2} extraction.

Insights from the switch between moderate and severe acute hypoxia

One major finding of the present study is that the gain in VO\textsubscript{2}\text{max} following rhEpo vanished within 1000 m of altitude, i.e. that rhEpo was able blunt VO\textsubscript{2}\text{max} decrement efficiently during moderate hypoxia (F\textsubscript{I\textsubscript{O}}\textsubscript{2} = 0.134) but failed to do so during slightly more severe hypoxia (F\textsubscript{I\textsubscript{O}}\textsubscript{2} = 0.115). One previous study is highly relevant to the present one, because it showed a dominance of central fatigue over peripheral muscle fatigue in influencing exercise performance, only if arterial saturation was below 70–75% [39]. Accordingly, we suggest that until the simulated altitude of 3500 m, corresponding to SaO\textsubscript{2} of ~78%, task failure in our subjects was likely more dependent on peripheral than central factors, thus enabling increased CaO\textsubscript{2} to exert its maximal...
influence on VO\textsubscript{2max}. By contrast, during exposure to a higher altitude of 4500 m, SaO\textsubscript{2} of \textasciitilde70\% was likely low enough to trigger central nervous system (CNS) hypoxia and central fatigue in our subjects, therefore accounting – at least in part – for our results. Nevertheless, since this previous study [39] did not manipulate CaO\textsubscript{2} independently of PaO\textsubscript{2} (as in the present one), we cannot draw a definitive conclusion.

Study limitations

The timing difference for the non-invasive study (5 weeks) and the invasive study (14 weeks) during the course of rhEpo administration (see Fig. 1) raises the question of the comparability of the two studies, because of a potential rhEpo time effect. Our previously published data with the same subjects indicate that the hematological changes induced by our rhEpo treatment were of the same magnitude at weeks 5 and 13, as reflected by the similar increases in red blood cell volume and CaO\textsubscript{2} [6]. Furthermore, the similar gain in pulmonary VO\textsubscript{2max} seen either after 5 or after 14 weeks of rhEpo treatment, both in normoxia and hypoxia (4500 m), suggests the attainment of a plateau in the VO\textsubscript{2max} response within the first month of treatment, as previously shown during 8 weeks of rhEpo administration [3].

Another point to be mentioned is the validity of the non-invasive impedance technique we used in the present study. We acknowledge that the measurement of cardiac output during strenuous exercise with the thoracic cardiac impedance method is a questionable technique [40], however a recent report testing the same impedance device against the direct Fick method found that cardiac impedance was reproducible (95% confidence interval <400 ml.min\textsuperscript{-1}) and provided a clinically acceptable evaluation of cardiac output in healthy subjects during an incremental exercise [18]. Our own comparison of the impedance method against the cardio-green dye dilution technique showed values for bias and 95% confidence interval (see Methods and Fig. 2) that were very similar to those previously published [18].

Conclusion

What was already known in the field is that prolonged rhEpo treatment boosts VO\textsubscript{2max} in normoxic conditions but not during severe hypoxia. What the present study adds to the established knowledge is that rhEpo efficiently blunted the decrement of VO\textsubscript{2max} up to the altitude of 3500 m, supporting the idea that CaO\textsubscript{2} is a limiting factor of VO\textsubscript{2max} during moderate hypoxia. Furthermore, our study demonstrates that rhEpo-stimulating effect on VO\textsubscript{2max} was mediated not only by increased CaO\textsubscript{2} but also by preferential blood flow redistribution toward exercising muscle tissue. We do not exclude the possibility that this advantageous blood flow redistribution also occurred at moderate altitude, therefore explaining why VO\textsubscript{2max} was substantially improved in this condition. That such cardiovascular changes did not take place during severe hypoxia would aim to protect some vital organs from deleterious hypoxemia.

Acknowledgments

We are grateful to Pr. Bengt Saltin for his expert comments on this study.

Author Contributions

Conceived and designed the experiments: PR JALC RB CL. Performed the experiments: PR JALC JTT RB PM PR CL. Analyzed the data: PR JALC JTT RB PM PR CL. Contributed reagents/materials/analysis tools: JALC RB PM CL. Wrote the paper: PR JALC CL.

References

1. Audran M, Gareau R, Matecki S, Durand F, Chenuard C, et al. (1999) Effects of erythropoietin administration in training athletes and possible indirect detection in doping control. Med Sci Sports Exerc 31: 639–645.
2. Birckland KL, Strez-Gunderson J, Hemmersbach P, Hallen J, Hauag E, et al. (2000) Effect of rhEPO administration on serum levels of ET and exercise performance. Med Sci Sports Exerc 32: 1238–1241.
3. Russell G, Gore CJ, Aschenby MJ, Parisotto R, Hahn AG (2002) Effects of prolonged low doses of recombinant human erythropoietin during submaximal and maximal exercise. Eur J Appl Physiol 86: 442–449.
4. Ekblom B, Berglund B (1991) Effect of erythropoietin administration on maximal aerobic power. Scand J Med Sci Sports 1: 88–93.
5. Thomsen JJ, Rentsch KL, Rohbach P, Calbet JA, Boushel R, et al. (2007) Prolonged administration of recombinant human erythropoietin increases submaximal performance more than maximal aerobic capacity. Eur J Appl Physiol 101: 481–486.
6. Lundby C, Thomsen JJ, Boushel R, Koskolou M, Warberg J, et al. (2007) Erythropoietin treatment elevates haemoglobin concentration by increasing red cell volume and depressing plasma volume. J Physiol 578: 309–314.
7. Parisotto R, Gore CJ, Emslie KR, Aschenby MJ, Brugnara C, et al. (2000) A novel method utilising markers of altered erythropoiesis for the detection of recombinant human erythropoiesin abuse in athletes. Haematologica 85: 564–572.
8. Kanstrup IL, Ekblom B (1984) Blood volume and hemoglobin concentration as determinants of maximal aerobic power. Med Sci Sports Exerc 16: 256–262.
9. Lundby C, Damsgaard R (2006) Exercise performance in hypoxia after novel erythropoiesis stimulating protein treatment. Scand J Med Sci Sports 16: 35–40.
10. Young AJ, Sawka MN, Mizu SR, Boushel R, Lyon T, et al. (1996) Effects of erythrocyte infusion on VO\textsubscript{2max} at high altitude. J Appl Physiol 81: 252–259.
11. Bender PR, Groves BM, McCullough RE, McCullough RG, Huang SY, et al. (1988) Oxygen transport to exercising leg in chronic hypoxia. J Appl Physiol 65: 2592–2597.
21. Andersen P, Saltin B (1985) Maximal perfusion of skeletal muscle in man. J Physiol 366: 233–249.
22. Boushel R, Calbet JA, Radegran G, Søndergaard H, Wagner PD, et al. (2001) Parasympathetic neural activity accounts for the lowering of exercise heart rate at high altitude. Circulation 104: 1765–1791.
23. González-Alonso J, Calbet JA (2003) Reductions in systemic and skeletal muscle blood flow and oxygen delivery limit maximal aerobic capacity in humans. Circulation 107: 824–830.
24. Kuipers H, Verschuren FT, Keizer HA, Greuten P, van Kraanenburg G (1985) Variability of aerobic performance in the laboratory and its physiologic correlates. Int J Sports Med 6: 197–201.
25. Lundby C, Rosbach P, Boushel R, Thomsen JJ, Rasmussen P, et al. (2006) Does recombinant human epon increase exercise capacity by means other than augmenting O2 transport? J Appl Physiol; doi:10.1152/japplphysiol.90484.2006.
26. Robertson RJ, Gilcher R, Metz KF, Caspersen CJ, Allison TG, et al. (1988) Effect of simulated altitude erythrocytosis in women on hemoglobin flow rate during exercise. J Appl Physiol 64: 1644–1649.
27. Robertson RJ, Gilcher R, Metz KF, Skirnar GS, Allison TG, et al. (1982) Effect of induced erythrocytosis on hypoxia tolerance during physical exercise. J Appl Physiol 53: 490–495.
28. Schuler B, Thomsen JJ, Gassmann M, Lundby C (2007) Timing the arrival at 2340 m altitude for aerobic performance. Scand J Med Sci Sports 17: 588–594.
29. Lundby C, Calbet JA, van Hall G, Saltin B, Sander M (2004) Pulmonary gas exchange at maximal exercise in Danish lowlanders during 8 wk of acclimatization to 4,100 m and in high-altitude Aymara natives. Am J Physiol Regul Integr Comp Physiol 287: R1202–1208.
30. Aragonès J, Schneider M, Van Geyte K, Fraid P, Dresselaers T, et al. (2008) Deficiency or inhibition of oxygen sensor Phd1 induces hypoxia tolerance by reprogramming basal metabolism. Nat Genet 40: 170–178.
31. Vogel JA, Hartley LH, Cruz JC, Hogan RP (1974) Cardiac output during exercise in sea-level residents at sea level and high altitude. J Appl Physiol 36: 160–172.
32. Stenberg J, Ekblom B, Møesén R (1966) Hemodynamic response to work at simulated altitude, 4,000 m. J Appl Physiol 21: 1589–1594.
33. Mollard P, Woorons X, Lebourel M, Lambert M, Favret F, et al. (2007) Determinants of maximal oxygen uptake in moderate acute hypoxia in endurance athletes. Eur J Appl Physiol 100: 663–673.
34. Calbet JA, Jensen-Urstad M, van Hall G, Holmberg HC, Rosdahl H, et al. (2004) Maximal muscular vascular conductances during whole body upright exercise in humans. J Physiol 558: 319–331.
35. Hansen J, Sander M, Thomas GD (2000) Metabolic modulation of sympathetic vasoconstriction in exercising skeletal muscle. Acta Physiol Scand 168: 489–505.
36. Calbet JA, Radegran G, Boushel R, Søndergaard H, Saltin B, et al. (2002) Effect of blood haemoglobin concentration on VO2max and cardiovascular function in lowlanders acclimatized to 3,260 m. J Physiol 545: 715–728.
37. Heidenreich S, Rahm KH, Zidek W (1991) Direct vasoconstrictor effect of recombinant human erythropoietin on renal resistance vessels. Kidney Int 39: 250–265.
38. Hansen J, Sander M, Hald CF, Vøhrer RG, Thomas GD (2000) Metabolic modulation of sympathetic vasoconstriction in human skeletal muscle: role of tissue hypoxia. J Physiol 527: 387–396.
39. Amann M, Romer LM, Subudhi AW, Pegelow DF, Dempeya J (2007) Severity of arterial hypoxaemia affects the relative contributions of peripheral muscle fatigue to exercise performance in healthy humans. J Physiol 581: 389–403.
40. Warburton DE, Haykowsky MJ, Quinney HA, Humen DP, Teo KK (1999) Reliability and validity of measures of cardiac output during incremental to maximal aerobic exercise. Part II: Novel techniques and new advances. Sports Med 27: 241–260.