Does recombinant human Epo increase exercise capacity by means other than augmenting oxygen transport?

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1Copenhagen Muscle Research Centre, Rigshospitalet, Copenhagen, Denmark; 2Department of Sport Science, University of Århus, Århus, Denmark; 3Ecole Nationale de Ski et d’Alpinisme, Chamonix, France; 4Department of Exercise Science, Concordia University, Montreal, Quebec, Canada; 5Copenhagen Muscle Research Centre, Department of Anaesthesi, Rigshospitalet, Copenhagen, Denmark; 6School of Physical Education, Department of Sports Medicine and Exercise Science, University of Athens, Athens, Greece; and 7Department of Physical Education, University of Las Palmas de Gran, Canaria, Spain

Lundby C, Robach P, Boushel R, Thomsen JJ, Rasmussen P, Koskolou M, Calbet JA. Does recombinant human Epo increase exercise capacity by means other than augmenting oxygen transport? J Appl Physiol 105: 581–587, 2008. First published June 5, 2008; doi:10.1152/japplphysiol.90484.2008.—This study was performed to test the hypothesis that administration of recombinant human erythropoietin (rHuEpo) in humans increases maximal oxygen consumption by augmenting the maximal oxygen carrying capacity of blood. Systemic and leg oxygen delivery and oxygen uptake were studied during exercise in eight subjects before and after 13 wk of rHuEpo treatment and after isovolumic hemodilution to the same hemoglobin concentration observed before the start of rHuEpo administration. At peak exercise, leg oxygen delivery was increased from 1,777.0 ± 102.0 ml/min before rHuEpo treatment to 2,079.8 ± 120.7 ml/min after treatment. After hemodilution, oxygen delivery was decreased to the pretreatment value (1,710.3 ± 138.1 ml/min). Fractional leg arterial oxygen extraction was unaffected at maximal exercise; hence, maximal leg oxygen uptake increased from 1,511.0 ± 130.1 ml/min before treatment to 1,793.0 ± 148.7 ml/min with rHuEpo and decreased after hemodilution to 1,428.0 ± 111.6 ml/min. Pulmonary oxygen uptake at peak exercise increased from 3,950.0 ± 160.7 before administration to 4,254.5 ± 178.4 ml/min with rHuEpo and decreased to 4,059.0 ± 161.1 ml/min with hemodilution (P = 0.22, compared with values before rHuEpo treatment). Blood buffer capacity remained unaffected by rHuEpo treatment and hemodilution. The augmented hematocrit did not compromise peak cardiac output. In summary, in healthy humans, rHuEpo increases maximal oxygen consumption due to augmented systemic and muscular peak oxygen delivery.

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were encouraged not to change physical activity pattern or dietary habits, but this was not controlled. The study was approved by the local ethics committee of the communities of Copenhagen and Frederiksberg and conformed to the Declaration of Helsinki. All subjects gave written, informed consent to participate. Before any experiments were initiated, all subjects performed several VO₂ max tests to become accustomed to this type of exercise. The subjects participated in studies besides the one presented here (4, 17, 21, 23, 35).

*rHuEpo administration and hemodilution. rHuEpo administration was designed to increase and maintain the hematocrit to ~50% throughout the study period. The subjects were not blinded toward the treatment. Two weeks before rHuEpo treatment, all subjects received 100 mg iron/day orally, and this was maintained throughout the entire study period. After baseline measurements were made, 5,000 IU of rHuEpo (NeoRecormon, Roche, Mannheim, Germany) was injected as follows: during first 2 wk, one injection every second day; during 3rd week, three injections on 3 consecutive days; and from week 4 to 15, one injection every week. Intravascular volumes were measured by CO rebreathing and have been reported elsewhere (23). To perform isovolemic hemodilution, the extra red blood cell volume gained with rHuEpo treatment was withdrawn, and the volume was replaced by infusing the amount of 5% human albumin solution needed to achieve the circulating volume and red blood cell volume recorded before rHuEpo. Because in some subjects (n = 5) rHuEpo administration decreased plasma volume more than red blood cell mass was increased (23), these subjects received a volume of 5% albumin solution that was greater than the volume of cell mass removed.

Experimental preparation. On the experimental day, the subjects reported to the laboratory at 8:00 AM, and catheters were inserted while participants were under local anesthesia (2% lidocaine) (2). A 20-gauge catheter (ES-14150; Arrow, Reading, PA) was inserted percutaneously using the Seldinger technique into the right femoral artery 2 cm below the inguinal ligament and advanced 5–10 cm in the proximal direction. This catheter was connected to a blood pressure transducer positioned at the height of the fourth intercostal space (Ti00209A; Baxter, Unterschleissheim, Germany) and was also used to sample arterial blood. In the right 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 iced physiological saline solution (1). A thin polyethylene-coated thermistor (model 94-030-2.5F TD probe; Edwards Edwards, Baxter, Irvine, CA) was inserted through the venous catheter for blood flow measurements by the constant infusion thermodilution technique (1). A flow-through chamber (model 93-505, Edwards) was connected to the entry of this catheter to measure infusedate temperature during ice-cold saline infusion. In the same vein, an additional 20-gauge catheter (Hydrocath, Ohmeda, Wiltshire, UK) was also inserted 2–3 cm below the inguinal ligament and advanced 7–10 cm in the distal direction, beyond the merger with the saphenous vein. This catheter was connected to another blood pressure transducer positioned at the height of the fourth intercostal space (Ti00209A, Baxter) and used to measure femoral venous pressure and to obtain femoral venous blood samples. An additional venous catheter was inserted into an antecubital vein to inject indocyanine green (Akorn) when measuring cardiac output, as explained below.

A three-lead ECG was displayed on a monitor during catheterization and during 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 infusedate temperatures, were recorded simultaneously with the data-acquisition system (MacLab 8s; ADInstruments, Sydney, Australia).

All exercise tests were performed upright on a cycle ergometer (Monark, Varberg, Sweden). The exercise protocol started with a warm up of 15 min at 100 W, and then the load was increased by 40 W every 1.5 min until exhaustion. At each exercise intensity, measurements started after 45 s with the assessment of blood flow, followed immediately by the withdrawal of blood samples from the femoral vein and from the femoral artery for the determination of blood gas status and acid-base balance. Pulmonary oxygen consumption (VO₂), heart rate, and arterial and femoral vein pressures were measured continuously during the exercise trials. Heart rate and blood pressures were averaged during 15 s around the blood flow measurements. During the incremental exercise test, VO₂ was averaged every 15 s. The VO₂ corresponding to each load was calculated as the mean VO₂ of the last four consecutive 15-s VO₂ averages. Peak VO₂ was defined as the maximal 15-s VO₂ value recorded during the test. The exercise load reached at exhaustion was considered to be the maximal exercise intensity. This test was completed before the rHuEpo administration period (pre-rHuEpo), as well as after the 13 wk of administration. On this occasion, two tests were completed: one control trial (post-rHuEpo) and one hemodiluted (hemodilution) trial performed after ~2 h of supine recovery following the first test.

Blood flow. Femoral venous blood flow was measured by constant-infusion thermodilution, as described in detail elsewhere (1). Briefly, iced saline was infused (Harvard pump; Harvard Apparatus, Millis, MA) through the femoral vein at flow rates sufficient to decrease blood temperature at the thermistor by 0.5–1°C. At rest, saline infusions were continued for at least 60 s, whereas, during exercise, 15- to 20-s-long infusions were used until femoral vein temperature had stabilized at its new lower value. Blood flow was calculated on thermal balance principles, as detailed by Andersen and Saltin (2).

Respiratory variables. Pulmonary VO₂, CO₂ production (VCO₂), and expired minute ventilation (Ve) were measured continuously with an automated metabolic cart (Quark b2; Cosmed, Rome, Italy). Before each test, ambient conditions were measured, and the gas analyzer and the flowmeter were calibrated with high-precision gases.

Vascular conductances. Leg vascular conductance was calculated as the quotient between leg blood flow and the pressure difference between the femoral artery and the femoral vein. Systemic vascular conductance was calculated as the quotient between cardiac output and mean arterial pressure.

Cardiac output. Cardiac output was measured with the dye-dilution method using indocyanine green as previously reported (20).

Blood samples and analytic procedures. Arterial and venous blood were sampled anaerobically in heparinized syringes and immediately analyzed for Hb, hematocrit (%), oxygen saturation (OSM 3 he- moxymeter; Radiometer, Copenhagen, Denmark), blood pH, base excess, plasma glucose, plasma lactate, and PCO₂ and PO₂ (ABL700; Radiometer). Femoral venous blood was also measured with the ABL700. Blood gases were corrected for measured femoral vein blood temperature. From these values, plasma HCO₃⁻ and actual base excess were determined as described by Siggaard-Andersen (33). Because reduced Hb has a higher buffer capacity than fully oxygenated Hb, base excess was adjusted in each blood sample to fully oxygenated Hb (33).

Blood O₂ content (CaO₂ and femoral vein O₂ content) was computed from the saturation and [Hb], i.e., (1.34 × [Hb] × oxygen saturation) + (0.003 × PO₂).

Capillary muscle O₂ conductance and mean capillary PO₂. To calculate capillary muscle O₂ conductance (D₂O), an iterative numerical integration procedure was used to find the value of O₂ conductance (i.e., in ml·min⁻¹·Torr⁻¹) that yields the measured femoral muscle venous PO₂ (6, 36, 37). The calculation of D₂O assumes (1) that the intracapillary PO₂ is negligibly small at VO₂ max (12, 30; 2) that the O₂ remaining in the femoral and subclavian venous blood is wholly accountable for by diffusion limitation of O₂ from the microcirculation to the mitochondria; and (3) that perfusion/VO₂ heterogeneity and perfusional or diffusional shunt are negligible. Mean capillary PO₂ is the numerical average of all computed PO₂ values, equally spaced in time, along the capillary from the arterial to venous end. The “eleva- tion index” Y, proposed by Piiper (27) [Y = D₂O/QL(Y)], where QL is the leg blood flow and γ is the mean slope of the oxygen dissociation curve of the Hb. With decreasing Y, diffusion limitation increases and perfusion limitation decreases (Y > 3 indicates predom-
inant perfusion limitation; 3 $> 1 > 0.1$ indicates combined perfusion and diffusion limitation, $Y < 0.1$ indicates prevailing diffusion limitation.

Statistics and calculations. One-way ANOVA for repeated measures with Student-Newman-Keuls post hoc test to locate differences was applied. Statistical difference was set at $P < 0.05$. All values reported are means ± SD. For submaximal data analysis, the final minutes of the 15-min 100-W warm-up were used, assuming a high degree of steady state at this easy workload.

RESULTS

Measurements during supine rest before and after rHuEpo administration. [Hb] was increased ($P < 0.05$) from 142 ± 4 to 156 ± 4 g/l after rHuEpo treatment and decreased to 142 ± 4 after hemodilution. In accordance, hematocrit was 43.7 ± 1.3, 47.7 ± 1.2 ($P < 0.05$), and 43.8 ± 1.3% in the three conditions. $\text{CaO}_2$ was increased ($P < 0.05$) from 190.4 ± 5.9 to 209.0 ± 5.9 ml/l after rHuEpo administration and decreased to 190.6 ± 5.8 ml/l with hemodilution. Resting pulmonary $\text{Ve}$ (9.9 ± 0.8 vs. 10.8 ± 0.6 l/min), $\text{VCO}_2$ (364 ± 22 vs. 388 ± 22 ml/min), and $\text{VCO}_2$ (300 ± 20 vs. 340 ± 22 ml/min) were not altered with prolonged rHuEpo administration. Resting heart rate (62.7 ± 3.1 vs. 61.6 ± 2.8 beats/min), stroke volume (111.1 ± 7.7 vs. 119.7 ± 7.3 ml), and cardiac output (6.8 ± 0.2 vs. 7.7 ± 0.5 l/min) remained unchanged after the rHuEpo treatment period. In contrast, resting systolic (135.1 ± 2.2 to 143.8 ± 4.3 mmHg), diastolic (76.2 ± 1.2 to 80.1 ± 1.2 mmHg), and mean blood pressure (96.7 ± 1.3 to 102.0 ± 2.1 mmHg) were all increased ($P < 0.05$) compared with before the treatment. The rate-pressure product (RPP) was increased ($P < 0.05$) from 8,477 ± 463 to 8,839 ± 414 mmHg·beats⁻¹·min⁻¹.

Measurements during exercise. All submaximal data reported here were obtained in the final minute during the warm-up period at 100 W. Maximal data were obtained as close to maximal effort as possible and correspond to 337.0 ± 13.1 W pre-rHuEpo treatment and to 368.4 ± 11.7 ($P < 0.05$) and 339.4 ± 12.0 W post-rHuEpo and after hemodilution, respectively (Table 1).

During submaximal exercise, pulmonary $\text{Ve}$, $\text{VO}_2$ (Fig. 1D), and $\text{VCO}_2$ did not differ between all three experimental conditions but were all increased ($P < 0.05$) at maximal exercise after rHuEpo administration (9, 8, and 6%, respectively), returning to pretreatment values after hemodilution. After rHuEpo treatment, Hb, hematocrit, and $\text{CaO}_2$ (Fig. 1A) were all increased during submaximal (12, 12, and 12%, respectively) and maximal (12, 12, and 11%, respectively) exercise, and all three were normalized with hemodilution. In contrast heart rate, stroke volume, and cardiac output remained unaffected, except after hemodilution where stroke volume and cardiac output were decreased.

Table 1. Measurements during exercise

<table>
<thead>
<tr>
<th>Submaximal Exercise (100 W)</th>
<th>Maximal Exercise</th>
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<tbody>
<tr>
<td></td>
<td>Pre-rHuEpo</td>
</tr>
<tr>
<td>Pulm Ve</td>
<td>442 ± 1.6</td>
</tr>
<tr>
<td>Pulm $\text{VO}_2$</td>
<td>1,829 ± 10.7</td>
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<tr>
<td>Pulm $\text{VCO}_2$</td>
<td>1,729 ± 9.7</td>
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<tr>
<td>$\text{Ve}/\text{VO}_2$</td>
<td>24.2 ± 0.6</td>
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<tr>
<td>$\text{Ve}/\text{VCO}_2$</td>
<td>26.1 ± 1.6</td>
</tr>
<tr>
<td>[Hb]</td>
<td>14.1 ± 0.4</td>
</tr>
<tr>
<td>Hct</td>
<td>43.3 ± 1.3</td>
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<tr>
<td>$\text{SaO}_2$</td>
<td>98.0 ± 0.4</td>
</tr>
<tr>
<td>$\text{CaO}_2$</td>
<td>189.8 ± 5.3</td>
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<tr>
<td>HR</td>
<td>126.2 ± 5.0</td>
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<tr>
<td>SV</td>
<td>128.1 ± 7.5</td>
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<tr>
<td>Q</td>
<td>15.9 ± 0.4</td>
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<tr>
<td>SBP</td>
<td>120.5 ± 4.7</td>
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<tr>
<td>DBP</td>
<td>65.4 ± 2.8</td>
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<tr>
<td>MBP</td>
<td>87.5 ± 2.2</td>
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<tr>
<td>RPP</td>
<td>15.264 ± 1.023</td>
</tr>
<tr>
<td>SVC</td>
<td>182.8 ± 8.0</td>
</tr>
<tr>
<td>FV VC</td>
<td>48.5 ± 2.4</td>
</tr>
<tr>
<td>Leg flow</td>
<td>4,886 ± 251.8</td>
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<tr>
<td>Leg $\text{O}_2$ del</td>
<td>879.8 ± 48.2</td>
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<tr>
<td>$%\text{Extra}$</td>
<td>70.8 ± 1.7</td>
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<tr>
<td>(a-$\text{VCO}_2$</td>
<td>134.1 ± 3.9</td>
</tr>
<tr>
<td>Leg $\text{VCO}_2$</td>
<td>656.5 ± 43.5</td>
</tr>
<tr>
<td>Arterial Lac</td>
<td>1.0 ± 0.1</td>
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<tr>
<td>Venous Lac</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Venous pH/AU</td>
<td>7,412 ± 0.0</td>
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<tr>
<td>Venous pH/AU</td>
<td>7,325 ± 0.0</td>
</tr>
<tr>
<td>Arterial NE, mmol/l</td>
<td>0.242 ± 0.04</td>
</tr>
</tbody>
</table>

Values are means ± SD. rHuEpo, recombinant human erythropoietin; Pulm Ve, pulmonary minute ventilation (l/min); Pulm $\text{VO}_2$, pulmonary O2 consumption (l/min); Pulm $\text{VCO}_2$, pulmonary CO2 production (l/min); [Hb], hemoglobin concentration (g/dl); Hct, hematocrit (%); $\text{SaO}_2$, arterial O2 saturation (%); $\text{CaO}_2$, arterial O2 content (ml/l); HR, heart rate (beats/min); SV, stroke volume (ml); Q, cardiac output (l/min); SBP, systolic blood pressure (mmHg); DBP, diastolic blood pressure (mmHg); MBB, mean blood pressure (mmHg); RPP, rate-pressure product (mmHg·beats⁻¹·min⁻¹); SVC, systemic vascular conductance (ml·mmHg⁻¹·min⁻¹); FVC, femoral vein vascular conductance (ml·mmHg⁻¹·min⁻¹); Leg flow, femoral vein blood flow (ml/min); Leg $\text{O}_2$ del, femoral arterial O2 delivery (ml/min); Leg $\text{O}_2$ extraction fraction (%); (a-$\text{O}_2$, arteriovenous O2 difference (ml); leg $\text{VO}_2$, leg O2 consumption (ml/min); arterial Lac, arterial lactate (mmol/l); venous Lac, venous lactate (mmol/l); AU, arbitrary units; NE, norepinephrine. Measurements were taken during 15 min at 100 W and at maximal exercise intensity. *$P < 0.05$ between pre-rHuEpo and post-rHuEpo; †$P < 0.05$ between post-rHuEpo and hemodilution.

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output were elevated \((P < 0.05)\) by 6 and 6\%, respectively. Systolic and mean blood pressures were increased \((P < 0.05)\) during submaximal (10 and 7\%, respectively) and also at maximal exercise, where diastolic blood pressure was also increased (16, 16, and 30\%, respectively). After rHuEpo treatment, RPP was increased \((P < 0.05)\) at maximal exercise and normalized after hemodilution. Systemic leg blood flow during submaximal exercise was unaffected by rHuEpo treatment but increased \((P < 0.05)\) compared with that shown post-rHuEpo with hemodilution. At maximal exercise, leg blood flows values were similar \((P < 0.05)\) with rHuEpo treatment and normalized with hemodilution.

Muscle \(O_2\) diffusing capacity (muscle \(O_2\) conductance). Muscle \(O_2\) conductance was similar before and after rHuEpo administration \((32.2 \pm 3.1\) and \(36.3 \pm 4.1\) ml\(\cdot\)min\(^{-1}\)\(\cdot\)mmHg\(^{-1}\), respectively; \(P = 0.17\)) and was not affected by hemodilution \((28.6 \pm 2.0\) ml\(\cdot\)min\(^{-1}\)\(\cdot\)mmHg\(^{-1}\); \(P = 0.10\), compared with pre-rHuEpo and post-rHuEpo). Mean capillary \(P_{O_2}\) was 2.8 Torr higher with rHuEpo \((47.3 \pm 1.0\) and \(50.1 \pm 1.1\) Torr, pre- and post-rHuEpo, respectively; \(P < 0.05\)) and remained unchanged with hemodilution \((49.9 \pm 1.2\) Torr; \(P < 0.05\) compared with pre-rHuEpo treatment). The parameter \(Y\) was significantly reduced after rHuEpo treatment \((1.75 \pm 0.04\) to \(1.66 \pm 0.03\), pre- and post-rHuEpo, respectively; \(P < 0.05\)). Hemodilution did not alter \(Y\) compared with that shown.
before rHuEpo treatment (1.67 ± 0.03; P < 0.05 compared with pre-rHuEpo). Thus, after rHuEpo treatment, there was a marginal increase in the diffusive limitation to blood-tissue O₂ transfer in muscle, which could not be attributed to the increased blood [Hb].

Femoral venous lactate, arterial glucose, and arterial norepinephrine. Femoral venous and arterial lactate, pH, glucose, and K⁺ were unaffected by rHuEpo treatment and hemodilution at rest and at submaximal and maximal exercise. Norepinephrine concentration was approximately doubled at maximal exercise after the rHuEpo treatment and hemodilution.

**Discussion**

The main finding in the present study is that rHuEpo exerts its main effects on VO2 max through an increase in red blood cell mass and thereby a higher CaO₂ and peak O₂ delivery. In addition, the data demonstrate 1) that rHuEpo treatment increases mean arterial pressure during exercise and that this does not restrain maximal cardiac output and 2) that VO2 max is primarily limited by the capacity of the cardiovascular system to deliver O₂ to the exercising muscles.

Effects of rHuEpo treatment and hemodilution on VO2 max. In the present study, rHuEpo administration increased [Hb] by 11.6%. As a result, O₂ delivery and VO2 max were both increased by ~300 ml. This demonstrates that all the gained O₂ delivering capacity with rHuEpo treatment was made available and used by the active muscles during maximal exercise. Therefore, the ergogenic effect of rHuEpo on VO2 max can entirely be explained through the enhancement of the O₂-carrying capacity of blood. In addition, this supports the notion that VO2 max is primarily limited by the capacity of the cardiovascular system to deliver O₂ to the exercising muscles (6, 10). Interestingly, after rHuEpo treatment, pulmonary VO2 reached a value very close to VO2 max already at ~80% of maximal work rate. In support of this finding, femoral arterial O₂ delivery and leg VO2 reached peak values at this workload. After isovolumic hemodilution, VO2 max decreased by ~200 ml/min, a finding that is in agreement with previous research [see Calbet et al. (9) for a recent review]. The match between the removed Hb/O₂ and the concomitant reduction in VO2 max again strongly shows that increased erythropoiesis is the main mechanism by which rHuEpo enhances VO2 max.

The increase in stroke volume and cardiac output with hemodilution is in line with previous research (18). In that study, it was argued that stroke volume was enhanced by increasing preload and hence relied on the Frank-Starling mechanism. Because of the increase in cardiac output, VO2 max was maintained despite a reduction in [Hb]. In the present study, however, the increase in cardiac output after hemodilution was not sufficient to counteract the decrease in [Hb], and oxygen delivery to the exercising limbs was reduced. Because we did not observe differences in total blood volume after the rHuEpo treatment period (~82 ml; P = 0.43), the mechanism for the increased cardiac output may also be different from that proposed previously (18). Although we only quantified blood volume before and not after hemodilution (impossible because of the long half life of CO that will bind to Hb and hence affect oxygen binding during the last exercise test), we are certain that the procedure was performed isovolumically. When calculated from the [Hb] and oxygen content obtained after the procedure, the ~1 h given the subjects to recover after hemodilution was not sufficient to induce potential fluid movement from inter- to intravascular compartments. Hence, the increase in cardiac output after hemodilution was most likely not the result of an altered preload. Because systolic and diastolic blood pressures were reduced after hemodilution, most likely afterload was reduced (7). This is usually associated with an increased ejection fraction and hence increased stroke volume and cardiac output.

An alternative explanation, such as an increased myocardial contractility, is unlikely since sympathetic nervous activity at maximal exercise, as reflected by plasma norepinephrine concentration, was not influenced by hemodilution.

**Hemodynamic effects.** When the hematocrit is increased, the viscosity of the blood is also increased (28), and this is the most likely candidate for the observed increases in mean arterial blood pressure in the present study. This possibility is strongly supported by the reduction in exercise mean arterial blood pressure after isovolemic hemodilution. The changes in blood pressures, however, could also be the result of an increased CaO₂-dependent vasoconstriction. Breathing 100% O₂ has been demonstrated to induce vasoconstriction in the femoral artery during exercise (39). It remains to be elucidated whether the constrictor effects of O₂ are PO₂ or CaO₂ dependent. Nevertheless, it has been speculated that, at some critical hematocrit, the increased load on the heart due to augmented blood viscosity will force a reduction in maximal cardiac output and hence limit exercise capacity. However, the present investigation disproves this classic explanation, at least up to a hematocrit value of ~50%. Moreover, the fact that blood pressures and the RPP were increased with the elevation of the hematocrit shows that, at the normal hematocrit (i.e., before rHuEpo treatment) at exhaustion, the heart has not reached its maximal working capacity. In agreement with this is the previously reported finding that VO2 max is increased with the same magnitude over a variety of [Hb] values (11). The finding that maximal cardiac output was maintained at ~25 l/min before and after the rHuEpo treatment period despite an increase in RPP argues against the “central governor theory” as the main limiting factor for VO2 max (14, 15). The central governor theory argues that the circulation is controlled by the central nervous system primarily to protect the heart muscle from becoming ischemic and that VO2 max is only a consequence of the amount of work that the heart is allowed to perform (14, 15). Thus, because similar peak cardiac outputs were achieved with markedly different RPPs, this argues against the “central governor theory.” In a recent study performed by Ekblom and colleagues (5), it was elegantly demonstrated that maximal oxygen uptake and cardiac output are not limited by a central nervous system governor because close to identical oxygen uptakes and cardiac outputs were reported in two different exercise protocols eliciting different RPP. Interestingly, we have recently demonstrated that this is also true at altitude (20). Because cardiac output was increased after hemodilution without a concomitant decrease in the RPP, this suggests that maximal cardiac output is established by a regulatory mechanism and not by a ceiling in the working capacity of the heart. Accordingly, for a given maximal oxygen uptake, cardiac output may be different (the arteriovenous oxygen...
difference is adjusted accordingly). Examples include short exercise trials (\(V_{O_2} = 4.9 \text{ l/min} \text{ and cardiac output} = 25.8 \text{ l/min}\)) vs. longer trials (\(V_{O_2} = 4.9 \text{ l/min} \text{ and cardiac output} = 28.7 \text{ l/min}\)) (13).

**Impact of Epo on blood-tissue \(O_2\) transfer.** This study provides further experimental evidence showing that, in healthy humans, \(V_{O_2 \text{ max}}\) is not limited by the muscle diffusing capacity (31). Because the rHuEpo administration did not induce angiogenesis (22) or augment the oxidative capacity of the skeletal muscle (17), no enhancement of muscle \(O_2\) diffusing capacity was expected. Mathematically, \(V_{O_2} = D_{O_2} \times \Delta P_{O_2}\), where the coefficient \(D_{O_2}\) is a lumped estimation of the muscle diffusing capacity and \(\Delta P_{O_2}\) is the gradient between the mean capillary \(P_{O_2}\) and the \(P_{O_2}\) in cytochrome \(c\), which is assumed to be close to 0 at \(V_{O_2 \text{ max}}\) (32). rHuEpo injections resulted in a higher \(V_{O_2 \text{ max}}\), without structural changes that could explain an increase in muscle diffusing capacity and hence \(D_{O_2}\) (6). Therefore, this finding implies that, before rHuEpo treatment, not all the available muscle \(O_2\) diffusing capacity was recruited; i.e., \(V_{O_2 \text{ max}}\) was not limited by muscle diffusing capacity before the treatment with rHuEpo. In agreement with our calculations and our interpretation, fractional \(O_2\) capacity was recruited; i.e., \(V_{O_2 \text{ max}}\) was not limited by muscle diffusing capacity in healthy humans. The latter could explain a greater flow of \(O_2\) despite similar \(AaDO_2\). In summary, rHuEpo administration increases red blood cell mass and \(O_2\) transport capacity, and this is the main reason for \(V_{O_2 \text{ max}}\) to increase. The healthy heart can tolerate higher RPP than observed at peak exercise in normoxia, suggesting the main mechanism causing exhaustion during an incremental exercise test to exhaustion is not a failure or attainment of the maximal working capacity of the heart. \(V_{O_2 \text{ max}}\) is not limited by muscle diffusion capacity in healthy humans.

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