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

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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 isovolemic 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.

hemoglobin concentration

RECOMBINANT HUMAN ERYTHROPOIETIN (rHuEpo) became commercially available in 1987; since then, several studies have investigated the effects of Epo on hemoglobin concentration ([Hb]) and maximal oxygen consumption ($\dot{V}O_{2\,max}$). Ekblom and Berglund (11) performed the first study, injecting 20–40 IU/kg body mass for a period of up to 6 wk, and found an increase in [Hb] and $\dot{V}O_{2\,max}$. More recently, our group demonstrated that the effects of rHuEpo are even more pronounced at submaximal exercise intensities (35) and that [Hb] is augmented by decreasing plasma volume concomitantly to stimulating erythropoiesis (23). Although it may seem obvious that the main effects of rHuEpo on exercise capacity are mediated through increases in Hb mass, several other mechanisms have been proposed in recent years.

Epo has been reported to exert effects similar to VEGF on the angiogenic process, and one of the mechanisms by which Epo appears to promote angiogenesis is by enhancing the level of VEGF in tissue. A close association between VEGF and Epo in angiogenesis has been proposed (1, 3), and Epo treatment has been found to enhance the release of VEGF from marrow stromal cells (40) and to increase levels of VEGF in brain (19, 38). Considering the importance of VEGF in skeletal muscle capillary growth (29), it is therefore plausible that one of the angiogenic effects of Epo is mediated by promoting VEGF levels in the muscle. In a recent training study, rodents were reported to change muscle fiber type toward a more oxidative phenotype when treated with Epo during the training period (10). However, in the same subjects as in the present study, we were not able to confirm skeletal muscle angiogenesis or a shift in fiber type (21), and also VEGF mRNA levels remained unaffected by the rHuEpo treatment. Metabolic enzymes analyzed from the same biopsies have also been reported to be unaffected by the rHuEpo treatment (17). In line with this, it has recently been demonstrated that rHuEpo may also have effects on brain function [for review, see Jelkmann (16)], and healthy subjects treated with rHuEpo are reported to feel improvements in mood (24) and perceived physical conditioning (26).

In the present study, the hypothesis to be tested was that the main mechanism by which treatment with rHuEpo induces an enhancement of $\dot{V}O_{2\,max}$ is by increasing systemic and muscular oxygen delivery, primarily from a higher Hb mass and arterial oxygen content (Ca_{O_2}). This hypothesis was tested in eight healthy men who first received rHuEpo during 3 mo and then were hemodiluted isovolumically to reduce the extra cell mass gained with the rHuEpo treatment.

METHODS

Subjects. Eight healthy male volunteers (age: 27 ± 7 yr; height: 180 ± 4 cm; weight: 83 ± 7 kg) participated in the study. All subjects were university students and did not participate in organized sports. Most of them were engaged in recreational sports, such as jogging and commuting by bike. Throughout the study period of 4 mo, the subjects

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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 $\dot{V}O_{2\,max}$ 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 (T100209A; 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 Edslab, 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, Edslab) was connected to the entry of this catheter to measure infusate 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 (T100209A, Baxter) and used to measure femoral vein 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 infusate temperatures, were recorded simultaneously with the data-acquisition system (MacLab 8/s; 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 ($\dot{V}O_2$), 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, $\dot{V}O_2$ was averaged every 15 s. The $\dot{V}O_2$ corresponding to each load was calculated as the mean $\dot{V}O_2$ of the last four consecutive 15-s $\dot{V}O_2$ averages. Peak $\dot{V}O_2$ was defined as the maximal 15-s $\dot{V}O_2$ 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) trail 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 $\dot{V}O_2$, CO_2 production ($\dot{V}CO_2$), and expired minute ventilation ($\dot{V}E$) were measured continuously with an automated metabolic cart (Quark b^2; 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 (OSM3 hemoxymeter; Radiometer, Copenhagen, Denmark), blood pH, base excess, plasma glucose, plasma lactate, and P_{CO_2} and P_{O_2} (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 HCO_3^- 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 O_2 content (Ca_{O_2} and femoral vein O_2 content) was computed from the saturation and [Hb], i.e., $(1.34 \times [Hb] \times \text{oxygen saturation}) + (0.003 \times P_{O_2})$.

Capillary muscle O_2 conductance and mean capillary P_{O_2}. To calculate capillary muscle O_2 conductance ($\dot{D}O_2$), an iterative numerical integration procedure was used to find the value of O_2 conductance (i.e., in $ml \cdot min^{-1} \cdot Torr^{-1}$) that yields the measured femoral muscle venous P_{O_2} (6, 36, 37). The calculation of $\dot{D}O_2$ assumes *1*) that the intracellular P_{O_2} is negligibly small at $\dot{V}O_{2\,max}$ (12, 30); *2*) that the O_2 remaining in the femoral and subclavian venous blood is wholly accountable for by diffusion limitation of O_2 from the microcirculation to the mitochondria; and *3*) that perfusion/$\dot{V}O_2$ heterogeneity and perfusional or diffusional shunt are negligible. Mean capillary P_{O_2} is the numerical average of all computed P_{O_2} values, equally spaced in time, along the capillary from the arterial to venous end. The "equilibration index" Y, proposed by Piiper (27) [$Y = \dot{D}O_2/(\dot{Q}L\gamma)$], where $\dot{Q}L$ 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; $3 > Y > 0.1$ 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 to $P < 0.05$. 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 ± 1.2% in the three conditions. Ca_{O_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 $\dot{V}_E$ (9.9 ± 0.8 vs. 10.8 ± 0.6 l/min), $\dot{V}_{O_2}$ (364 ± 22 vs. 388 ± 22 ml/min), and $\dot{V}_{CO_2}$ (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 $\dot{V}_E$, $\dot{V}_{O_2}$ (Fig. 1*D*), and $\dot{V}_{CO_2}$ 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 Ca_{O_2} (Fig. 1*A*) 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

Table 1. *Measurements during exercise*

	Submaximal Exercise (100 W)			Maximal Exercise		
	Pre-rHuEpo	Post-rHuEpo	Hemodilution	Pre-rHuEpo	Post-rHuEpo	Hemodilution
Pulm $\dot{V}_E$	44.2±1.6	49.8±1.2	51.8±2.2	150.3±8.2	163.4±6.5*	159.9±6.7*
Pulm $\dot{V}_{O_2}$	1,829.4±53.5	1,906.0±31.7	1,845.8±57.2	3,950.0±160.7	4,254.5±178.4*	4,059.6±161.1†
Pulm $\dot{V}_{CO_2}$	1,726.9±97.9	1,761.2±41.6	1,735.6±45.4	4,583.4±96.2	4,865.8±202.1*	4,633.0±181.8†
$\dot{V}_E/\dot{V}_{O_2}$	24.2±0.6	25.6±0.9	26.3±0.9	37.9±0.7	38.6±1.1	39.4±0.9
$\dot{V}_E/\dot{V}_{CO_2}$	26.1±1.6	27.7±1.0	27.9±1.0	32.8±0.8	33.7±1.1	34.5±0.8
[Hb]	14.1±0.4	15.8±0.4*	14.2±0.4†	14.7±0.4	16.4±0.3*	14.8±0.3†
Hct	43.3±1.3	48.3±1.1*	43.6±1.1†	44.9±1.1	50.3±0.9*	45.4±1.0†
Sa_{O_2}	98.4±0,1	98.1±0.1	97.2±0.5	96.9±0.4	96.5±0.4	96.4±0.4
Ca_{O_2}	189.8±5.3	212.1±5.1*	190.0±4.8†	191.7±4.0	212.9±5.1*	193.6±4.8†
HR	126.2±5.0	123.3±5.4	127.6±3.7	177.1±5.4	180.1±2.8	181.0±2.2
SV	128.1±7.5	123.1±7.0	130.0±6.4	141.8±6.3	140.4±6.8	148.1±6.1*†
Q	15.9±0.4	15.0±0.5	16.5±0.8	25.0±1.0	25.2±1.2	26.8±1.1*†
SBP	120.5±4.7	132.3±2.2*	120.8±4.2†	119.4±4.6	139.1±10.4*	116.6±11†
DBP	65.4±2.8	67.3±2.0	62.2±3.1†	53.3±4.8	69.4±8.3*	56.0±8.3†
MBP	87.9±2.2	93.8±1.6*	88.1±3.1†	83.7±2.1	97.1±8.8*	82.1±9.0†
RPP	15,264±1,023	16,270±606	15,379±550	21,145±1193	25,027±1916*	21,157±2157†
SVC	182.4±8.0	159.9±6.5*	188.9±10.9†	308.4±18.8	276.2±31.8*	346.5±31.3†
FV VC	48.5±2.4	50.0±2.3	60.8±3.2*†	88.2±6.7	90.0±13.0	127.7±14.5*§
Leg flow	4,886.8±251.8	4,698.6±174.1	5,236.9±237.7†	9,077.7±514.1	9,999.9±528.2	10,575.7±609.5
Leg O_2 del	879.8±48.2	994.0±36.3*	992.5±61.7*	1,777.0±102.0	2,079.8±120.7*	1,710.3±138.1†
%Extrac	70.8±1.7	72.1±1.5	70.9±1.3	84.9±2.6	83.9±1.8	83.1±1.0
(a-v)O_2	134.1±3.9	152.6±3.0*	132.3±3.4†	166.4±8.0	182.3±6.7*	161.7±3.2†
Leg $\dot{V}_{O_2}$	656.5±43.5	715.1±23.1	691.6±44.8	1,511.0±130.1	1,793.0±148.7*	1,428.0±111.6†
Arterial Lac	1.0±0.1	1.2±0.1	0.9±0.1	10.8±1.4	11.6±0.5	10.4±0.7
Venous Lac	1.0±0.1	1.2±0.1	0.9±0.1	12.8±1.9	12.5±0.7	11.4±0.7
Arterial pH,AU	7.412±0.0	7.399±0.0	7.3941±0.0	7.284±0.0	7.270±0.0	7.284±0.0
Venous pH,AU	7.325±0.0	7.304±0.0	7.306±0.0	7.072±0.0	7.083±0.0	7.092±0.0
Arterial NE, mol/l	0.244±0.040	0.262±0.0	0.384±0.1	1.042±0.343	2.136±0.6*	2.557±0.6*

Values are means ± SD. rHuEpo, recombinant human erythropoietin; Pulm $\dot{V}_E$, pulmonary minute ventilation (l/min); Pulm $\dot{V}_{O_2}$, pulmonary O_2 consumption (l/min); Pulm $\dot{V}_{CO_2}$, pulmonary CO_2 production (l/min); [Hb], hemoglobin concentration (g/dl); Hct, hematocrit (%); Sa_{O_2}, arterial O_2 saturation (%); Ca_{O_2}, arterial O_2 content (ml/l); HR, heart rate (beats/min); SV, stroke volume (ml); $\dot{Q}$, cardiac output (l/min); SBP, systolic blood pressure (mmHg); DBP, diastolic blood pressure (mmHg); MBP, mean blood pressure (mmHg); RPP, rate-pressure product (mmHg·beats^{-1}·min^{-1}); SVC, systemic vascular conductance (ml·mmHg^{-1}·min^{-1}); FV VC, femoral vein vascular conductance (ml·mmHg^{-1}·min^{-1}); Leg flow, femoral vein blood flow (ml/min); Leg O_2 del, femoral arterial O_2 delivery (ml/min); %Extrac, O_2 extraction fraction (%); (a-v)O_2, arteriovenous O_2 difference (ml); leg $\dot{V}_{O_2}$, leg O_2 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.

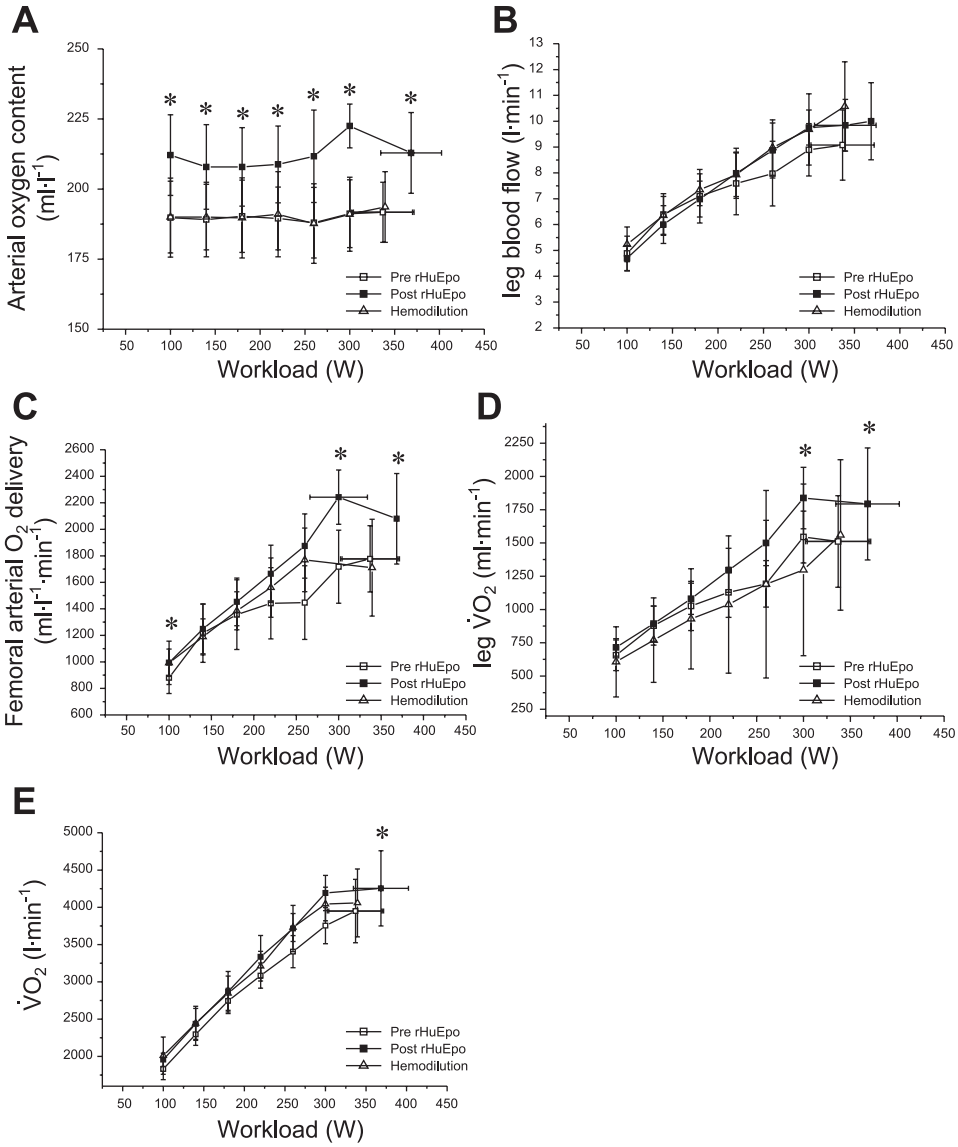

Fig. 1. Arterial oxygen content (ml/l; *A*). leg blood flow (l/min; *B*), femoral arterial oxygen delivery (ml/min; *C*), leg oxygen consumption ($\dot{V}O_2$) (ml/min; *D*), and pulmonary $\dot{V}O_2$ (ml/min; *E*) during maximal exercise tests performed before recombinant human erythropoietin (rHuEpo) treatment (□), after rHuEpo treatment (■), and after hemodilution (△). *$P < 0.05$ compared with pre-rHuEpo; §$P < 0.05$ between post-rHuEpo and posthemodilution.

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 (Fig. 1*B*). Oxygen delivery to the exercising limb was unaltered at submaximal exercise with rHuEpo treatment and hemodilution. At maximal exercise, however, oxygen delivery was increased ($P < 0.05$) by 17% after rHuEpo administration and reduced to the level observed before rHuEpo treatment after hemodilution (Fig. 1*C*). The response of the fractional leg O_2 extraction during exercise was not altered as a result of rHuEpo treatment or by hemodilution. The leg arteriovenous oxygen difference was increased ($P < 0.05$) at submaximal (14%) and maximal exercise (10%) with rHuEpo but normalized with hemodilution. This resulted in an unchanged leg $\dot{V}O_2$ during submaximal exercise but a 19% increase ($P < 0.05$) during maximal exercise after the rHuEpo treatment period, which was normalized after hemodilution (Fig. 1*D*). Systemic and femoral vein vascular conductances were decreased ($P < 0.05$) 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 ± 3.1 and 36.3 ± 4.1 ml·min^{-1}·mmHg^{-1}, respectively; $P = 0.17$) and was not affected by hemodilution (28.6 ± 2.0 ml·min^{-1}·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 ± 1.0 and 50.1 ± 1.1 Torr, pre- and post-rHuEpo, respectively; $P < 0.05$) and remained unchanged with hemodilution (49.9 ± 1.2 Torr; $P < 0.05$ compared with pre-rHuEpo treatment). The parameter *Y* was significantly reduced after rHuEpo treatment (from 1.75 ± 0.04 to 1.66 ± 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 O_2 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 $\dot{V}O_{2\,max}$ through an increase in red blood cell mass and thereby a higher Ca_{O_2} and peak O_2 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 $\dot{V}O_{2\,max}$ is primarily limited by the capacity of the cardiovascular system to deliver O_2 to the exercising muscles.

Effects of rHuEpo treatment and hemodilution on $\dot{V}O_{2\,max}$. In the present study, rHuEpo administration increased [Hb] by 11.6%. As a result, O_2 delivery and $\dot{V}O_{2\,max}$ were both increased by ~300 ml. This demonstrates that all the gained O_2 delivering capacity with rHuEpo treatment was made available and used by the active muscles during maximal exercise. Therefore, the ergogenic effect of rHuEpo on $\dot{V}O_{2\,max}$ can entirely be explained through the enhancement of the O_2-carrying capacity of blood. In addition, this supports the notion that $\dot{V}O_{2\,max}$ is primarily limited by the capacity of the cardiovascular system to deliver O_2 to the exercising muscles (6, 10). Interestingly, after rHuEpo treatment, pulmonary $\dot{V}O_2$ reached a value very close to $\dot{V}O_{2\,max}$ already at ~80% of maximal work rate. In support of this finding, femoral arterial O_2 delivery and leg $\dot{V}O_2$ reached peak values at this workload. After isovolumic hemodilution, $\dot{V}O_{2\,max}$ 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/O_2 and the concomitant reduction in $\dot{V}O_{2\,max}$ again strongly shows that increased erythropoiesis is the main mechanism by which rHuEpo enhances $\dot{V}O_{2\,max}$.

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, $\dot{V}O_{2\,max}$ 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 Ca_{O_2}-dependent vasoconstriction. Breathing 100% O_2 has been demonstrated to induce vasoconstriction in the femoral artery during exercise (39). It remains to be elucidated whether the constrictor effects of O_2 are Po_2 or Ca_{O_2} 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 $\dot{V}O_{2\,max}$ 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 $\dot{V}O_{2\,max}$ (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 $\dot{V}O_{2\,max}$ 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 ($\dot{V}O_2$ = 4.9 l/min and cardiac output = 25.8 l/min) vs. longer trials ($\dot{V}O_2$ = 4.9 l/min and cardiac output = 28.7 l/min) (13).

Impact of Epo on blood-tissue O_2 transfer. This study provides further experimental evidence showing that, in healthy humans, $\dot{V}O_{2\,max}$ 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, $\dot{V}O_2 = \dot{D}O_2 \times \Delta PO_2$, where the coefficient $\dot{D}O_2$ is a lumped estimation of the muscle diffusing capacity and ΔPO_2 is the gradient between the mean capillary PO_2 and the PO_2 in cytochrome *c*, which is assumed to be close to 0 at $\dot{V}O_{2\,max}$ (32). rHuEpo injections resulted in a higher $\dot{V}O_{2\,max}$, without structural changes that could explain an increase in muscle diffusing capacity and hence $\dot{D}O_2$ (6). Therefore, this finding implies that, before rHuEpo treatment, not all the available muscle O_2 diffusing capacity was recruited; i.e., $\dot{V}O_{2\,max}$ was not limited by muscle diffusing capacity before the treatment with rHuEpo. In agreement with our calculations and our interpretation, fractional O_2 extraction was similar before and after rHuEpo treatment, implying that the muscle was able to extract more O_2 when it was made available by the cardiovascular system. Although the PO_2 gradients driving $\dot{D}O_2$ from the capillaries to the mitochondria was slightly higher after rHuEpo treatment, this increase could only account for 35% of the increase in peak leg $\dot{V}O_2$ observed after Epo treatment. A question to be answered is why during maximal exercise not all O_2 is utilized from the arterial blood by the apparently capable skeletal muscle. The answer could include a critical value for PO_2 or some degree of heterogeneity in the distribution of blood flow to match O_2 demand with O_2 delivery at the microcirculatory level (8).

Pulmonary effects. It has recently been demonstrated that transgenic mice overexpressing Epo have an altered regulation of ventilation when exposed to hypoxia (34). However, although the treatment with rHuEpo in our subjects resulted in increased maximal ventilation, this seems to be the result of a higher $\dot{V}O_{2\,max}$. Increasing exercise capacity and hence power output resulted in a higher $\dot{V}CO_2$, which in turn likely contributed to enhance the ventilatory response to exercise after the rHuEpo treatment. This is also supported by the $\dot{V}E/\dot{V}O_2$ and $\dot{V}E/\dot{V}CO_2$ data.

Of note is that the degree of arterial desaturation from rest to maximal exercise was similar in the three experimental conditions (~2% units). In case of an increase in acid buffering power, arterial oxygen saturation (Sa_{O_2}) may be increased due to the left-shift in the O_2-Hb dissociation curve (25). However, this effect was not observed, which is in agreement with the lack of a change of the buffering power of blood in vivo after rHuEpo treatment.

Because the maximal flow of O_2 through the lungs was 300 ml/min higher after Epo treatment and because Sa_{O_2} and alveolar-arterial oxygen tension difference ($AaDO_2$) were not affected by rHuEpo treatment, it can be concluded that lung O_2 diffusing capacity was if anything increased by Epo. Epo treatment increased [Hb]; hence, the carrying capacity for O_2 was increased, which could maintain the PO_2 gradient and facilitate $\dot{D}O_2$, while maintaining maximal cardiac output and likely pulmonary transit time unaltered. An elevation of the O_2 solubility in blood should facilitate $\dot{D}O_2$ in the lungs. 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 $\dot{V}O_{2\,max}$ 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. $\dot{V}O_{2\,max}$ is not limited by muscle diffusion capacity in healthy humans.

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