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Effects of erythropoietin administration on cerebral metabolism and exercise capacity in men

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Rasmussen P, Foged EM, Krogh-Madsen R, Nielsen J, Nielsen TR, Olsen NV, Petersen NC, Sørensen TA, Secher NH, Lundby C. Effects of erythropoietin administration on cerebral metabolism and exercise capacity in men. *J Appl Physiol* 109: 476–483, 2010. First published June 3, 2010; doi:10.1152/jappphysiol.00234.2010.—Recombinant human erythropoietin (EPO) increases exercise capacity by stimulating erythropoiesis and subsequently enhancing oxygen delivery to the working muscles. In a large dose, EPO crosses the BBB and may reduce central fatigue and improve cognition. In turn, this would augment exercise capacity independent of erythropoiesis. To test this hypothesis, 15 healthy young men (18–34 years old, 74 ± 7 kg) received either 3 days of high-dose (30,000 IU/day; $n = 7$) double-blinded placebo controlled or 3 mo of low-dose (5,000 IU/wk; $n = 8$) counter-balanced open but controlled administration of EPO. We recorded exercise capacity, transcranial ultrasonography-derived middle cerebral artery blood velocity, and arterial-internal jugular venous concentration differences of glucose and lactate. In addition, cognitive function, ratings of perceived exertion, ventilation, and voluntary activation by transcranial magnetic stimulation-induced twitch force were evaluated. Although EPO in a high dose increased cerebrospinal fluid EPO concentration ~ 20 -fold and affected ventilation and cerebral glucose and lactate metabolism ($P < 0.05$), 3 days of high-dose EPO administration had no effect on cognition, voluntary activation, or exercise capacity, but ratings of perceived exertion increased ($P < 0.05$). We confirmed that 3 mo of administration of EPO increases exercise capacity, but the improvement could not be accounted for by other mechanisms than enhanced oxygen delivery. In conclusion, EPO does not attenuate central fatigue or change cognitive performance strategy, suggesting that EPO enhances exercise capacity exclusively by increased oxygen delivery to the working muscles.

central fatigue; cerebrospinal fluid; cognition; lactate; respiration

RECOMBINANT HUMAN ERYTHROPOIETIN (EPO) augments hemoglobin concentration ([Hb]) by decreasing plasma volume concomitantly with a stimulating effect on erythropoiesis (25) leading to an increase in maximal oxygen uptake ($\dot{V}O_{2\max}$) and exercise capacity (24). Besides the well known effects of EPO on blood parameters, EPO may also exert an effect on brain function. Miskowiak et al. (28, 30, 31) observed that EPO increases activation in brain areas associated with working memory, long-term memory, executive functions, and fear processing. Similarly, Ehrenreich et al. (11) found improvement in memory and executive performance in schizophrenic patients treated with EPO. Furthermore, EPO-treated rats

change the search strategy in a water-maze environment (32), whereas healthy subjects treated with EPO are reported to feel improved mood (29) and physical conditioning (33) as well as increased ventilation (43). The feeling of improved physical condition could influence exercise capacity by modulating central fatigue or by enhancing the competition strategy (8, 34). Previously, we tested that hypothesis by hemodiluting healthy subjects following 13 wk of EPO treatment and assessed $\dot{V}O_{2\max}$ (24). Following the hemodilution procedure, $\dot{V}O_{2\max}$ was normalized to pre-EPO treatment values, indicating that the main mechanism by which EPO increases $\dot{V}O_{2\max}$ is by increasing systemic oxygen transport (24). One concern remains, however. For EPO to have any effects on brain function, it may be that the applied dose (5,000 IU/wk) (24, 25) was too small to exert any neurological effects since EPO crosses the blood-brain barrier (BBB) in a dose-dependent manner (47).

The main aim of this study was to provide EPO in sufficient dosages for it to pass the BBB (without increasing [Hb]) and, subsequently, to confirm its presence in the cerebrospinal fluid (CSF). We hypothesized that EPO in the CSF, and hence in the brain, would improve exercise capacity by improving cerebral blood flow (CBF) and metabolism, cognitive function, ratings of perceived exertion (RPE), and voluntary activation. Since EPO protects against hypoxia-induced cell damage (9, 12), it may enhance the hypoxia-induced increase in ventilation (43) and, in turn, arterial hemoglobin saturation. We further hypothesized that a non-erythropoietic performance-enhancing effect of EPO would be more evident in hypoxia compared with normoxia. For comparison, we performed the same measurements following a 3-mo low-dose EPO administration, demonstrated to increase exercise capacity (24, 25).

METHODS

Fifteen young males aged of 18–34 years participated in this study (74 ± 7 kg, $\dot{V}O_{2\max}$ 4.2 ± 0.7 l min^{-1} ; mean \pm SD). The subjects provided informed consent to the study as approved by the Ethical Committee of Copenhagen and Frederiksberg (KF 01257177).

EPO Treatment

Three-days group. On two occasions separated by 3 mo, seven subjects received 30,000 IU/day of EPO (NeoRecormon, Roche, Mannheim, Germany) or placebo (saline) during 3 consecutive days in a randomized, double-blinded counter-balanced crossover study design. This dose was considered because it has a neuroprotective effect (10), and injection of either 40,000 or 1,500 IU/kg EPO in humans increases the CNS EPO concentration (47). The EPO was dissolved

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into 0.3 ml of saline per 5,000 IU of EPO and injected for the last time 20–24 h before the subjects reported to the laboratory for the exercise test.

Three-months group. Eight subjects reported twice to the laboratory. One visit served as control, whereas before the other visit the subject received EPO for 13 wk (25) in an open-study design. The order of the trials were counter-balanced by a crossover design, randomized, and separated by 3 mo. In brief, 5,000 IU/day of EPO was administered subcutaneously every second day for the first 2 wk; thereafter, three injections on 3 consecutive days were followed by one injection every week for the remaining time. The total EPO dose was ~100,000 IU, and the last dose was delivered 5 days before the experimental trial.

Experimental Design

On a separate day before the experiments, the subjects were familiarized with the protocol and performed 1) incremental cycling to determine $\dot{V}O_{2\max}$, 2) 20 min of cycling at a normal inspiratory oxygen fraction ($F_{I_{O_2}}$; 0.21), and 3) at a reduced $F_{I_{O_2}}$ (0.10, equivalent to 5,500-m altitude) with the subjects instructed to complete as much work as possible. The workload for the submaximal part of the main experiment was then calculated individually as 95% of the workload that the subject could sustain for 20 min at a $F_{I_{O_2}}$ of 0.10 (124 ± 21 W).

On the day of the study, a catheter was placed in the brachial artery (1.1 mm, 20 gauge) of the nondominant arm. Furthermore, a catheter was placed retrograde under local anesthesia (2% lidocaine) with Seldinger technique in the right internal jugular vein (1.6 mm, 14 gauge; ES-04706, Arrow International) with the tip at the bulb of the vein. Placement of the catheter was guided by ultrasound imaging.

Following instrumentation, the subjects exercised at the predetermined submaximal workload for 20 min at a normal $F_{I_{O_2}}$. This is subsequently referred to as the “low-intensity workload.” Following 10 min of recovery, the subjects exercised at the same absolute workload for 20 min but at a $F_{I_{O_2}}$ of 0.10. To avoid the development of a vasovagal syncope, hypoxia was introduced immediately at the onset of exercise. The subjects then rested for ~90 min to perform graded exercise until exhaustion. The workload was increased 10% every fifth minute and the third increment was set at the intensity that the subjects could sustain for 20 min at a normal $F_{I_{O_2}}$. The second increment (5–10 min of exercise) is hereafter referred to as “medium intensity,” and we denote the final increment as high-intensity exercise. We did not expect the protocol to elicit a $\dot{V}O_{2\max}$. Time to exhaustion was recorded, and exercise capacity was calculated as the total work (in kJ) performed over the trial, i.e., time \times load.

Blood and CSF Sampling

Blood was drawn immediately before all exercise bouts, after 10 min, and at the end of the exercise. Blood was sampled in preheparinized syringes, kept anaerobic, and analyzed within 30 min for O_2 , glucose, and lactate content using an ABL 725 (Radiometer, Copenhagen, Denmark). Approximately 10 min after termination of exercise, CSF was obtained in three subjects from the 3-day group and in four subjects in the 3-mo group (7).

Ventilation

The $\dot{V}O_2$, respiratory frequency, and tidal volume were assessed breath by breath (Medgraphics, St. Paul, MN).

EPO Assay

Blood for EPO analysis was immediately mixed with EDTA and spun (3,500 revolutions/min; 15 min), and the supernatant was stored at -80°C until analyzed. EPO was measured on unconcentrated 100- μl samples of serum and CSF by means of the Quantikine IVD ELISA kit (R&D Systems), using the recommended procedure and

supplied standards. Reading was done at 450 nm with 595 nm as reference on ELISA (Bio-Rad, Richmond, CA). The detection limit of the system was 0.1 mU/ml.

CBF and Metabolism

The cerebral oxygen/glucose (OGI) and oxygen/carbohydrate (OCI) uptake indexes were calculated (18), and the middle cerebral artery (MCA) mean velocity (V_{mean}) was evaluated by transcranial Doppler (Transcan, EME, Überlingen, Germany). Depending on the position with the best signal-to-noise ratio, the proximal part of the MCA was insonated at a depth of 40–60 mm from the temporal bone, and the probe was secured with a headband. Data were interfaced with a Dialogue-2000 (IBC-Danica, Copenhagen, Denmark), sampled at 200 Hz (DI-720, Dataq), and stored.

Changes in CBF, oxygen delivery, and the cerebral metabolic rate for oxygen (CMRO_2) were derived from the MCA V_{mean} and the cerebral arterial-venous difference (a-v diff) for O_2 , assuming an unchanged internal diameter of the MCA (4, 14) with resting mean CBF set at 46 ml·100 $\text{g}^{-1}\cdot\text{min}^{-1}$ (26). The average capillary oxygen saturation ($S_{\text{cap}O_2}$) was then calculated (15, 35, 36):

$$S_{\text{cap}O_2} = S_aO_2 \left(1 - \frac{E_{O_2}}{2} \right) \quad (1)$$

The O_2 extraction fraction (E_{O_2}) is expressed as

$$E_{O_2} = \frac{c_aO_2 - c_vO_2}{c_aO_2} \quad (2)$$

and combining Eqs. 1 and 2 yields:

$$S_{\text{cap}O_2} = S_aO_2 \left(1 - \frac{c_aO_2 - c_vO_2}{2 \cdot c_aO_2} \right) \quad (3)$$

assuming that the O_2 extraction rises linearly with distance as blood traverses the capillary network (17, 20). The dissolved oxygen accounts for <2% of the arterial oxygen (0.12 mM at a P_{O_2} of 100 Torr) and was disregarded. Equation 3 can then be simplified to

$$S_{\text{cap}O_2} = \frac{S_aO_2 + S_vO_2}{2} \quad (4)$$

Solution of the Hill equation of O_2 saturation yielded the mean $P_{\text{cap}O_2}$

$$P_{\text{cap}O_2} = P_{50_a}^{\text{Hb}} \sqrt[4]{\frac{S_{\text{cap}}}{1 - S_{\text{cap}}}} \quad (5)$$

where $P_{50_a}^{\text{Hb}}$ is the P_{O_2} when hemoglobin is half-saturated and h_a is Hill's coefficient for arterial blood; P_{50} was estimated by the Radiometer ABL 725 machine, and h_a and h_v were subsequently calculated using similar formalism as for h_a

$$h_a = \frac{\log \left(\frac{S_aO_2}{100} \right)}{\log \left(\frac{P_aO_2}{P_{50_a}^{\text{Hb}}} \right)} \quad (6)$$

Because of the absence of capillary recruitment within the brain, O_2 diffusibility (L) was assumed to remain stable at 4.4 $\mu\text{mol}\cdot 100 \text{g}^{-1}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$ (35). Changes in mitochondrial oxygen tension ($P_{\text{mito}O_2}$) relative to rest were then calculated

$$P_{\text{mito}O_2} = P_{\text{cap}O_2} - \frac{\text{CMRO}_2}{L} \quad (7)$$

Values for cerebral hemoglobin oxygenation obtained by NIRS tracks changes in P_{mitoO_2} across the physiological range of arterial PO_2 and PCO_2 (35, 36), providing support for the approach used.

Electrophysiological Recordings

To determine isometric elbow flexion force, the right elbow was flexed 90° and fully supinated with the wrist secured to a strain gauge dynamometer (14-bit analog-to-digital conversion) when the subject was sitting on the cycling ergometer. In short, electromyographic (EMG) activity was recorded with surface electrodes (Cleartrace, 1700-030, Conmed, NY) over the muscle belly and tendons of biceps, triceps, and brachialis muscles. We performed motor point (MP) stimulation of the intramuscular nerve fibers in the biceps muscle via a computer triggering a double 1-ms electrical stimulus at constant current (interstimuli interval of 10 ms; S7A Digitimer) and transcranial magnetic stimulation of the motor cortex with a circular coil (Magstim Rapid Rate, Magstim, Dyfed, UK) positioned over the vertex to evoke MEPs in biceps brachii. Voluntary activation was calculated by twitch interpolation with the direction of the current in the coil preferentially activating the left motor cortex. The stimulation intensity was maintained throughout the protocol (70–90% of maximum) and developed a large MEP in the biceps and only a small MEP in the triceps muscle. To estimate resting biceps brachii transcranial magnetic stimulation (TMS) twitch force, the setup included 1) three maximal voluntary contractions (MVC) of which the largest was taken to represent muscle strength, 2) determination of MP and TMS stimulation intensity, and 3) twitch amplitude at 25, 60, 80, and 100% of MVC to extrapolate to the “resting” value. Voluntary activation was quantified by the force responses. Using motor nerve stimulation (twitch interpolation), any increment in elbow flexion force evoked during a MVC (superimposed twitch) was expressed as a fraction of the amplitude of the maximal response evoked by the same stimulus in the relaxed muscle:

$$\text{Voluntary activation (\%)} = 100 - \left(\frac{\text{superimposed twitch}}{\text{resting twitch}} \right) \times 100$$

RPE

For every 5 min of exercise, we obtained RPE on the Borg 6–20 scale (3). The RPE provides a subjective estimate of the sensation of the exercise that depends on both sensory information and cortical output. The subjects were asked, “How hard do you find the exercise right now?” and indicated so by pointing to a scale held by an investigator.

Cognitive Evaluation

Subjects were tested on a wide range of cognitive functions with a battery of neuropsychological tests: The Rey-Osterrieth complex figure, which determines visual perceptual organization and visual memory (23). Subjects were first asked to copy the figure in three different colors, shifting approximately one-third and two-thirds into the figure. Recall trials followed after 3 and 30 min, respectively. The scoring system allocated 2 points for correctly drawn and placed lines; 1 point for correctly drawn, but misplaced lines and incomplete, but correctly placed lines; 0.5 point for incomplete and misplaced, but recognizable lines; and 0 points for missing or nonrecognizable lines. *Trail makings A and B*, which measure visuomotor tracking, divided attention and cognitive flexibility (23). The time taken to complete the two individual tests was recorded. The d2 test measures a range of cognitive functions including selective attention, mental concentration, visual perception, visual scanning abilities, and perceptual speed (5). Total scores, error scores, test scores, and their ranges were noted for each subject. The Stroop test is a measure of attention and response inhibition (23). The time taken to name all colors in the congruent and incongruent versions was noted, as well as errors in color naming. The test was a modified version of the original using

only three colors; red, blue, and green. The symbol digits modalities test is an evaluation of sustained attention that expresses processing speed (21). The number of correct answers in 90 s and time spent on the task were recorded. The block design test in a modified version measured visuospatial constructional abilities (23). The number of correct designs and the time taken to complete each pattern were noted. Letter fluency tests had subjects generate as many words as possible with the letters F, A, and S. Subjects were given 1 min for each letter to test the speed and ease of verbal production (23). The total number of generated words for each letter was noted. Design fluency, a nonverbal fluency test, measured executive functions (23) within 5-point templates. For this modified version of design fluency task, the total number of novel figures, repeating figures, and errors were recorded. Iowa gambling task was used in which a probabilistic version was programmed to make a computer-based test that resembles real-life decision making and measured risk aversion in a gambling situation (2).

Experimenter bias may influence the results of cognitive evaluations, but the investigators responsible for the cognitive evaluation were kept naive to the EPO treatment of the subjects until completion of the study and data analysis. For analysis, we ranked the scores of the subjects so that a positive effect of the EPO treatment was given the rank “1,” no effect the rank “0,” and a negative effect the rank “–1.” These ranks were then entered into the statistical analysis.

Statistical Analysis

A statistical package (SAS, 9.1, SAS Institute, Cary, NC) was used, and the two groups were analyzed separately. For the RPE and blood- and TMS-derived parameters, a two-way repeated-measures ANOVA with the Tukey post hoc procedure identified statistical significance ($P < 0.05$). For the CSF data, a paired Student’s *t*-test was used. The values are presented as means \pm SD. For the cognitive tests, a signed rank test was used to identify differences with EPO treatment and to detect a possible training effect from the first to the second report to the laboratory. The values are presented as median rank with first and third quartiles.

RESULTS

EPO and the Blood Brain Barrier

Administration of high, but not low, doses of EPO increased the arterial EPO concentration \sim 100-fold ($P < 0.01$; Table 1). Concomitantly, the CSF EPO concentration increased from 0.6 ± 0.2 to 13.9 ± 4.0 mIU/ml ($P < 0.05$) with high-dose, short-term EPO administration, whereas no change was observed with low-dose, prolonged EPO administration.

Pulmonary Ventilation and Arterial Oxygen Saturation

During exercise in hypoxia, pulmonary ventilation increased with high-dose, short-term EPO administration ($P < 0.01$; Fig. 1). In contrast, no change in pulmonary ventilation was observed with the low-dose, prolonged EPO ad-

Table 1. Erythropoietin in plasma and CSF and its effect on Hct

	EPO, \pm	Arterial, mIU/ml	CSF, mIU/ml	Hct, %
3-day treatment	–	11 \pm 3	0.6 \pm 0.2 ^a	45.6 \pm 1.3
	+	1,102 \pm 1,388*	13.9 \pm 4.0**	47.3 \pm 2.3
3-mo treatment	–	16 \pm 14	0.7 \pm 0.3 ^b	42.5 \pm 3.7
	+	13 \pm 12	0.6 \pm 0.2 ^b	47.6 \pm 4.1*

Values are means \pm SD for $n = 7$, except ^a $n = 3$ and ^b $n = 4$. EPO, erythropoietin; CSF, cerebrospinal fluid; Hct, hematocrit. *Significantly different from control value ($P < 0.05$).

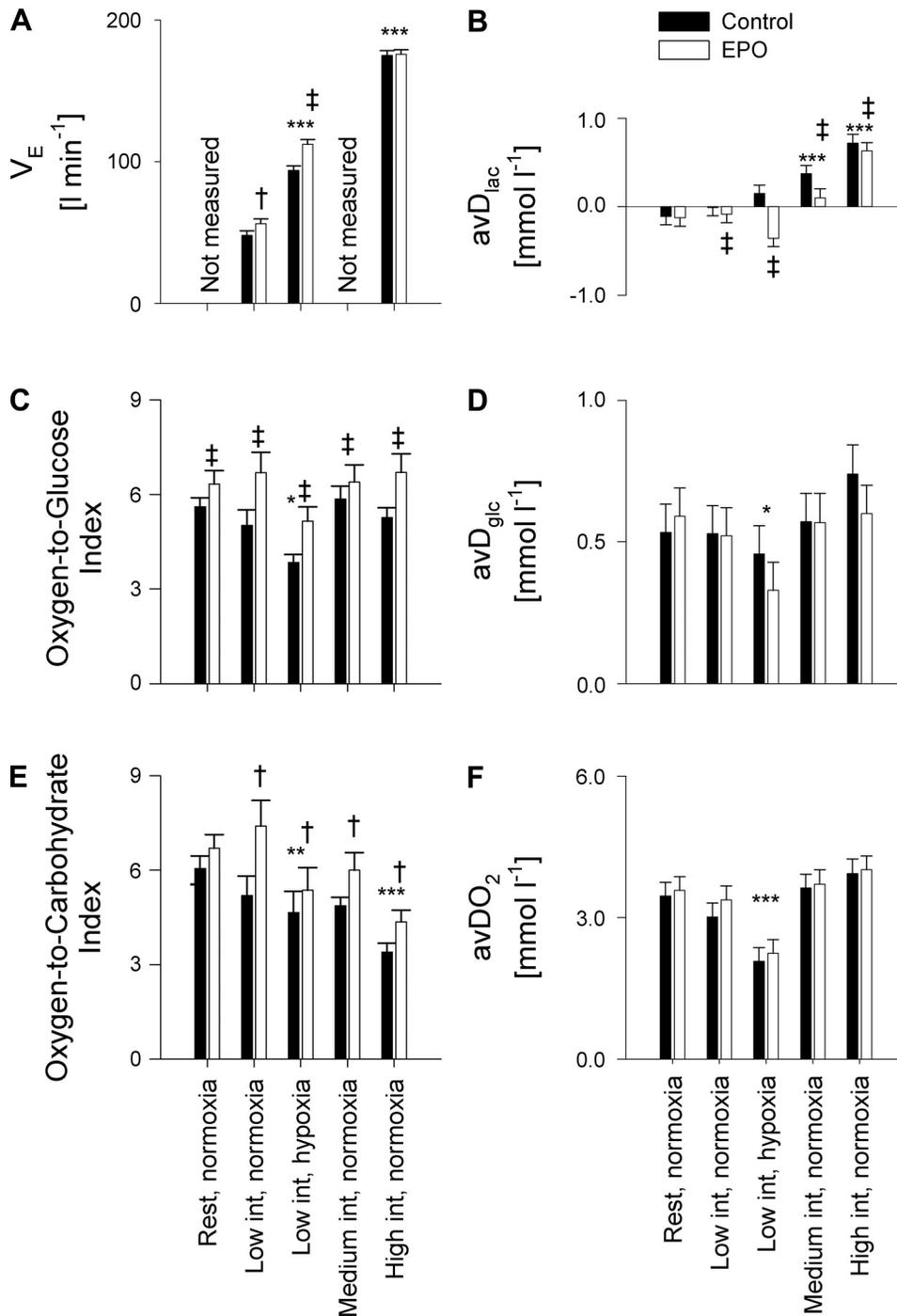


Fig. 1. The effects of 3 days of high-dose erythropoietin (EPO) administration on ventilation and cerebral metabolism at rest and during cycling exercise at varying intensities (int) with or without hypoxia (10% oxygen fraction). A: administration of 30,000 IU of EPO per day for 3 days increase pulmonary ventilation (\dot{V}_E) compared with placebo. B and C: exercise in hypoxia decreases the cerebral oxygen-to-glucose index (OGI; $P < 0.05$), whereas the cerebral oxygen-to-carbohydrate index (OCI) is affected by strenuous exercise (rest vs. exercise in hypoxia, medium- and high-intensity exercise in normoxia; all $P < 0.001$). For clarity the P values are not shown on the figure. Three days of high-dose EPO administration increases OGI and OCI during rest and exercise. The difference in OGI and OCI was due to EPO administration decreasing the cerebral arterio-venous difference (avD) for lactate (lac, d) but not for glucose (glc, e), or for oxygen (O_2 , f). Values are means \pm SE ($n = 7$). Significant difference vs. rest: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Significant difference of EPO vs. control: † $P < 0.01$; ‡ $P < 0.001$.

ministration. This increase was the product of an increase in both tidal volume (from 2.8 ± 0.3 to 3.0 ± 0.3 liters) and respiration frequency (34 ± 5 to 38 ± 6 breaths/min). The increase in pulmonary ventilation was accompanied by an increase in arterial oxygen saturation from 58 ± 5 to $62 \pm 4\%$ ($P < 0.01$). Tidal volume also increased with the high-dose, short-term EPO administration during low-intensity normoxic exercise (from 2.3 ± 0.2 to 2.8 ± 0.5 liters; $P < 0.001$); however, no change was observed in arterial oxygen saturation in this trial. No changes in pulmonary ventilation or arterial oxygen saturation were observed with low-dose, prolonged EPO administration.

Across the trials, \dot{V}_{O_2} was not affected by EPO treatment in either group.

Cerebral Oxygenation

In both EPO groups the combined effect of $\sim 10\%$ increased MCA V_{mean} ($P < 0.05$) and blood hemoglobin concentration (Table 1) lead to an increased oxygen delivery to the brain ($P < 0.01$ for both groups). At rest, however, capillary oxygen saturation and tension ($S_{\text{cap}O_2}$ and $P_{\text{cap}O_2}$) were 79% and 45 Torr in both groups and similar with and without EPO treatment. During exercise, both cerebral arterio-venous differences

(avD) for oxygen (avDO₂) and MCA V_{mean} tended to increase ($P < 0.01$). EPO treatment in both groups widened the avDO₂ and increased MCA V_{mean} , leading to an increase in cerebral metabolic rate for oxygen (CMRO₂) with EPO treatment. This was, on the other hand, compensated by the increase in oxygen delivery with EPO administration, and thus no changes in $\Delta P_{\text{mitoO}_2}$ were observed in either group with EPO treatment.

EPO and Cerebral Carbohydrate Metabolism

In normoxia, avD for glucose (avD_{glc}) remained stable both at rest and during exercise, whereas it decreased during exercise in hypoxia ($P < 0.05$). No significant changes with EPO administration were observed in cerebral avD_{glc} (Fig. 1) despite an increase in the arterial glucose concentration during exercise in hypoxia that was independent of EPO administration. OGI, however, increased with short-term, high-dose EPO administration ($P < 0.01$) and that effect was independent of the arterial level of glucose or the avDO₂. No effect of low-dose, prolonged EPO administration was observed.

The control arterial lactate concentration at rest was 1.26 ± 0.28 mM and 1.17 ± 0.25 mM during high-dose, short-term, and low-dose, prolonged EPO administration, respectively, and was unchanged regardless of EPO treatment. Low-intensity exercise did not produce a significant systemic lactate response, whereas exercise in hypoxia and maximal exercise increased systemic lactate. We observed no differences with EPO administration. In contrast to cerebral glucose metabolism, cerebral avD for lactate (avD_{lac}), decreased ~ 0.3 mM during exercise with high-dose, short-term EPO administration ($P < 0.05$; Fig. 1), whereas no change in cerebral avD_{lac} was observed with low-dose, prolonged EPO administration. As a consequence of the changes in lactate metabolism, OCI was higher during exercise following short-term, high-dose EPO administration ($P < 0.05$). The changes in OCI were independent of changes in the arterial lactate concentration.

Perceived Exertion

RPE was lowest with low-intensity exercise and increased with addition of hypoxia or when exercise was maximal ($P < 0.05$; Fig. 2). RPE decreased with prolonged low-dose EPO

administration ($P < 0.05$) but increased with high-dose, short-term EPO administration ($P < 0.05$).

Voluntary Activation

Voluntary activation of the elbow flexors decreased during exercise in both groups ($P < 0.001$); however, EPO did not have a significant effect. Voluntary activation ranged with high-dose, short-term EPO administration from 96.2 ± 1.6 to $98.1 \pm 3.9\%$ (control) and from 95.2 ± 1.8 to $98.2 \pm 4.2\%$ (treated). For low-dose, prolonged EPO administration, voluntary activation ranged from 93.3 ± 2.4 to $98.1 \pm 7.9\%$ and from 94.8 ± 2.4 to $97.2 \pm 5.9\%$, control and treated, respectively.

Cognitive Function

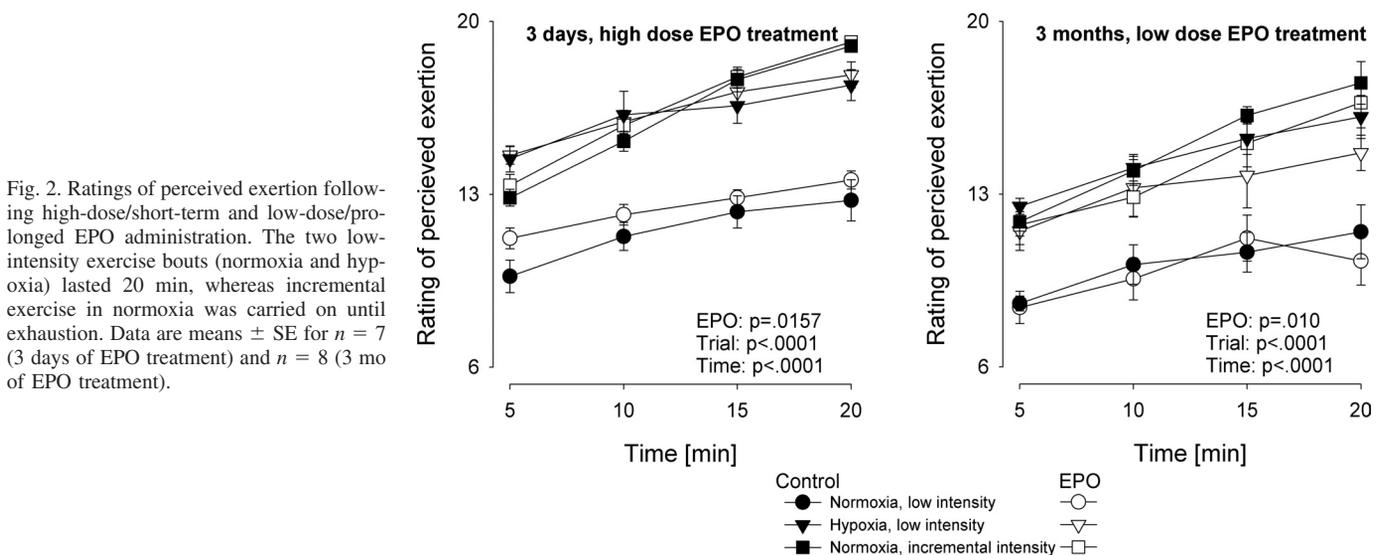
There were 33 evaluations of cognitive function and strategy, and, of those variables, no one was found to change significantly with EPO treatment in either group. We did, however, find a training effect (0.5 rank) from the first to the second report to the laboratory ($P < 0.001$), which is a common phenomenon in cognitive testing. This was corrected for by adding the rank "0.25" to the first visit to the laboratory and the rank "-0.25" to the last. Following this procedure, persistently, EPO treatment did not affect cognition, both per individual test and in the overall score. The median score for the short-term, high-dose group was -0.25 (-0.75 to 0.75) and for the prolonged, low-dose group was 0.75 (-0.75 to 0.75).

Exercise Performance

Exercise performance increased $\sim 15\%$ with 3 mo of prolonged EPO administration from 511 ± 129 to 600 ± 175 kJ ($P < 0.01$; Fig. 3). In contrast, exercise performance was unchanged with high-dose, short-term EPO administration (423 ± 90 vs. 436 ± 55 kJ).

DISCUSSION

The main findings in the present study were that when EPO is administered to healthy humans at a high dose ($3 \times 30,000$



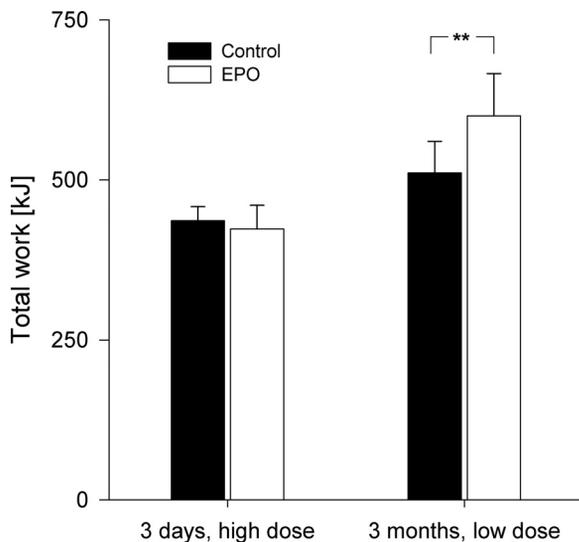


Fig. 3. Exercise capacity following 3 days of high-dose or 3 mo of low-dose EPO administration. Data are means \pm SE for $n = 8$ (low dose) and $n = 7$ (high dose). **Significant difference for control vs. EPO treatment ($P < 0.01$).

IU) it crosses the blood brain barrier (BBB) since the CSF values for EPO became elevated. At lower concentrations (5,000 IU), this was not the case, supporting that only high doses of EPO, potentially, exert an effect on the brain. When present in the CSF, EPO increased net cerebral lactate release and increased both OGI and OCI. In addition, high-dose EPO treatment increased ventilation and arterial saturation. Despite these effects, however, EPO in a high dose did not enhance exercise performance or influenced cognitive function undertaken before exercise. Rather, EPO increased RPE, especially during low-intensity exercise. These findings suggest that EPO does not exert an additional effect on exercise performance besides increasing blood hemoglobin mass in the aforementioned doses and within the given timeframe.

That EPO crosses the BBB was demonstrated in rodents (6) and subsequently confirmed in humans. In a randomized study of patients with strokes because of MCA occlusion, treatment with recombinant human EPO increased CSF EPO and also demonstrated a clinical benefit (10). In patients provided with an Ommaya reservoir that allows for serial CSF sampling and subsequently for determination of the pharmacokinetics of EPO in blood and CSF after single and multiple doses, it was demonstrated that EPO crosses the BBB in a dose-dependent manner (47). Although EPO crosses the BBB when injected in high concentrations, the transport mechanism needs to be established. Mechanisms proposed for the transport across the BBB include: 1) receptor-mediated transport; 2) carrier-mediated transport; 3) fluid phase endocytosis; 4) nonspecific or receptor-mediated absorptive endocytosis; and 5) transmembrane diffusion (16). The experimental data of Ref. 47 are consistent with first-order transmembrane transport EPO or a similar non-receptor-mediated/nonsaturable mechanism.

EPO may influence ventilation at rest (42, 43). We demonstrated that this is also the case during submaximal exercise, especially under hypoxic conditions (Fig. 1). During maximal exercise, however, the stimulating effects of EPO on ventilation was diminished, most likely because exercise-induced changes in especially pH overrule the effects of EPO. During

submaximal exercise in acute hypoxia, the EPO-induced increase in ventilation could increase the arterial oxygen saturation and hence also arterial oxygen content. Soliz et al. (42) demonstrated an EPO receptor both on the main brain stem respiratory centers and within the carotid bodies and proposed that the regulatory effects of EPO on ventilation are mediated through activation of the main brain stem respiratory centers (42). These authors have also demonstrated that the effect of EPO on ventilation is large in females and thus may be related to an effect of sexual hormones (43). As seen from our data, the respiratory response to the high EPO dose, which crossed the BBB, was higher compared with the smaller dose. This could be interpreted as either 1) that the effect of EPO on carotid body stimulation is dose-dependent or 2) that the effect of EPO on ventilation is mediated through brain stem stimulation. Also, differences in the EPO receptor density could play a role. Alternatively, the changes in MCA V_{mean} and thus CBF may directly affect ventilation via regulating washout of H^+ from brain tissue and thus tissue pH (1). However, ventilation would be expected to increase in response to a decrease in tissue pH, whereas conversely the observed increase in MCA V_{mean} with a high-dose EPO administration would be expected to remove H^+ , in turn lowering pH and thereby decreasing ventilation. Thus it is not clear from the present data how EPO interacts with regulation of ventilation. Despite the effects of EPO on ventilation, in healthy subjects, an increase in ventilation during exercise does not concomitantly increase $\dot{V}O_2$ or exercise capacity.

Central Factors

RPE increased during exercise following high-dose EPO administration. Also, there were no changes in cognitive performance with high-dose EPO administration, although EPO does affect brain areas related to memory, executive functions, and emotional processing (28, 30, 31). As such, central effects of EPO have previously been reported in both humans (see references by Miskowiak et al.) and rodents (32); however, to our knowledge, no study has previously examined the impact of EPO on central function in relation to exercise capacity in young healthy men. EPO may have an effect in patients with preexisting cerebral dysfunction or trauma. In contrast, however, in patients without cognitive dysfunction, EPO does not appear to have any measurable effect on cognition (13). We, however, acknowledge that the change in activation may be too small to have any effect on behavior; another possibility is that EPO does change behavior but that the applied cognitive tests were not sensitive enough to detect such an effect and maybe

Table 2. Exercise capacity and cerebral oxygenation ($S_{\text{cap}O_2}$) in the volunteers receiving 3 days of high-dose EPO in whom CSF EPO was measured

Subject	EPO, \pm	CSF EPO, mIU/ml	Exercise Capacity, kJ	$S_{\text{cap}O_2}$, %
AFB	—	0.72	362	75.0
	+	13.52	253	73.8
KG	—	0.36	688	75.9
	+	10.03	390	76.1
JML	—	0.82	408	71.7
	+	18.06	465	74.2

$S_{\text{cap}O_2}$, cerebral oxygenation.

that a significant change cannot be expected in healthy young people. However, if more sensitive tests are necessary to elucidate the effect of EPO on cognitive performance in relation to competition strategy, we consider it unlikely that the effect will have a large impact on exercise and competition performance.

Voluntary activation measures central fatigue that develops during maximal exercise (37). Here, the exertion was reported to be maximal, and we confirmed central fatigue by the inability to fully activate the elbow flexors. Administration of EPO in high doses, however, did not influence that expression of central fatigue. EPO may only influence the areas directly involved in the exercise, but we were unable to detect any increase in exercise capacity with high-dose administration. Furthermore, prolonged EPO administration increased exercise performance without changing voluntary activation, which supports that EPO does not affect central motor drive but only oxygen-carrying capacity.

Cerebral metabolism was seemingly affected by EPO administration. Without EPO administration, OCI decreases to ~3 or even lower (8, 46), as confirmed here. The reduction in OCI is primarily driven by a large uptake of lactate that is in proportion to the arterial lactate concentration (45). With EPO administration, the reduction in OCI and the lactate uptake by the brain were attenuated, suggesting an impact of EPO on cerebral metabolism. The reduction in OCI may implicate development of central fatigue; however, we noted no change in performance even with a higher OCI. We acknowledge that reductions in OGI and OCI may be driven by adrenaline (22, 40). However, it remains to be established whether EPO affects the sympathetic nervous system. Even with an apparent effect on cerebral metabolism, EPO in high dose did not improve performance through central factors. This lack of a central effect of EPO is supported by the observation that prolonged administration of EPO improved performance and reduced RPE but without having an effect on cerebral metabolism.

Low cerebral oxygenation has been implicated in central fatigue (27, 38, 39, 44). Arterial oxygenation increased with both high-dose and prolonged EPO administration. This was, however, not manifested in increased capillary and mitochondrial oxygen tension; rather, there was a tendency for a decrease in $P_{\text{mito}}\text{O}_2$ with high-dose EPO administration. It does not appear that EPO causes significant changes in cerebral oxygenation, and thus cerebral oxygenation does not offer an explanation for the effect of EPO on work capacity.

Limitations

We cannot exclude that a lack of effect on exercise performance in the short-term, high-dose group may relate to training state. On the other hand, $\dot{V}\text{O}_{2\text{max}}$ in the volunteers ranged from 3.2 (43) to 5.7 (67) l/min ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), and we saw no relation between initial $\dot{V}\text{O}_{2\text{max}}$ and performance-enhancing effect of EPO.

We found that high-dose EPO administration increased CSF EPO content. This was, however, only measured in three subjects due to the problems recruiting volunteers for the lumbar punctures. On the other hand, all three subjects in the high-dose group increased the EPO CSF concentration (Table 2), and we have no reason to assume that the remaining subjects did not show a similar response (47). Furthermore, the effect size

of the treatment is very large (~20-fold increase) and the power of the paired *t*-test was 0.83, conforming to the accepted limit of 80%.

We used TCD to estimate flow for evaluation of cerebral oxygenation. An important assumption in the interpretation of the Doppler velocity data is an unchanged internal diameter of the insonated vessel. CBF is regulated distally to the basal cerebral vessels (14, 41), and, hence, the MCA diameter would not be expected to change (19). Some caution must, however, be exerted, since EPO may act both as vasodilator and vasoconstrictor, and the potential effect on cerebral circulation is unknown.

In conclusion, acute high-dose EPO administration increases the CSF EPO concentration without having any measurable effect on exercise or cognitive performance, suggesting that the ergogenic effect is primarily, if not exclusively, by means of increased oxygen-carrying capacity by blood. This in turn implicates that high-dose EPO administration should not be considered as a viable way of enhancing exercise capacity or reducing perceived exertion in, e.g., renal failure patients. Rather, long-term administration aimed primarily at increasing the oxygen-carrying capacity of the blood above anemic values should be considered.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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