The Performance Effects of Microdose Recombinant Human Erythropoietin Administration and Carbon Monoxide Rebreathing

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Abstract

Frequent, low doses of recombinant human erythropoietin (rHuEpo) have been shown to increase the oxygen carrying capacity of an athlete and enhance endurance performance, although its effect on repeated sprint ability (RSA) remains unknown. If the mechanisms behind improved RSA performance reside within the augmented O_2 carrying capacity, then carbon monoxide (CO) inhalation should inhibit RSA. **Purpose:** The aim of this study was to assess the effects on maximal oxygen uptake ($\dot{V}O_{2max}$) and RSA of two interventions known to differentially influence blood oxygen carrying capacity. **Methods:** Fourteen endurance-trained individuals were administered microdoses of rHuEpo (20–40 IU·kg^{-1}) or placebo twice per week for 7 wk using a randomized, crossover design. $\dot{V}O_{2max}$ and RSA were measured at baseline and after rHuEpo administration. Total hemoglobin mass (tHb-mass) was measured twice at baseline (14 and 7 d before the first injection), three times during rHuEpo administration (10, 24, and 38 d after the first rHuEpo injection) and twice after the cessation of rHuEpo administration (7 and 21 d after the final injection) using the optimized CO rebreathing method. $\dot{V}O_{2max}$ and RSA also were assessed in a separate cohort of 11 trained subjects who underwent CO or placebo rebreathing. **Results:** $\dot{V}O_{2max}$ was increased following rHuEpo in comparison to baseline by an average of 3.9% (54.5 ± 5.1 mL·kg^{-1}·min^{-1} vs 56.6 ± 5.8 mL·kg^{-1}·min^{-1}, $P < 0.01$) and decreased following CO rebreathing compared with placebo by 4.8% (49.6 ± 5.5 mL·kg^{-1}·min^{-1} vs 47.2 ± 5.3 mL·kg^{-1}·min^{-1}, $P < 0.01$). tHb-mass was increased by 10.8% (13.0 ± 1.7 vs 14.4 ± 1.7 g·kg^{-1}, $P < 0.01$) compared with baseline 24 d into rHuEpo administration and remained elevated 21 d after the final injection (13.6 ± 1.2 g·kg^{-1}, $P = 0.01$). The ventilatory threshold did not increase significantly following rHuEpo administration compared with baseline, whereas CO rebreathing resulted in a significant decrease compared with placebo (36.8 ± 5.4 mL·kg^{-1}·min^{-1} vs 38.6 ± 4.7 mL·kg^{-1}·min^{-1}, $P < 0.01$). Repeated sprint ability was not affected by either rHuEpo or CO rebreathing. **Conclusions:** Despite dramatic alterations in the O_2 carrying capacity of the blood and marked changes in $\dot{V}O_{2max}$, neither CO rebreathing nor rHuEpo administration influenced RSA. This suggests that $\dot{V}O_{2max}$ has limited importance in RSA and rHuEpo may not provide any ergogenic effect to improve RSA.

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Introduction

The theoretic concept that there exists an maximal oxygen uptake ($\dot{V}O_{2max}$) in an exercising human was developed by Hill and Lupton more than 90 years ago (1). Over these decades of scientific research, it remains one of the most important factors in exercise physiology and one of the most widely used methods to assess performance. Current literature suggests that this limit is primarily caused by the body's ability to deliver oxygen (O_2) to the working muscles (2); however, this is disputed (3). To overcome this limitation, several methods have been developed and extensively researched to enhance the body's ability to deliver O_2 at a greater rate. For example, altitude training can be used to enhance an individual's total hemoglobin mass (tHb-mass) and increase the O_2 carrying capacity of the blood during exercise (4). This augmented O_2 delivery permits a greater aerobic contribution to the total energy production during an exercise task and improves performance when exercising at sea level (5). However, the improvements in tHb-mass and subsequent improvement sea level endurance performance have not always been consistent (6). Due to the expense and potential for only marginal improvements in performance with altitude training, some athletes have been searching for more effective methods to enhance tHb-mass and endurance performance.

There is overwhelming scientific evidence that rHuEpo increases exercise performance by augmenting tHb-mass and therefore transport O_2 to active muscles (7). The administration of rHuEpo for 4 to 6 wk has been shown to increase $\dot{V}O_{2max}$ by 6%–8% (7–11) as well as submaximal cycling capacity at 80% of $\dot{V}O_{2max}$ by approximately 54% using time to exhaustion (12). Similarly, increases in submaximal running performance by approximately 6% during a 3000-m time trial have also been observed following rHuEpo administration (11). Due to this clear, performance-enhancing effect, the use of rHuEpo by athletes has been banned by the World Anti-Doping Agency (WADA). Conversely, several recent studies have suggested that the improvements seen in laboratory-based assessments (*e.g.,* $\dot{V}O_{2max}$) do not translate to real-world improvements in performance (13,14). Athletes have now evolved doping practices to use low doses of rHuEpo to elicit small hematological responses and reduce the presence of rHuEpo in their urine (15). It has been known for some time that microdoses of rHuEpo are difficult to detect using the current tests available (16) and evidence quantifying the performance-enhancing effect of microdoses of rHuEpo is lacking.

Despite the interest in quantifying the effects of rHuEpo on athletic performance, relatively few studies have investigated the effects of rHuEpo on high intensity repeated sprint ability (RSA). The majority of the energy turnover during a single sprint lasting up to approximately 10 s is gained from the degradation of phosphocreatine (PCr) (17), while the contribution from aerobic sources is small, this contribution increases with each progressive sprint with insufficient recovery periods (*e.g.,* <5 min) (18). The enhanced O_2 delivery achieved through rHuEpo administration may facilitate a greater resynthesis of PCr, as this process is known to be O_2-dependant (19) and subsequently enhance recovery between sprints, resulting in improved RSA performance. While rHuEpo is known to increase the O_2 carrying capacity of blood via an augmented tHb-mass, carbon monoxide (CO) inhalation impairs the ability to transport O_2 via a reduced O_2 carrying capacity of the blood, and therefore reduces $\dot{V}O_{2max}$ (20). While there is some insight into the effects of altering O_2 delivery following rHuEpo in repeated sprint protocol, performance has not been assessed (21). In addition, the physiology of multiple sprints is influenced by alterations in the exercise protocol, in particular, studies including work-to-rest ratios of 1:5, with larger number of sprints (*i.e.,* >6) and sprint durations (*i.e.,* >6 s) are lacking (22).

With this in mind, the aim of this investigation is to assess numerous performance indicators including $\dot{V}O_{2max}$ and RSA in a cohort that regularly received injections of microdoses of rHuEpo and in a separate cohort that has been administered CO.

Methods

General Overview

Participants were recruited for the purpose of this specific analysis from two separate studies. Study 1 is part of a wider research initiative focusing on identifying a gene expression profile of microdose rHuEpo abuse, detailed procedures and results are available elsewhere (23) and study 2 is a separate investigation using a different cohort of participants to eluicidate the effect of reducing O_2 availability on RSA. The purpose of the current manuscript is to compare the effects of acutely altering the O_2 carrying capacity on $\dot{V}O_{2max}$ and RSA between these two studies. Both studies were approved by the University of Glasgow ethics committee in accordance with the Declaration of Helsinki and written informed consent was obtained from all participants.

Study 1

Fourteen endurance-trained males volunteered to participate (age, 30 ± 4 yr; height, 178.8 ± 4.5 cm; weight, 72.1 ± 4.4 kg). Every participant received a subcutaneous injection of rHuEpo (NeoRecormon; Roche, Welwyn Garden City, UK) or placebo (Saline solution, NaCl 0.9%; Baymed Healthcare Limited, Glasgow, UK) twice per week for 7 wk. The injection regime involved 20 IU·kg^{-1} doses at weeks 3 and 9, 30 IU·kg^{-1} doses at weeks 4 and 8 and 40 IU·kg^{-1} at weeks 5, 6, and 7. Every participant received daily iron (105 g of elemental iron) tablets or daily lactose tablets (placebo). The average washout period between the end of the rHuEpo phase and the initial testing of the placebo phase was 62 ± 10 d. tHb-mass was measured twice at baseline (14 and 7 d before the first injection) three times during administration, (10, 24, and 38 d after the first injection) and twice postadministration (52 and 66 d after the first injection). The experimental design is depicted in Figure 1.

Study 2

Eleven healthy, recreationally active males volunteered to participate in a CO rebreathing study (age, 25.9 ± 3 yr; height, 176.7 ± 4.8 cm; weight, 73.9 ± 7.2 kg). Participants were required to make five visits in total. Visits 1 and 2 was either CO rebreathing or placebo (air) rebreathing in random order, shortly (12 min) followed by an incremental cycle test. Visit 3 was a RSA familiarization, visits 4 and 5 were either CO or placebo rebreathing in random order followed by the RSA test (Fig. 2). The RSA test was identical to the one used in study 1

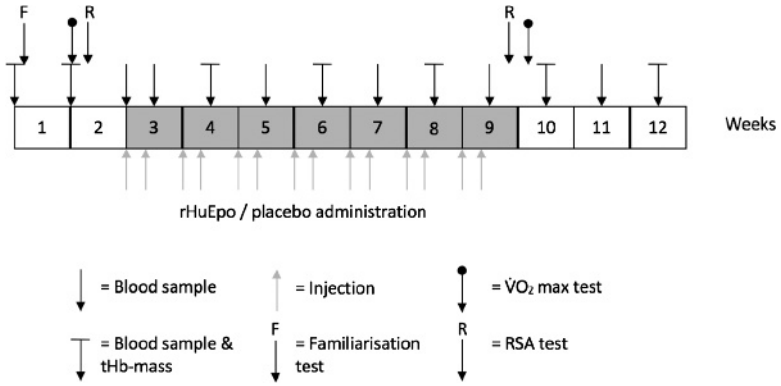

Figure 1: Study 1 experimental design, 14 subjects were administered microdoses of rHuEpo or placebo twice per week for 7 wk. tHb-mass was measured continuously with $\dot{V}O_{2max}$ and RSA measured at the start and end of the administration phase.

but with a reduced cycling resistance (see section Repeated sprint ability below).

Incremental exercise tests

$\dot{V}O_{2max}$ was determined for participants in study 1 using an incremental running test performed at baseline and at the end of rHuEpo administration (Fig. 1). Testing was performed on a motorized treadmill (PPS55 Med; Woodway, Weil Am Rhein, Germany) with a running speed starting at 9 $km \cdot h^{-1}$ with 1% gradient which increased by 2 $km \cdot h^{-1}$ every 3 min until 17 $km \cdot h^{-1}$ was reached. Once 3 min at 17 $km \cdot h^{-1}$ was completed, the elevation was increased by 1% after every minute until volitional exhaustion with the speed remaining constant. Expired gases and heart rate (HR) were both measured using an automated metabolic analyzer system (Quark CPET, Cosmed, Rome, Italy), calibrated according to the manufacturer's instructions.

In study 2, participants performed incremental exercise tests on a cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands). Participants started cycling at 20 W and the resistance was increased at 25 $W \cdot min^{-1}$ until volitional exhaustion, participants were instructed to cycle at 70 to 80 rpm continuously throughout the test. Power, HR, and expired gases were recorded during the test, gases were analyzed using an automated metabolic analyzer system (K4b^2; Cosmed, Rome, Italy) calibrated according to the manufacturer's instructions.

In both studies, $\dot{V}O_{2max}$ was considered achieved when meeting at least two of the following criteria; 1) $\dot{V}O_2$ did not increase greater than 150 mL despite an increase in work load, 2) the participant was within 10% of their age-predicted HR maximum, and 3) the RER >1.15. The breath by breath data were averaged every 20 s with the highest value reported as $\dot{V}O_{2max}$.

Ventilatory threshold (VT) was determined via visual identification technique in both studies. Three independent researchers separately determined VT without prior knowledge of the intervention applied. VT was obtained by meeting the following criteria: 1) increase in $\dot{V}O_2$ with a nonlinear increase in $\dot{V}CO_2$ and ventilation and 2) increase in end-tidal $\dot{V}O_2$ without a decrease in end-tidal $\dot{V}CO_2$ (23). The average $\dot{V}O_2$ between the three investigators was reported, in the case that the variance between the investigators exceeded 4%, an average of the two closest investigators was taken.

Repeated sprint ability

In study 1 after a familiarization period, RSA was assessed at baseline and at the end of the week after the last rHuEpo injection (Fig. 1). Following a standard warm-up on a cycle ergometer, participants performed 10 maximal effort of 10-s sprints, each separated by 50-s rests. Each sprint was performed against a resistance of 0.9 $N \cdot kg^{-1}$. During each sprint, peak power, mean power, minimum power, peak power to body mass, time to peak power, mean power to body mass, and rate to fatigue was recorded using computer software (Version 1; Lode, Groningen, The Netherlands). Venous blood samples were taken before the warm-up, after sprint 5, sprints 10 and 5, 10, and 30 min after the completion of the RSA test. Samples were immediately analyzed using a blood gas analyzer (ABL 725, Radiometer, Copenhagen, Denmark) for lactate (La), potassium concentration (K^+) and pH. Participants of study 2 performed an identical RSA test and procedures

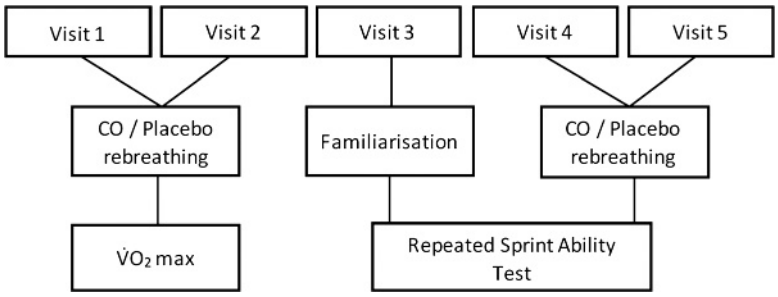

Figure 2: Study 2 experimental design, 11 subjects were administered air or CO in random order followed by a $\dot{V}O_{2max}$ test or RSA test.

with a reduced cycling resistance (0.8 $N \cdot kg^{-1}$) due to the lower training status of the subjects. In both studies, all testing sessions were separated by at least 48 h. Throughout the study period, participants were requested to maintain their usual dietary intake, to refrain from alcohol and to abstain from hard training for at least 24 h before testing.

tHb-mass determination/CO administration

Determination of tHb-mass in study 1 was carried out via the optimized CO rebreathing procedure which is outlined in detail elsewhere (24). Briefly, 1 $mL \cdot kg^{-1}$ of CO is injected into a spirometer and is rebreathed by the participant with 4 L of chemically pure O_2 for 2 min. A 2-mL venous blood sample is taken before and 8 min after rebreathing for determination of the percentage of carboxyhemoglobin (HbCO%). Blood samples were analyzed using a blood gas analyzer (ABL 725; Radiometer, Copenhagen, Denmark). Change in HbCO% from before to after rebreathing, volume of CO administered and the binding capacity of CO to hemoglobin (1.39 $mL \cdot g^{-1}$) were used to calculate tHb-mass using the following calculation:

$$tHb-(g) = K \times MCO \times (\Delta HbCO\% \times 1.39)^{-1}$$

where K is barometric pressure $\times$ 760^{-1} $\times$ (0.003661 $\times$ temperature), MCO is CO administered $-$ (CO in system + lungs + CO exhaled), ΔHbCO% is differences between HbCO% before and after CO administration and 1.39 is mL CO $\times$ $g \cdot Hb^{-1}$.

After the rebreathing protocol, participants were observed for signs of early CO toxicity. When determining HbCO%, the first repeated value among multiple measures was taken, otherwise an average was calculated of all values. The differences observed were approximately 0.01% to 0.03% from the original reading.

During study 2, the optimized CO rebreathing protocol as outlined above was used to administer CO or air 12 min prior to the incremental cycle test or RSA test. During the incremental cycling tests, 95 μL capillary blood samples were collected immediately before, 5 and 7 min after rebreathing whereas in the RSA tests, a 2-mL venous blood sample was taken immediately before and 6 min after rebreathing. The different methods are believed to have a negligible effect on tHb-mass measurement (25). HbCO% also was measured directly after the RSA test but not after the incremental exercise test and so an estimation of the HbCO% during the last 2 min was made (26). High precision of the optimized CO rebreathing protocol our group has been previously shown with a typical error <2% (27), similar to that reported by others (24,28,29).

Statistical analysis

All data in the studies 1 and 2 were considered statistically significant at Bonferroni corrected $P \leq 0.05$, all data are presented as mean ± SD. All correlations in both studies were assessed using Pearson's Product Moment correlation coefficient in SPSS (SPSS Inc, Chicago, IL). Statistical analysis was performed on $\dot{V}O_{2max}$, VT and tHb-mass with changes across time points compared with baseline in study 1 and on $\dot{V}O_{2max}$ and VT between CO rebreathing and placebo in study 2 using R (The R Foundation for Statistical Computing, version 3.4.1) lme4 (30) and phia (31) packages using the mixed model. Repeated sprint performance and blood gas variables were also assessed using R in studies 1 and 2 with three within-subject variables (Group: rHuEpo/placebo, Trial: Pre/Post and Sprints: 10 sprints, study 1) and with two within-subject variables (Group: CO rebreathing/placebo and Sprints: 10 sprints, study 2), respectively.

Results

Maximal Oxygen Uptake

There was a significant increase in $\dot{V}O_{2max}$ by 3.9% in the rHuEpo trial (54.5 ± 5.1 $mL \cdot kg^{-1} \cdot min^{-1}$ vs 56.6 ± 5.8 $mL \cdot kg^{-1} \cdot min^{-1}$; $P < 0.01$) (Fig. 3A). In contrast, there was a significant reduction in $\dot{V}O_{2max}$ by 4.8% after CO rebreathing compared with placebo (49.6 ± 5.5 $mL \cdot kg^{-1} \cdot min^{-1}$ vs 47.2 ± 5.3 $mL \cdot kg^{-1} \cdot min^{-1}$; $P < 0.01$) (Fig. 3B). There were no significant differences in maximal HR between rHuEpo, and CO trials and their respective placebo trials.

Ventilatory Threshold

The $\dot{V}O_2$ at which VT was attained did not significantly change following rHuEpo or placebo administration (Fig. 3C). Conversely, CO rebreathing resulted in a 4.7% reduction in the $\dot{V}O_2$ at VT compared with placebo (36.8 ± 5.4 $mL \cdot kg^{-1} \cdot min^{-1}$ vs 38.6 ± 4.7 $mL \cdot kg^{-1} \cdot min^{-1}$; $P < 0.01$) (Fig. 3D).

tHb-Mass

In the rHuEpo group, relative tHb-mass significantly increased from baseline 24 d after the first injection (13.0 ± 1.5 $g \cdot kg^{-1}$ vs 14.4 ± 1.7 $g \cdot kg^{-1}$, $P < 0.01$, Table), this trend was maintained after 38 d resulting in a 14.2% increase compared with baseline (14.8 ± 1.7 $g \cdot kg^{-1}$, $P < 0.01$). Following the cessation of rHuEpo supplementation, tHb-mass remained significantly elevated 7 (14.6 ± 1.1 $g \cdot kg^{-1}$, 52 d after first injection, $P < 0.01$, Table 1) and 21 d after the final injection (13.6 ± 1.2 $g \cdot kg^{-1}$, 66 d after the first injection, $P = 0.01$). There were no significant changes in the placebo group.

A significant correlation was found between $\dot{V}O_{2max}$ and tHb-mass following rHuEpo administration but not following placebo administration ($r = 0.63$, $P < 0.05$ and $r = 0.37$, $P = 0.28$, respectively). As in the rHuEpo study, tHb-mass was also significantly correlated with $\dot{V}O_{2max}$ in study 2 ($r = 0.51$, $P < 0.05$) (Fig. 4). There was no correlation between change in tHb-mass and change in $\dot{V}O_{2max}$ following rHuEpo administration.

In study 2, the mean ΔHbCO% prior to the maximal incremental test measured resulting from CO rebreathing was 4.7%, ranging between 4% and 5.7%. Despite not directly measured, the estimated HbCO% during the last 2 min (*i.e.*, at exhaustion) of the incremental exercise test was ~2.8%. Baseline HbCO% values during the incremental and RSA tests were consistent and minimal (1.1% ± 0.2% vs 1.0% ± 0.2%, respectively). There was a significant reduction in tHb-mass following CO rebreathing (980 ± 87 g vs 934 ± 7 g, $P < 0.05$).

Repeated Sprint Ability

There was a significantly higher blood pH following rHuEpo administration 5 min after the completion of the RSA test (7.1 ± 0.1 vs 7.2 ± 0.1, $P < 0.01$). Following rHuEpo administration there was a trend toward elevated

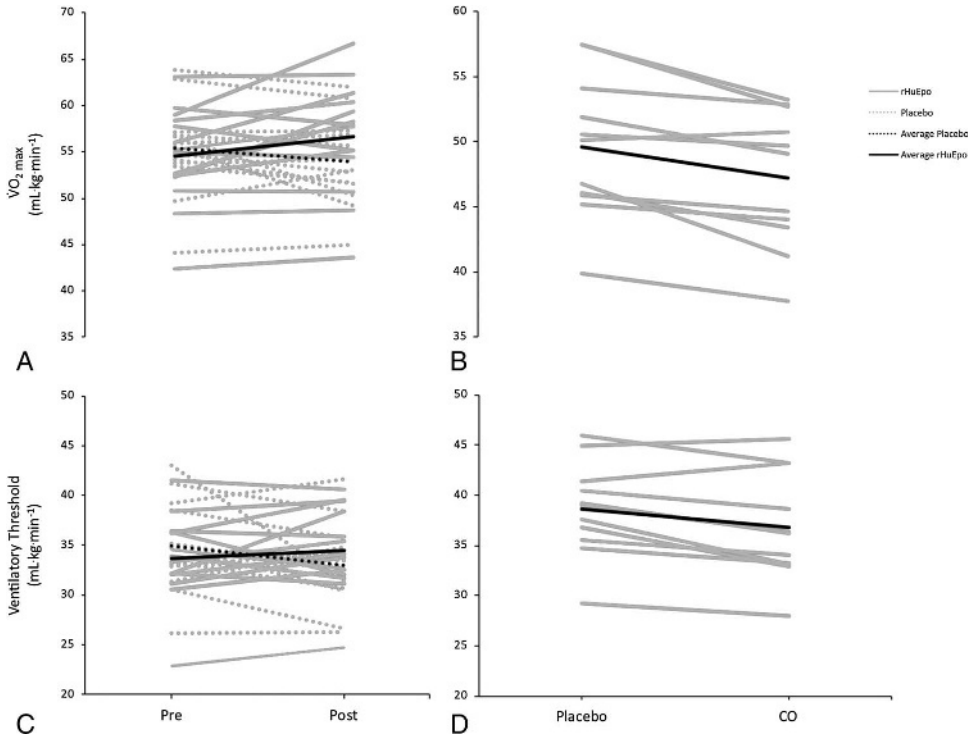

Figure 3: Individual changes in $\dot{V}O_{2max}$ and VT in studies 1 and 2. Each gray line represents one subject in one experimental condition. Black lines represent the mean for all subjects. (A) Changes in $\dot{V}O_{2max}$ following rHuEpo administration, (B) $\dot{V}O_{2max}$ following placebo and CO rebreathing, (C) Changes in VT following rHuEpo administration and (D) Changes in VT following placebo or CO rebreathing.

blood K^+ concentration prior to the onset of the RSA test (3.7 ± 0.3 mmol·L $^{-1}$, vs 4.1 ± 0.7 mmol·L $^{-1}$, $P = 0.06$). There also was a trend toward lower blood La following rHuEpo administration throughout the RSA test which reached significance after the fifth sprint compared with baseline (10.7 ± 2.8 mmol·L $^{-1}$, vs 9.1 ± 2.8 mmol·L $^{-1}$, respectively, $P < 0.05$). No significant differences were observed following placebo administration in study 1, similarly, in study 2, there were no significant differences following CO or placebo administration in any blood variable.

There were no significant differences in any of the measures of anaerobic performance following placebo or rHuEpo administration with the exception of time to peak power during the seventh sprint which was 0.67 s shorter post-rHuEpo ($P < 0.05$, Fig. 5). No other significant differences in any measures of performance between CO rebreathing and placebo groups were observed (Fig. 6).

Discussion

The main finding of this analysis is that altering the blood oxygen carrying capacity via rHuEpo or CO rebreathing significantly influenced $\dot{V}O_{2max}$ but had no effect on RSA. Regular, subcutaneous microdoses of rHuEpo augmented the oxygen carrying capacity of blood through an increased production of red blood cells, resulting in an elevation of tHb-mass by 1.8 g·kg^{-1}, 38 d after the first injection. This increase of 14.2% is comparable to a similar study investigating regular doses (5000 IU three to four times per week for 3 wk, followed by 5000 IU once a week for 11 wk) of rHuEpo and found tHb-mass increased by 3.8% to 18.8% (32), demonstrating the effectiveness of this low dose rHuEpo regime to enhance the oxygen carrying capacity of blood. The augmented tHb-mass led to an increase in $\dot{V}O_{2max}$ of 3.9% (Fig. 3A), which is a smaller change than seen in other rHuEpo studies where the increase typically ranges from 6% to 8% (7–11). However, the majority of these rHuEpo studies use high doses (>10,000 IU·wk^{-1}) to elicit a large effect, whereas the present study used smaller, regular doses to achieve the increase (2500–5500 IU·wk^{-1}). This rHuEpo administration protocol was conceived to mimic the "microdosing" regiment allegedly being used by athletes wishing to avoid detection. When compared with a similar regime, where 50 IU·kg^{-1} was administered for 3 wk followed by 20 IU·kg^{-1} for the subsequent 5 wk, a 4.7% change in $\dot{V}O_{2max}$ was observed (7), similar to 3.9% in the present study. Conversely, it was found that acute rebreathing of CO reduced the O_2 carrying capacity of blood by 4.7% resulting in a decrease in $\dot{V}O_{2max}$ by 4.8%. Using equations based on several studies performed in the 1970s, the predicted decrease in $\dot{V}O_{2max}$ should be within the range of 6.6% to 7.6% (26,33). The discrepancy between the observed and predicted decrease could be due to the methods used during previous studies investigating HbCO%, with shorter (2–5 min) or longer (22–25 min) trials while keeping HbCO% constant (33–35) compared with the approximately 12 min trial with a single bolus of CO in the present study. The decrease in $\dot{V}O_{2max}$ also is lower than achieved through cigarette smoking, which has been reported to reduce $\dot{V}O_{2max}$ by approximately 7% (20).

It has been suggested that a 1-g change in tHb-mass induces a change in $\dot{V}O_{2max}$ of approximately 4 mL·min^{-1} (36). The increase of 96 g in tHb-mass after rHuEpo administration in study 1 would theoretically result in an increase of 384 mL·min^{-1}, far higher than the 137-mL·min^{-1} measured. This mismatch between calculated and measured

Table.
tHb-mass changes following rHuEpo or placebo injections in relative ($g \cdot kg^{-1}$) and absolute (g) terms.

	Baseline		Administration Phase			Post	
	−14	−7	7	24	38	+7	+21
rHuEpo ($g \cdot kg^{-1}$) (SD)	13.0 (1.5)	13.3 (1.4)	13.3 (1.4)	*14.4 *(1.7)	*14.8 *(1.7)	*14.6 *(1.1)	*13.6 *(1.2)
rHuEpo (g) (SD)	942.4 (134.6)	967.0 (136.5)	966.5 (136.7)	*1036.7 *(153.3)	*1072.9 *(161.1)	*1061.5 *(131.0)	*984.2 *(122.1)
Placebo ($g \cdot kg^{-1}$) (SD)	13.6 (1.3)	13.4 (1.2)	13.5 (1.2)	13.1 (1.2)	13.1 (1.2)	13.2 (1.4)	13.1 (1.1)
Placebo (g) (SD)	978.9 (119.3)	964.8 (117.4)	962.3 (119.7)	944.7 (121.3)	941.2 (120.2)	951.4 (128.2)	946.7 (109.7)

Significant differences from baseline are indicated by $^*P < 0.05$.

increase of $\dot{V}O_{2max}$ could be due to the muscle being unable to fully utilize the increased O_2 delivery and therefore much is underused. Something analogous can be seen in studies investigating the Live High, Train Low (LHTL) method of altitude training. When subjects train at a low altitude but live at a higher altitude while maintaining their "normal" training regime, the muscle, in particular the degree of capillarization, may not change but tHb-mass will be augmented by living at altitude. For example, a study investigating the mechanism of LHTL reported an increase in tHb-mass of 42 g (calculated increase in $\dot{V}O_{2max}$ of 168 $mL \cdot min^{-1}$) but resulting in an increase in $\dot{V}O_{2max}$ of only 34 $mL \cdot min^{-1}$ (37). In contrast, the change in $\dot{V}O_{2max}$ following CO rebreathing is in line with the calculated decrease (reduction of 169 $mL \cdot min^{-1}$, study 2). There was no correlation between the change in $\dot{V}O_{2max}$ and change in tHb-mass, which also may explain part of the lack of agreement with previous studies. This lack of correlation may have arisen due to the large individual variation in tHb-mass in response rHuEpo within study 1 (4.8%–30.6% increase in tHb-mass). Large individual variations may be, in part, due to the relatively common (5%–10% of anemic patients treated with rHuEpo) occurrence of "nonresponders" to rHuEpo (38) and is a common occurrence in other studies investigating rHuEpo (*e.g.,* 3.8%–18.8% increase in tHb-mass, 33).

Following rHuEpo administration, there was a nonsignificant increase in VT (2.2%). Conversely, the $\dot{V}O_2$ at which VT was reached was found to be significantly reduced by 4.8% after CO rebreathing in study 2. The reduction in $\dot{V}O_2$ at the onset of VT after CO rebreathing could be related to the utilization of carbohydrate (glucose or glycogen) as seen with individuals exercising at altitude (39). An increased glycolytic activity and increased sympathetic nervous activity could increase the efflux of lactate, H^+ and subsequently increase the ventilatory drive, resulting in an earlier onset of VT (40).

Mechanisms other than the hemopoietic effects of rHuEpo have recently been the focus of a study that evaluated the effect of three differing doses of rHuEpo on $\dot{V}O_{2max}$ and time to exhaustion in trained individuals (41), where it was found that rHuEpo administration improved $\dot{V}O_{2max}$ by 6% and increased time to exhaustion by 10% to 70%. The authors speculate that the increased red blood cell volume was not the

Figure 4: Relationship between tHb-mass and $\dot{V}O_{2max}$ following rHuEpo administration represented by black markers ($r = 0.63$, $P < 0.02$) and CO rebreathing trial represented by gray markers ($r = 0.51$, $P < 0.05$).

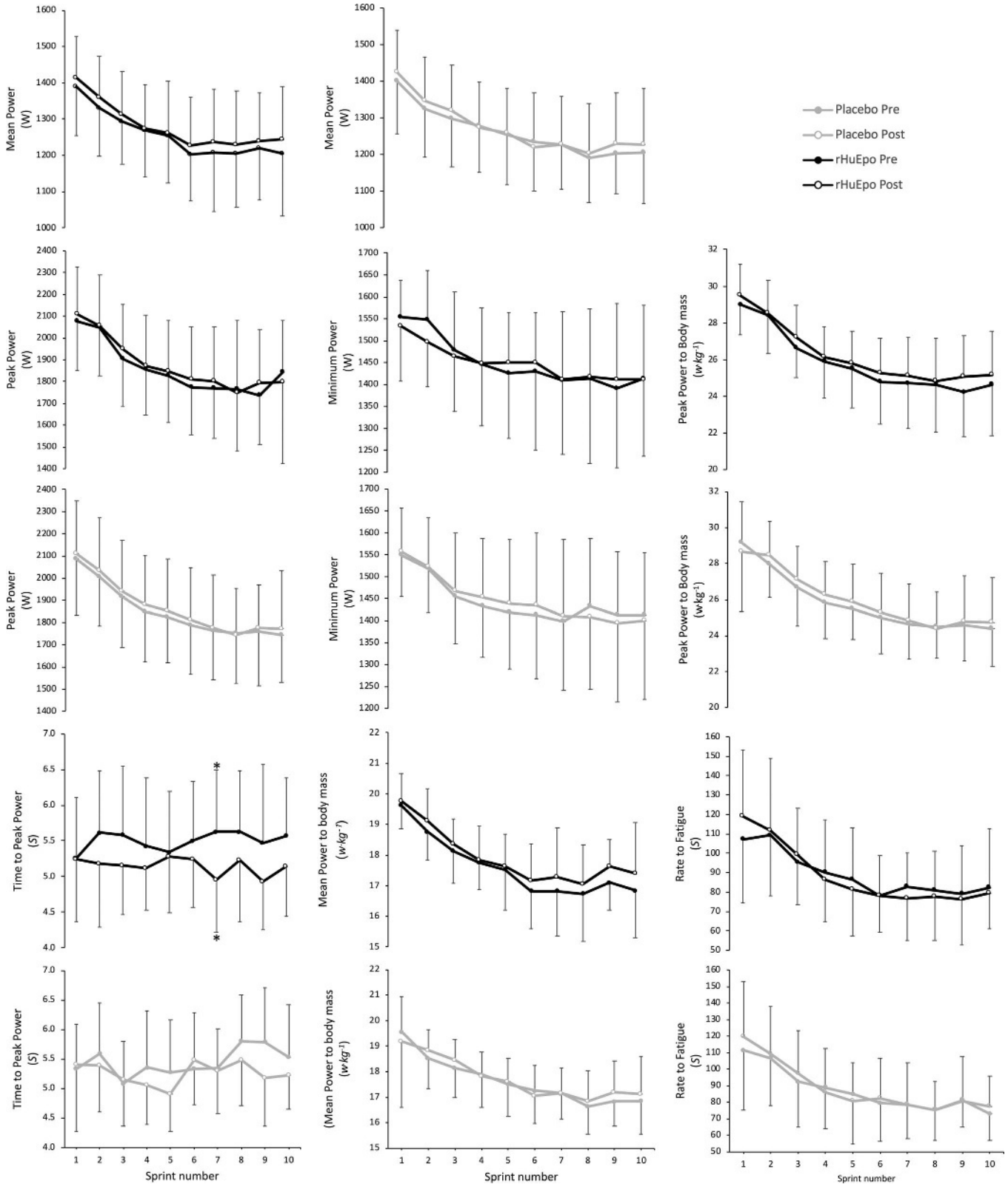

Figure 5: RSA during rHuEpo and placebo trials. * Significant difference pre- vs post-rHuEpo or placebo administration ($P < 0.05$).

cause of the increased time to exhaustion due to the lack of any correlation between the two ($r = 0.033$, $P = 0.26$) but rather increased mitochondrial enzyme activity resulting in increased fat oxidation and therefore reduced glycogen use. Another recent rHuEpo study found that while there was a clear improvement in laboratory-based measures such as $\dot{V}O_{2max}$ after rHuEpo administration, there was little to no improvement in a "real-life" racing situation (13). In this

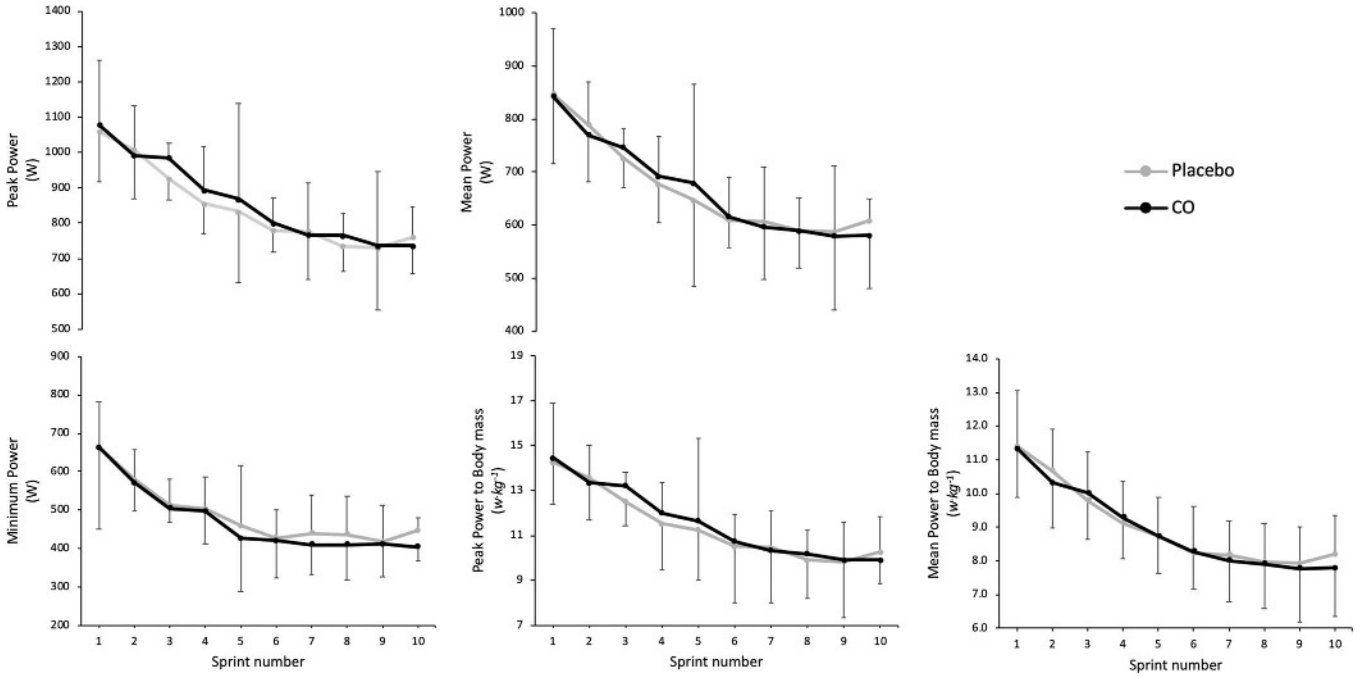

Figure 6: RSA during CO rebreathing and placebo trials.

study, participants were administered injections varying from 5000 to 8000 IU per dose to elicit a 10% to 15% increase in hemoglobin concentration. $\dot{V}O_{2max}$ was increased by 10% but this did not translate into significant improvement in performance compared with the matched control group during a fixed 100-km group cycle followed by a mountain climb. Given the lack of a tightly controlled crossover design including the uncontrolled nature of the 100-km cycle prior to a mountain race which had wind speeds varying from slight 40 $km \cdot h^{-1}$ to a more intense 85 $km \cdot h^{-1}$, the outcome of this study should be interpreted with caution.

Repeated sprint ability was not improved post-rHuEpo administration or hindered after CO rebreathing compared with placebo (Fig. 5 and 6). The primary energy system used for muscular work during short, high-intensity exercise is the degradation of PCr, providing approximately 55% of the ATP usage during an all-out 3 s sprint while only approximately 3% of the ATP used is produced through aerobic glycolysis (17). As multiple sprints are combined with little rest (approximately 50 s), the contribution of the aerobic system increases but still remains lower than the contribution from PCr. During intense exercise, PCr stores are decreased and can take up to 5 min to replenish (18). If the recovery period between bouts of intense exercise is less than 5 min (such as in studies 1 and 2), PCr stores may not replenish fully and decrease continually across the exercise duration (19). This process is exacerbated when under hypoxic conditions, as demonstrated in a study where participants performed 10 × 6 s sprints with 30 s rest in between in either simulated altitude of 3000 m or under normobaric conditions (21). It was found that RSA was impaired in the hypoxic condition, the authors suggest that hypoxia reduced O_2 delivery and therefore PCr resynthesis was inhibited during the brief recovery periods. This is contradictory of study 2, which showed no change in sprint performance despite a reduced O_2 availability (similar to exercising in a hypoxic environment). After rHuEpo administration and subsequent increased O_2 delivery, RSA did not improve. This also is contrary to the concept that RSA is O_2 dependent as RSA should have increased following rHuEpo administration or decreased following CO rebreathing; however, no such changes were observed (Fig. 5).

A significant decrease in time to peak power post-rHuEpo was observed during the seventh sprint; however, this is the only performance parameter which appears to have been moderately influenced by rHuEpo administration. It is possible that this improvement is due to the nonhemopoietic effects of rHuEpo such as increased mood (42), resulting in a lower perception of effort and therefore a more rapid time to peak power. It is also worth noting the differences during the seventh sprint observed here, while significant, are small (<1 s).

As RSA did not change in either experimental condition (CO rebreathing or rHuEpo) with exception of time to peak power, it suggests that O_2 delivery does not influence RSA performance but rather some other factors residing with the exercising muscles such as the ability of the muscle to utilize O_2. For example, an increased O_2 extraction by the muscles during repeated sprints was reported in a hypoxic environment compared with normoxia has been found (21).

While PCr resynthesis is O_2 dependent and could represent a limiting step in RSA, there remain several other factors which may have an equally important role in RSA (*e.g.*, muscle excitability, neural drive and metabolite accumulation) (43). Other studies have investigated the effect of rHuEpo on high-intensity exercise and concluded that the increased O_2 delivery following rHuEpo administration increased the proportion of energy turnover from aerobic glycolysis during a RSA test (44). This conclusion is likely to have risen from an increase in aerobic glycolysis during the final sprint compared to the first (45). No performance

differences in the final sprint between rHuEpo or CO rebreathing and their respective placebo trials was observed in the present investigation (Fig. 5 and 6). It is in this final sprint that any differences would be apparent as the combined effect of potentially enhanced PCr resynthesis and the greater aerobic contribution would be largest following rHuEpo administration and most inhibited following CO rebreathing. The lack of any differences found during this period suggests that altering the O_2 delivery played a minimal role in attenuating fatigue during a RSA test. This is further evidenced by the lack of any significant differences in blood metabolites after CO rebreathing compared with placebo. If there was an increased reliance on the anaerobic system following CO rebreathing, there would be a concurrent increase in blood metabolites associated with enhanced anaerobic metabolism; however, there were no such changes in blood La, K^+ or pH. After rHuEpo administration, blood La concentration was significantly lower only after the fifth sprint compared with baseline (9.1 ± 2.8 mmol·L^{-1} vs 10.7 ± 2.8 mmol·L^{-1}; $P = 0.03$, respectively). This lower blood La concentration could reflect an increased O_2 delivery postsprint after rHuEpo administration and subsequent decreased reliance on ATP from anaerobic sources and subsequently less La is produced (18). Despite this modest effect on blood La, RSA performance was not affected by rHuEpo (Fig. 5).

The current investigation's primary limitation is the inclusion of two separate cohorts to elucidate the mechanisms of altering O_2 carrying capacity. The cohort used for study 2 was recreationally active compared to the endurance-trained athletes in study 1, however despite these differences in training status $\dot{V}O_{2max}$ was largely the same (49.6 ± 5.5 mL·kg^{-1}·min^{-1} vs 54.5 ± 5.1 mL·kg^{-1}·min^{-1}, respectively). Absolute differences in RSA between the baseline rHuEpo and CO groups were more pronounced, however the decrements in performance between the first and 10th sprints were similar (*e.g.*, mean Power; 184 W vs 239 W, respectively). The similarity between the decrease in performance seen suggests that the effects of differentially altering the O_2 carrying capacity between the two groups can be compared.

In conclusion, microdose rHuEpo administration improved $\dot{V}O_{2max}$ by increasing tHb-mass and O_2 delivery to the active muscles while CO rebreathing had the opposite effect, but these acute alterations in $\dot{V}O_{2max}$ did not affect RSA performance. Despite the weakness of comparing two studies with different subject populations and procedures, interpretation of the data can be made with caution. These findings imply that enhancement of aerobic capacity during sports involving repeated sprints might not be a critical aspect and subsequently, microdose rHuEpo administration may not confer any ergogenic effects in such sports. Nonetheless, the contribution of $\dot{V}O_{2max}$ is largely dependent on the RSA test used and thus caution should be taken when extrapolating these results to other RSA protocols and evaluating the relevance of $\dot{V}O_{2max}$ on intermittent sports performance. It remains to be determined whether during tasks involving a larger number of sprints and/or shorter sprints, as occurs during many team-based sports would benefit from an improved $\dot{V}O_{2max}$. These data also reiterate the need for advances in antidoping to allow the accurate detection of low doses of rHuEpo which can improve $\dot{V}O_{2max}$ and potentially endurance performance. Further research is required to investigate the extent to which factors other than augmented O_2 carrying capacity influence the improved performance seen with administration of rHuEpo.

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