Plasma-volume contraction and exercise-induced hypoxaemia modulate erythropoietin production in healthy humans

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A B S T R A C T

This study examined exercise-induced hypoxaemia (EIH) and plasma volume contraction as modulators of serum erythropoietin (Epo) production. Five athletes cycled for 3 min at supra-maximal power outputs, at each of two different elevations (1000 m and 2100 m). Five subjects were exposed to normobaric hypoxia ($F_IO_2 = 0.159$), seven subjects underwent plasmapheresis to reduce plasma volume and eight subjects were time controls for Epo levels. Oxyhaemoglobin saturation was significantly reduced during exercise and during normobaric hypoxia. The time period of haemoglobin oxygen saturation < 91% was 24±29 s (mean±S.D., $n = 5$) for exercise at 1000 m, 136±77 s (mean±S.D., $n = 5$) for exercise at 2100 m and 178±255 s (mean±S.D., $n = 5$) with resting hypoxic exposure. However, significantly increased serum Epo levels were observed only following exercise (24±3%; mean±S.D., $n = 5$ at 1000 m and 36±5%; mean±S.D., $n = 5$ at 2100 m). Volume contraction also resulted in increased serum Epo (35±6%; mean±S.D., $n = 7$) in spite of a significant rise in haematocrit of 2.2%. Despite similar degrees of arterial desaturation, only the hypoxaemia induced by exercise was associated with an increase in serum Epo. This finding indicates that other factors, in addition to hypoxaemia, are important in modulating the production of Epo in response to exercise. Volume depletion in the absence of exercise resulted in increases in Epo levels that were comparable with those observed in response to exercise. The paradoxical responses of the increased haematocrit and the increase in Epo in subjects undergoing plasmapheresis suggests that plasma volume may also modulate the production of Epo.

INTRODUCTION

The hypoxic exposure required to elicit an increase in serum erythropoietin (Epo) in resting subjects has been shown to be severe. More than 2 h of exposure to a partial pressure of oxygen, similar to that found at altitudes of 3000 m or more, are necessary to increase Epo levels [1–3]. Stimulation of Epo by exercise has also been investigated previously by a number of groups [4–7] with equivocal results. The variability in the results of these and other studies suggests that the production of Epo in healthy humans is a more complex process than has previously been thought.

Mild hypoxaemia has been reported to occur during intense exercise in some well-trained individuals [8], and the degree of hypoxaemia can be exacerbated by de-

Key words: Epo, plasma volume, exercise, hypoxia, plasmapheresis.

Abbreviations: Epo, erythropoietin; EIH, exercise-induced hypoxaemia; $\% SaO_2$, arterial haemoglobin saturation.

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creasing the partial pressure of oxygen in inspired air [9,10]. The development of hypoxaemia has been shown recently to be a requirement for stimulation of Epo with exercise [11], which may account for some of the variability of the post-exercise Epo responses reported in the literature. Evaluation of serum Epo levels in diseases such as congenital heart disease or chronic obstructive pulmonary disease support a relationship between elevated serum Epo levels and the degree of hypoxaemia [12]. However, information describing serum Epo levels and arterial haemoglobin saturation (% SaO_2) levels is scarce [3,11] and the effect of hypoxaemic episodes during supra-maximal exercise on Epo production has not been fully examined.

In addition to hypoxaemia, however, other factors such as blood volume [13], sodium reabsorption at the proximal tubule [14] and the activity of the renin/angiotensin system [15] have been implicated in the modulation of Epo production by the kidney. As volume depletion during exercise has been well documented [16,17], and the restoration of extracellular fluid volume is dependent upon increased active transport of sodium in the proximal tubule [18,19], some of the variability in the Epo response post-exercise may arise from changes in extracellular fluid volume.

Thus this study was undertaken to evaluate Epo production in response to exercise-induced hypoxaemia (EIH) compared with a resting hypoxic exposure which elicited a similar level of hypoxaemia. Further, the role of plasma-volume contraction in the discrepancy between the severity of stimuli required to induce Epo production during exercise compared with hypoxic exposure at rest was examined. Plasma-volume contraction, independent of hypoxic exposure or exercise, was investigated as a putative modulator of the post-exercise increase in Epo.

METHODS

Subjects

Five (2 male, 3 female) members of the Canadian national speed-skating team volunteered to perform the exercise protocols. Well-trained athletes were required to ensure that the intensity of exercise would be of such magnitude to generate EIH. In addition, five (2 male, 3 female) non-exercising control subjects underwent resting exposure to normobaric hypoxia ($F_IO_2 = 0.159$) to compare resting hypoxaemia with EIH. Seven male non-exercising subjects volunteered to undergo plasmapheresis. This protocol was used as a quantifiable model for volume depletion, generating a reduced plasma volume with an increased haematocrit. Finally, eight non-exercising male subjects acted as experimental time controls for Epo levels without treatment.

All subjects were normally resident at 1000 m. Volunteers were screened to ensure normal levels of haematological and iron parameters. Smokers and subjects with a history of renal or haematological disease were excluded. Subjects were required to abstain from the use of alcohol or drugs during the data collection periods, and were instructed to hydrate *ad libitum*.

Informed consent was obtained and the rights and privileges of the subjects were observed in accordance with the Declaration of Helsinki of the World Medical Association and the Conjoint Medical Ethics Committee, Office of Medical Bioethics, Faculty of Medicine, University of Calgary.

Exercise tests

All tests were performed at an elevation of 1000 m above sea level (barometric pressure = 87.9 ± 1.3 kPa), except for the altitude test at 2100 m (barometric pressure = 79.3 ± 0.3 kPa). Two weeks before the experimental period, each athlete underwent a screening exercise test. The protocol was a continuous, 2-min interval, incremental exercise test to peak oxygen consumption ($\dot{V}O_2$ peak) [20] using a 800 s cycle ergometer (Sensormedics Ergo-metrics; Yorba Linda, CA, U.S.A.). Measurements of oxygen consumption and expired CO_2 were made every 15 s using a Sensormedics Horizon Metabolic Cart (Summit Technologies Inc., Oakville, Ontario, Canada).

On testing days, subjects reported to the laboratory in the morning, at least 1 h after a light breakfast. Pre-exercise blood samples were collected, subjects were weighed and then performed a standard warm-up, consisting of two 2-min work bouts at a heart rate of 120 beats/min and 150 beats/min, 15 s at the target work load for the supra-maximal interval and an additional 2 min at 120 beats/min. The EIH protocol was a 3-min supra-maximal work bout, where power output was calculated as 115 % of the maximum load from the oxygen uptake ($ml \cdot kg^{-1} \cdot min^{-1}$) vs. power output (W) regression equation generated during the initial $\dot{V}O_2$ test. Target power outputs at altitude were calculated based upon a 5.5 % decrement in $\dot{V}O_2$ due to the elevation of 2100 m [21]. Three skaters performed the trial first at 2100 m, with a second trial at 1000 m carried out a minimum of 1 month later; two skaters performed the 1000 m trial first, followed by the 2100 m trial 2 weeks later. The altitude trials were carried out for between 1–4 h following ascent to 2100 m.

Resting hypoxic exposure

Subjects in the resting normobaric hypoxia group also reported to the laboratory in the morning, at least 1 h after a light breakfast, and were exposed to a N_2-balanced hypoxic gas mixture ($F_IO_2 = 0.159$) for 13 min while remaining seated. Gas was administered via a two-way non-rebreathing valve (Hans Rudolph Inc., Kansas City, MO, U.S.A.), from two 200 l Douglas bags positioned horizontally on a platform adjacent to the subject. Two

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8000 l gas tanks provided a continuous re-supply of gas to the Douglas bags.

Plasmapheresis

Plasmapheresis subjects reported to the plasmapheresis laboratory (Red Cross Society of Canada) after a light breakfast. A plume fistula needle was inserted into an anti-cubital vein under aseptic conditions. Subjects rested in a seated position with the feet elevated for approx. 30 min. Immediately pre- and post-plasmapheresis, 7 ml of whole blood was collected through a luer-lock connection. The plasmapheresis procedure consisted of approx. three draw and return cycles for the collection of 500 ml plasma. During the draw cycle, trisodium citrate (6 %/final plasma product) was immediately added to whole blood to prevent clotting. The blood then was centrifuged and the cellular components were re-infused during the return cycle.

Blood collection and analysis

Blood samples were collected from an anti-cubital vein pre-treatment and at 4, 7, 24 and 48 h post-intervention. Serum was immediately separated and then frozen at −20 °C until analysis, which was within 1 month of collection.

Epo analysis was by radioimmunoassay (DiaSorin Epo-trac, Stillwater, MN, U.S.A.). This method has been validated and described elsewhere [22]. All samples from each subject were analysed in a single assay run.

Whole blood in 7.5 % (w/v) EDTA was analysed for haemoglobin, haematocrit, erythrocyte and reticulocyte count using a Coulter STKS Haematology System (Coulter Counter, Miami, FL, U.S.A.). Percentage changes in blood and plasma volumes were calculated from haemoglobin and the haematocrit according to the method of Dill and Costill [23].

Blood samples for lactate analysis were collected three-min post-exercise from fingertip capillaries into 100 μl capillary tubes. The samples were immediately transferred into YSI 2372 preservative tubes (Yellow Springs Instruments, Yellow Springs, OH, U.S.A.), mixed well and stored at 4 °C until analysis, within 24 h (YSI model 1500 Sport Lactate Analyzer).

Estimated arterial O_2–haemoglobin saturation

Estimated arterial O_2–haemoglobin saturation was recorded every 5 s during the exercise or hypoxic-exposure period through to recovery of baseline values using a Nellcor N200 pulse oximeter with a RS-10 reflective forehead sensor and a three-lead electrocardiogram (Nellcor Inc., Pleasanton, CA, U.S.A.). Data from the N200 was recorded in real time using the SatMaster Computerized Pulse Oximetry software package (EMG Scientific, Beverly Hills, CA, U.S.A.). Validation of this instrument under exercise conditions has been described elsewhere [11].

Statistical analysis

Statistical analysis was performed using Wilcoxon signed rank test for paired data (before and after comparisons) or the Kruskal–Wallis test for the analysis of two independent groups. The relationship between the change in Epo and the degree of desaturation in the exercise protocols was assessed by regression analysis of ranks to maintain the non-parametric analysis for this data set (STATA 3; College Station, TX, U.S.A. and SYSTAT; SPSS Inc., Chicago, IL, U.S.A.). Statistical significance was set at $P < 0.05$.

RESULTS

Physical characteristics of the subjects are presented in Table 1. All haematological measures were within the normal range and no significant changes were observed in haemoglobin, haematocrit, plasma volume or erythrocyte and reticulocyte counts in the exercise, hypoxia or control groups throughout the experimental period (Table 2).

Although the peak O_2 consumption achieved was lower at 2100 m than at 1000 m [48.3 ± 2.7 ml·kg^{-1}·min^{-1} (mean ± S.D., $n = 5$) versus 55.5 ± 4.7 ml·kg^{-1}·min^{-1} (mean ± S.D., $n = 5$), $P < 0.05$] post-exercise lactate values were not different (Table 3). All measures of % SaO_2 indicated that a greater level of desaturation was experienced with exercise at the higher altitude; minimum % SaO_2 value was lower (82 ± 3 %; mean ± S.D., $n = 5$ versus 89 ± 2 %; mean ± S.D., $n = 5$, $P < 0.05$) and both the time of hypoxaemic period (136 ± 77 s; mean ± S.D., $n = 5$ versus 24 ± 29 s; mean ± S.D., $n = 5$, $P < 0.05$) and time to recover resting % SaO_2 (102 ± 27 s; mean ± S.D., $n = 5$ versus 4 ± 7 s; mean ± S.D., $n = 5$, $P < 0.05$) were longer at 2100 m than at 1000 m (Table 3). No significant difference was observed between resting % SaO_2 values at 2100 m (98 ± 2 %; mean ± S.D., $n = 5$) and 1000 m (98 ± 1 %; mean ± S.D., $n = 5$). However, exposure to the N_2-replaced gas at rest did result in a mean period of 178 ± 255 s (mean ± S.D., $n = 5$) where % SaO_2 values declined below 91 %.

No changes in serum Epo were observed in the resting hypoxic group or in the control group over the 48-h measurement period. In contrast, serum Epo concentrations increased significantly in the exercise protocol at 24 h (Figure 1). This increase was of even greater magnitude following exercise at 2100 m (Figure 1) compared with that at 1000 m (36 ± 5 %; mean ± S.D., $n = 5$ versus 24 ± 3 %; mean ± S.D., $n = 5$, $P < 0.05$). However, the hypothesized direct relationship between the increase

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Table 1 Physical characteristics of the subjects (means ± S.D.)

Subject group	*n*	Age (years)	Height (cm)	Body mass (kg)
Athletes	5	23 ± 3	173 ± 10	70.4 ± 10.9
Normobaric hypoxia	5	27 ± 2*	178 ± 5	74.1 ± 8.5
Plasmapheresis	7	32 ± 3*	179 ± 5	94 ± 14
Controls	8	30 ± 4*	182 ± 4	85 ± 10

* Significantly different from athletes $P < 0.05$.

Table 2 Haematological Profile

Haematological parameters pre- (Pre) and 24-h post-(Post)treatment for the normobaric hypoxia resting control (CNBH), exercise at 1000 m (1000 m), exercise at 2100 m (2100 m) and plasmapheresis (PV) groups (means ± S.D.). Hb, haemoglobin; δ PV, change in plasma volume at 4 h and 24 h post-exercise; RBC, erythrocyte count.

	CNBH		1000 m		2100 m		PV		Control	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Hb (g/dl)	15.7 ± 0.4	15.7 ± 0.5	15.1 ± 1.2	14.9 ± 1.0	15.2 ± 0.9	14.8 ± 0.9	15.2 ± 0.7	15.7 ± 0.7*	14.9 ± 0.6	14.8 ± 0.7
Haematocrit (%)	0.45 ± 0.01	0.45 ± 0.01	0.43 ± 0.04	0.43 ± 0.04	0.45 ± 0.03	0.44 ± 0.03	0.44 ± 0.02	0.46 ± 0.02*	0.43 ± 0.02	0.43 ± 0.03
RBC (10^{12}/l)	5.0 ± 0.2	5.0 ± 0.2	5.0 ± 0.6	4.8 ± 0.6	5.0 ± 0.4	4.9 ± 0.4	5.2 ± 0.4	5.4 ± 0.3*	5.0 ± 0.4	4.9 ± 0.3
Reticulocytes (%)	0.5 ± 0.3	0.6 ± 0.3	0.9 ± 0.5	0.9 ± 0.2	1.0 ± 0.3	0.8 ± 0.2	1.0 ± 0.4	1.4 ± 0.7	0.9 ± 0.6	1.1 ± 0.6
δ PV (%)										
4 h				2 ± 2		1 ± 2		−5 ± 2		0 ± 1
24 h		0 ± 2		1 ± 3		−1 ± 2		−2 ± 2		1 ± 2

* Significantly different from pre-plasmapheresis $P < 0.05$.

Table 3 Arterial haemoglobin saturation (SaO_2) and oxygen consumption ($\dot{V}O_2$) during exercise at 1000 m and 2100 m (means ± S.D.)

Parameter	1000 m	2100 m
Peak $\dot{V}O_2$ (ml · kg^{-1} · min^{-1})	55.5 ± 4.7	48.3 ± 2.7*
Lactate (mmol/l)	14.0 ± 1.2	13.3 ± 0.9
Minimum SaO_2 (%)	89 ± 2	82 ± 3*
Time at $SaO_2 < 91\%$ (s)	24 ± 29	136 ± 77*
Time at $SaO_2 < 91\%$ (% total time)	11 ± 12	52 ± 17*
Time to recover SaO_2 (s)	4 ± 7	102 ± 27*
Time at $\dot{V}O_2$ peak (s)	69 ± 27	99 ± 17

* Significantly different from 1000 m $P < 0.05$.

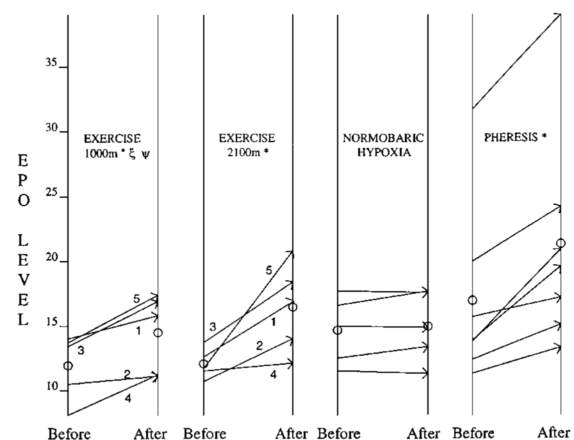

Figure 1 Serum Epo (units · l^{-1}) in response to supramaximal exercise at 1000 m, 2100 m, normobaric hypoxia or plasmapheresis

The individual subjects are denoted numerically. The open circles indicate the mean values; * indicates $P < 0.05$ for the before versus after comparisons; † indicates $P < 0.05$ for the post Epo during exercise or hypoxia versus plasmapheresis (pheresis); ‡ indicates $P < 0.05$ for the post Epo at 1000 m versus the post Epo at 2100 m.

in Epo levels and the magnitude of the hypoxaemic episode did not achieve statistical significance ($P < 0.10$).

Seven additional subjects at rest underwent volume depletion by plasmapheresis. The plasmapheresis group was significantly older than the exercise and resting hypoxia groups, however, pretreatment values were not different for any of the blood parameters and age has been shown previously not to influence Epo levels [22]. Post-plasmapheresis values for haemoglobin and haematocrit were significantly increased from pre-plasmapheresis, as indicated in Table 2. Plasma volume was significantly decreased by −5 ± 2% (mean ± S.D., $n = 7$) immediately post-plasmapheresis and serum Epo levels were found to be increased by 35 ± 6%

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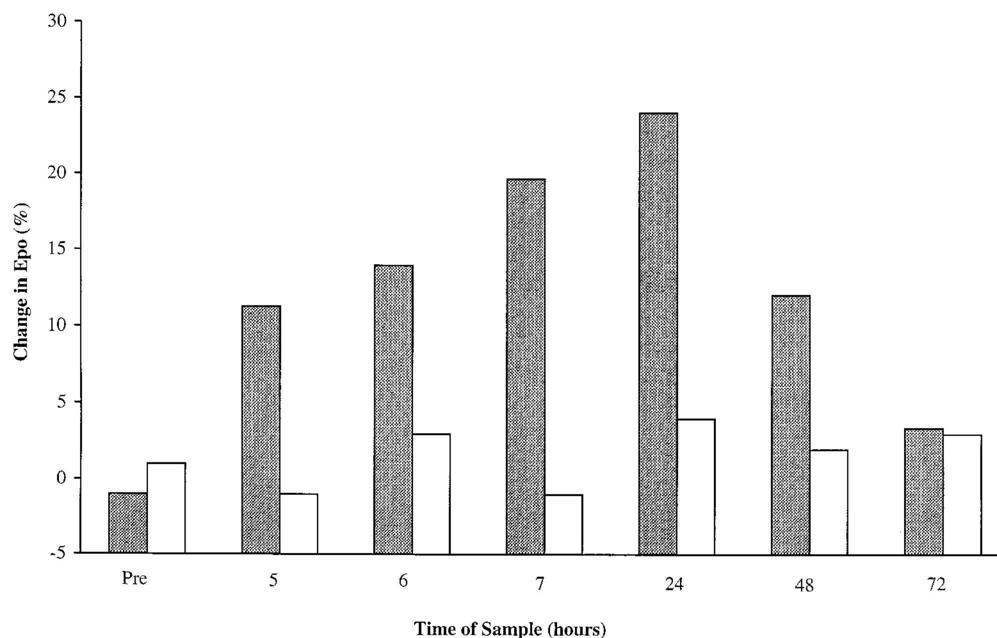

Figure 2 Changes in serum Epo concentration with plasmapheresis
At 7 h and 24 h, Epo was significantly different from pre-plasmapheresis levels.

(mean ± S.D., $n = 7$) 24 h post-plasmapheresis from 15.5 ± 3.9 units/l (mean ± S.D., $n = 7$) to 20.9 ± 5.6 units/l (mean ± S.D., $n = 7$), $P < 0.05$. The change in Epo levels was observed to be maximal at 24 h (Figure 2).

DISCUSSION

The present investigation confirms that extremely intense exercise provides a physiological stimulus to increase Epo production [11]. Notwithstanding the trend for increased magnitude of Epo response with increased severity of hypoxaemia following supra-maximal exercise, factor(s) in addition to hypoxaemia are implicated, as a similar degree of hypoxaemia in non-exercising control subjects was not associated with changes in serum Epo levels.

EIH has been defined as % $SaO_2 < 91$ % [24], and the development of hypoxaemia during severe exercise has been shown to have physiological limitations on work performance [9]. We hypothesized that, if the delivery of oxygen was being compromised by episodes of hypoxaemia during exercise, a dose-dependent physiological stimulus for adaptation of the oxygen carrying system may have been generated. The data presented in the present work appear to support this hypothesis. Increased Epo levels were observed following exercise (Figure 1) and the magnitude of this increase was observed to be significantly greater under conditions that elicited a more severe level of hypoxaemia. The relationship between Epo levels and the duration of hypoxaemia did not reach statistical significance ($P <$ 0.10), however, the possibility of such an interaction cannot be excluded, due to the small sample size ($n = 5$).

No comparisons between the physiological effects of EIH and resting hypoxaemia on serum Epo levels have been reported previously. In the present experiment, increased Epo levels were observed following hypoxaemia of only seconds to minutes during exercise (Table 3), however, a similar hypoxaemic exposure generated by a 13-min exposure to $F_IO_2 = 0.159$ at rest did not give rise to increased Epo levels. Post-treatment Epo levels following resting hypoxic exposure were not different from the pre-treatment levels in any of the experimental groups (Figure 1). Although the lack of Epo response to this level of resting hypoxaemia is supported by reports in the literature [1–3], the difference between the Epo response to similar hypoxaemic episodes generated by exercise or at rest suggests that there may be an additional factor(s) contributing to stimulation of Epo during exercise.

The degree of desaturation observed with exercise in the present work was of similar magnitude to that reported previously with comparable inspired O_2 levels, although the duration of the EIH episodes were not stated [8,10]. The magnitude of the hypoxaemia which we observed with the supra-maximal exercise protocol can also be compared with that reported previously for resting exposures to reduced inspired O_2 pressure. Using a gas mixture containing only 10.5 % O_2 (stimulating an altitude of more than 5000 m), which produced SaO_2 saturation values of 75–85 %, no elevation in serum Epo levels were observed within 5 h following either a 5-min or 60-min exposure to hypoxia [3]. A 120-min exposure period produced an increase of 48 % in serum Epo over

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baseline values after 240 min. These, and similar, results [1–3] confirm our observation that, in the absence of exercise, a much more severe hypoxic stimulus is required to elicit an increase in serum Epo.

A number of factors, other than hypoxaemia, have been suggested as contributing to renal Epo production [13–15]. The role of plasma volume in modulating serum Epo is of particular interest in view of the evidence indicating that exercise elicits acute reductions in plasma volume [16,17] followed by an 'overshoot' expansion 24–48 h later [25]. This short-lived fluctuation in plasma volume would be expected to produce a transient increase in the concentration of all serum components followed by a dilutional effect, and the lower haemoglobin concentration has been suggested to be a stimulus for Epo [4]. The dilutional hypothesis is not supported by our data. The calculated plasma volume values remained unchanged over the observation period, from 4–48 h post-exercise (Table 2). Furthermore, expansion of plasma volume did not result in increased Epo production in resting humans [26] or in conscious dogs [13]. Expansion of blood volume by 12% did not change Epo production, however, a 20% reduction in blood volume increased Epo levels by 1.5-fold in the absence of a change in haematocrit [13]. The effect of the reduction in plasma volume, however, warrants further consideration. In the present study, the first measurement of plasma volume was not made until 4 h post-exercise and the exercise-induced plasma volume depletion/repletion cycle may have been missed.

To further examine the possible role of volume depletion in the observed difference in the magnitude of hypoxaemia required for stimulation of Epo production at rest compared with exercise, seven resting subjects were studied after plasmapheresis. A significant increase in Epo production was observed (Figures 1 and 2). The paradoxical response of serum Epo compared with haemoglobin (i.e. where an increase in haemoglobin during plasmapheresis was associated with an increase in serum Epo) indicates that Epo production is sensitive to changes in plasma volume. The prevailing oxygen tension at the renal-tissue level is believed to control the *Epo* gene [27]. Furthermore, it is known that sodium reabsorption accounts for at least 90% of renal oxygen consumption [28], and that inhibition of sodium uptake in the proximal tubule attenuates the increased Epo production in response to a hypoxic stimulus [14]. Therefore, it may be that sodium reabsorption following plasma volume contraction may enhance Epo production, and that the stimulus for increased Epo synthesis following plasmapheresis (plasma volume depletion) is the increased O_2 utilization, rather than the decreased O_2 delivery at the renal interstitium. Altered levels of renal sodium uptake and O_2 consumption following volume contraction are also supported by the strong neuro-endocrine stimuli provided by the removal of 500 ml of plasma during plasmapheresis. The renin/angiotensin/aldosterone system and arginine/vasopressin hormone systems respond to volume depletion, resulting in increased sodium and water reabsorption in the renal proximal tubule and the distal nephron [29]. In addition, norepinephrine has also been shown to increase Na^+/K^+ ATPase function and O_2 consumption in the renal proximal tubule [30].

It is also possible that the reduction in renal blood flow associated with intense exercise [31] may have induced a hypoxic effect leading to increased Epo synthesis. However, Roberts and Smith [11] have not found increased Epo in the absence of EIH following supra-maximal exercise. It is also unlikely that the change in Epo levels observed was due to a diurnal rhythm, as, in the present study, no change in serum Epo secretion was observed in the non-exercising control group over 24 h. Also unlikely is the supposition that the observed increase in Epo was due to chronic training rather than the 3-min exercise protocol. No differences were observed in the development of a EIH or Epo response in athletes from different sports [11], and at least 24 h had elapsed between the previous training session and the test exercise protocol for all subjects.

In summary, the present study demonstrates the development of arterial desaturation and increased serum Epo levels in response to a single 3-min supra-maximal exercise bout in elite-level sprint-trained speed skaters. The increase in Epo post-exercise was greater at 2100 m than at 1000 m; both the duration of hypoxaemia and the minimum haemoglobin saturation levels indicate that the EIH episode was also more severe at 2100 m than at 1000 m. However, similar levels of arterial desaturation without exercise did not result in increased Epo levels, suggesting that other factor(s), in addition to hypoxaemia, may modulate Epo stimulation during exercise. Reduction of plasma volume by plasmapheresis resulted in a paradoxical but significant increase in Epo production, in spite of an increase in haemoglobin concentration. As hypoxaemia is necessary, though not sufficient, to give rise to the increase in Epo post-exercise, the increased renal O_2 consumption that occurs with increased sodium uptake following volume contraction may be another essential factor for increased Epo production under conditions of altered plasma volume. Further investigations are required to elucidate the putative role of enhanced proximal tubule O_2 consumption in this mechanism.

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