

# The time-course response of endogenous erythropoietin, IL-6 and TNF $\alpha$ in response to acute hypoxic exposures

G. Turner, O.R. Gibson, P.W. Watt, J.S.M. Pringle, A.J. Richardson and N.S. Maxwell

Environmental Extremes Laboratory  
Centre for Sport and Exercise Science and Medicine  
University of Brighton  
Welkin House, Denton Road  
Eastbourne  
BN20 7SN

Mr Gareth Turner                      Gareth.turner@eis2win.co.uk

Mr Oliver Gibson                      O.R.Gibson@brighton.ac.uk

Dr Peter Watt                          P.Watt@brighton.ac.uk

Dr Jamie Pringle

Dr Alan Richardson                  A.J.Richardson@brighton.ac.uk

Dr Neil Maxwell                      N.Maxwell@brighton.ac.uk

## Running head

EPO and inflammatory responses to hypoxia

## Abstract

Erythropoietin (EPO) rapidly decreases on return from chronic altitude exposure. Acute hypoxia may provide an additional stimulus to prevent this decline in EPO. Optimal normobaric hypoxic exposure has not been established; therefore, investigation of methods eliciting the greatest response, whilst not causing any additional stress is required. Eight physically active males (age  $27 \pm 4$  yrs, body mass  $77.5 \pm 9.0$  kg, height  $179 \pm 6$  cm) attended the laboratory on four separate occasions, in a randomised order, and rested passively in a hypoxic chamber for 2 h whilst exposed to four simulated altitudes [ $FiO_2$ : 0.209 (SL),  $FiO_2$ :  $\sim 0.135$  (3,600 m),  $FiO_2$ :  $\sim 0.125$  (4,200 m) and  $FiO_2$ :  $\sim 0.115$  (4,800 m)]. Venous blood samples were drawn immediately pre-exposure and then at 1, 2, 4, 6 and 8 h to assess changes in blood plasma erythropoietin concentration ([EPO]), interleukin-6 concentration (IL-6) and tumor necrosis factor alpha concentration (TNF $\alpha$ ). During 4,200 m and 4,800 m [EPO] increased from  $5.9 \pm 1.5$  to  $8.1 \pm 1.5$   $mU \cdot mL^{-1}$  ( $P = 0.009$ ) and  $6.0 \pm 1.4$  to  $8.9 \pm 2.0$   $mU \cdot mL^{-1}$  ( $P = 0.037$ ), respectively, with the mean increase in [EPO] peaking at 4h (2h post-exposure). Results indicate there were no differences found in IL-6 or TNF $\alpha$  during or post-exposure. An increase in endogenous [EPO] was found two hours post-hypoxic exposure as result of two hours of normobaric hypoxia, equivalent to 4,200 m and above. There was no dose-response relationship in [EPO] between the severity of simulated hypoxia.

**Key words:** hypoxia, EPO, altitude, cytokines, inflammation,

## 1 Introduction

2 Exercise performance and haematological responses have been shown to be variable in response to  
3 altitude exposures (Chapman et al. 1998; Chapman 2013). Practitioners and coaches should  
4 therefore consider individualising an athlete's post-altitude training strategy in order to fully  
5 optimise the benefits of the camp. Elite coach, Dick (1992), discussed training at altitude in practice  
6 and suggested that on return from altitude, time was needed to reach a stage where performance  
7 shows a clear sign of benefit. Although these assumptions were based upon the training status of  
8 the athlete, there is haematological evidence to suggest there is a 're-acclimatisation' that occurs  
9 when returning to sea level from altitude. Garvican et al. (2012) observed a 1.5% decrease in  
10 tHbmass within 3 days of descent from a 3 week natural altitude training camp, which persisted when  
11 measured 10 days after descent and Pottgiesser et al. (2012) found that 9 days after completing a 26  
12 day simulated altitude training camp there was a 3.0% decrease in tHbmass. Brugniaux et al. (2006)  
13 and Heinicke et al. (2005) both found tHbmass increased after 3 days at sea level but returned to  
14 baseline levels after 16 and 15 days, respectively.

15 Prommer et al. (2009) found that when natural altitude dwellers reside at sea level for sustained  
16 durations, a reduction in tHbmass occurs. The study found tHbmass remained stable within the first  
17 2 weeks at sea level followed by a reduction of ~2% per week before levelling off around 5-6 weeks  
18 post-altitude. The reduction in tHbmass was attributed to transiently suppressed erythropoietin  
19 (EPO) as a result of returning to a normoxic environment (Prommer et al. 2010). The removal of the  
20 altitude stimulus appear to result in a 're-acclimatization' to sea level (Garvican et al. 2012).  
21 Chapman et al. (2014) stated that, if an athlete completes a 4 week altitude training camp, followed  
22 by a short time (~7-14 days) at sea level to compete, returning to altitude even for a short time may  
23 mitigate or delay these effects by re-establishing EPO levels again.

24 Brief periods of severe hypoxia have been suggested as a potential method to prevent the sudden  
25 decrease in EPO (Pottgiesser et al. 2012). This in turn would preserve the haematological  
26 acclimatisation response for a longer time, thereby expanding the window for endurance training  
27 and optimal competition racing (Chapman et al. 2014). Figure 1 illustrates the previous  
28 investigations that have demonstrated an increase in EPO as a result of exposures lasting 5 minutes  
29 to 5.5 hours (Eckardt et al. 1989; Knaupp et al. 1992; Rodríguez et al. 2000; Ge et al. 2002; Niess et  
30 al. 2004; Friedmann et al. 2005; MacKenzie et al. 2008; Wahl et al. 2013). The chart bubble size  
31 represents the magnitude of EPO production in response to exposure time and simulated altitude  
32 (i.e. the larger the bubble, the greater the production of EPO). Collectively these findings suggest  
33 that a minimum time period of two hours at an altitude of >2,500 m elicit a significant increase in

1 EPO; however, differing methods (hypobaric vs. normobaric), physical status (rest vs. exercise) and  
2 timing of blood sampling have been investigated.

3 Stray-Gundersen et al. (2001) believed that when athletes attend altitude training camps it is  
4 possible that the presence of injury or infection, and therefore pro-inflammatory cytokines (such as  
5 IL-6 and TNF $\alpha$ ), could impair the erythropoietic response to altitude (Faquin et al. 1992; Jelkmann et  
6 al. 1992). Hypoxia is a stressor that alters homeostasis (subsequent to a reduction in arterial oxygen  
7 (O $_2$ ) saturation) and may result in an inflammatory response (Mazzeo et al. 2013). Previous research  
8 has found that in persons with acute mountain sickness, levels of interleukin-6 (IL-6) and C-reactive  
9 protein (CRP) were increased after 3 nights at elevations higher than 3400 m (Hartmann et al. 2000)  
10 and IL-6 was elevated after 12 nights at 4,300 m (Mazzeo et al. 2013). Conversely, Schobersberger et  
11 al. (2004) found in clinical trials of individuals with metabolic syndrome, there were no differences in  
12 IL-6 or tumor necrosis factor- $\alpha$  (TNF $\alpha$ ) after a 3-week sojourn (living and hiking) at moderate altitude  
13 (1,700 – 2,500 m). Consequently, if additional hypoxic exposures are to be considered post-altitude  
14 training then the role of pro-inflammatory cytokines and EPO production should be investigated.

15 Altitude training is a common practice undertaken by endurance athletes in pursuit of an  
16 enhancement of subsequent sea level performance (Fudge et al. 2012), however, identifying the  
17 best time to return to sea level prior to a major competition to optimise the haematological gains  
18 remains a question (Chapman et al. 2014). A minimum exposure of two hours is required to elicit an  
19 increase in EPO; however, less is understood about the effect of a two hour exposure of normobaric  
20 hypoxia on EPO and the time course of this response. Furthermore, the response of inflammatory  
21 cytokines to differing levels of hypoxia in healthy individuals is unclear. The aim of this study was to  
22 establish the dose-response relationship of EPO, alongside markers of inflammation IL-6 and TNF $\alpha$ ,  
23 during and following two hour exposures of normobaric hypoxia with the intention of mitigating  
24 neocytolysis. We hypothesized that [EPO] would increase following two hour normobaric hypoxic  
25 exposure, and the increase would be in accordance with the severity of simulated altitude. Secondly,  
26 basal levels of pro-inflammatory cytokines would inhibit the production of [EPO] in participants.

## 1 **Method**

### 2 **Participants**

3 Eight physically active, Caucasian males participated in the study (see table 1). Participants were well  
4 trained, completing  $8 \pm 3$  hours training per week. Institutional ethical approval was issued in  
5 accordance with the Helsinki declaration 1975 (revised 2013) and participants provided written  
6 informed consent. Participants were non-smokers and had not been exposed to altitudes above  
7 2,000 m in the preceding two months. Participants were instructed not to consume alcohol or  
8 caffeine during a period of at least 24 h immediately preceding each trial and maintained their  
9 normal training regimen during the testing period.

10 TABLE 1

### 11 **Preliminary Testing**

12 Prior to the assessment of aerobic capacity, anthropometric data was collected with body mass  
13 measured using digital scales (Adams Equipment, Model GFK 150; Milton Keynes, UK) and body fat  
14 assessed from four sites (biceps, triceps, subscapular and supra-iliac) as described by Durnin and  
15 Womersley (1974) using skinfold callipers (Harpندن Instruments, UK). Participants then  
16 performed a standardised stepwise incremental test on a cycle ergometer. Cycling started at 80 W,  
17 increasing by 24 W each minute, as previously described by (Gibson et al. 2015).

### 18 **Experimental design**

19 The outline of this single blind, randomised and controlled study is presented in Figure 2.  
20 Participants attended the laboratory in Eastbourne, UK (10 m altitude) on an individual basis on four  
21 separate occasions; three involved resting in a hypoxic environment at three different levels of  
22 hypoxia and once in normoxia. Participants were required to attend the laboratory for eight and a  
23 half hours for each visit. They were instructed to eat the same breakfast before each visit, were  
24 supplied with a standardised isocaloric lunch and drank water ad libitum throughout the testing  
25 procedure. The order of the trials was randomised, determined by a Latin squares design. Each trial  
26 was separated by a seven day wash out period (MacKenzie et al. 2008). All trials commenced at the  
27 same time for each individual participant between 07:30 and 09:30, to control for diurnal variations  
28 in EPO (Klausen et al. 1993; Klausen et al. 1996).

29 FIGURE 2

### 30 **Hypoxic Exposures**

1 Following a 15 min resting period at sea level, during which baseline measures were recorded,  
2 participants spent two hours resting in a normobaric hypoxic chamber achieved using a purposely  
3 built nitrogen-enriched chamber (Altitude Centre; London, UK), at four different simulated altitudes  
4 [ $\text{FiO}_2$ : 0.209, (SL),  $\text{FiO}_2$ : 0.135, (3,600 m),  $\text{FiO}_2$ : 0.125, (4,200 m) and  $\text{FiO}_2$ : 0.115, (4,800 m)]. Oxygen  
5 concentration was monitored and adjusted continually by automated computer feedback.  
6 Participants remained seated during the two hours exposure. The laboratory environmental  
7 conditions [temperature =  $22.8 \pm 0.7^\circ\text{C}$ , relative humidity =  $38.4 \pm 1.8\%$ , pressure =  $760 \pm 2\text{mmHg}$ ,  
8  $\text{FiCO}_2$  (range) = 0.05 – 0.1%] were maintained stable throughout. For the remaining six hours,  
9 participants rested in normoxic conditions in temperate laboratory conditions.

10  $\text{FiO}_2$  was measured in the chamber every 15 minutes.  $\text{SpO}_2$  was measured every 15 minutes, should  
11 a participant remain below 70% they were removed from the chamber (zero incidences). The Lake  
12 Louise Questionnaire was also completed every 30 mins to assess for symptoms of acute mountain  
13 sickness (AMS), should a participant remain at 6 or above. They were removed from the chamber  
14 (zero incidences).

#### 15 **Preliminary Measures**

16 Upon arrival participants weighed themselves (SECA 778; SECA UK, Birmingham, UK) and provided a  
17 urine sample. Urine specific gravity (Uspg) was assessed using a refractometer (Atago, USA) and  
18 urine osmolality (Uosm) was measured using an osmometer (Osmocheck; Vitech Scientific, West  
19 Sussex, UK). Euhydration was achieved when urine osmolality and urine specific gravity were below  
20  $700 \text{mOsmol.kg}^{-1} \text{H}_2\text{O}$  and 1.020, respectively (Sawka et al. 2007). Participants were required to  
21 consume 500 ml of water and wait 30 min before they entered the chamber if they were above the  
22 criteria (MacKenzie et al. 2008). Participants then sat and rested for 15 minutes for a resting heart  
23 rate (HR) and  $\text{O}_2$  saturation ( $\text{SpO}_2$ ) to be measured prior to entering the chamber.

#### 24 **Physiological measures**

25 Heart rate (Polar 810i heart rate monitor; Kempele, Finland) and  $\text{SpO}_2$  (Nonin 2500; Nonin Medical  
26 Inc, Minnesota, USA) were measured every 15 min whilst in the hypoxic chamber and every 30 min  
27 outside of the chamber. The measurement was recorded after 30 seconds.

#### 28 **Haematological measures**

29 For venous blood sampling a cannula (18G x 1.5" BD Venflon I.V. Cannula; BD Infusion Therapy AB,  
30 Helsingborg, Sweden) was positioned in the antecubital fossa. Blood samples were taken before  
31 entering the chamber, after one and two hours whilst in the chamber and at four, six and eight

1 hours outside the chamber. After discarding the first 1 ml, venous blood was collected (~8 ml) with a  
2 plastic syringe (10 ml BD Plastipak; Becton & Dickinson UK, Oxford, UK) and dispensed into two 5ml  
3 K-EDTA collection tubes (Sarstedt Ltd., Leicester, UK) prior to centrifugation at 2,200 rpm (Eppendorf  
4 Refrigerated Centrifuge Model 5702R; Eppendorf UK Ltd., Stevenage, UK) for 15 min to separate  
5 plasma. Plasma was pipetted (Eppendorf Research/Research Pro) into 1.5 ml microtubes (Western  
6 Laboratory Service, Hampshire, UK) and stored at a -85°C (Sanyo VIP Series; Sanyo Electric  
7 Biomedical Co, Ltd., Japan) until the samples were analysed.

8 [EPO], IL-6 and TNF $\alpha$  concentrations were measured in plasma for all four trials (SL, 3,600 m, 4,200  
9 m, and 4,800 m). Enzyme-linked immunosorbent assays were used in accordance with manufacturer  
10 instructions to determine concentrations for [EPO] (Roche Diagnostics Ltd., Lewes, UK) and for IL-6  
11 and TNF $\alpha$  (DuoSet ELISA Development System; R&D Systems Inc., Abingdon, UK). The technical error  
12 of measurement (TEM) between duplicate samples for [EPO] was 3.8%, with a unit error value of 0.7  
13 mU·mL<sup>-1</sup>, for IL-6 it was 7.1%, with a unit error value of 2.76 pg·mL<sup>-1</sup> and for TNF $\alpha$  it was 4.1%, with a  
14 unit error value of 518.7 pg·mL<sup>-1</sup>.

## 15 **Statistical Analysis**

16 Data was assessed for normality and sphericity and adjusted where necessary using the Huynh-Feldt  
17 method. Differences in [EPO], IL-6 and TNF $\alpha$  at each time point (e.g. Pre, 1h, 2h, 4h,6h and 8h.), HR  
18 and SpO<sub>2</sub> were analysed with a two-way repeated measures ANOVA (hypoxia x time), with  
19 Bonferroni correction used to determine differences between groups. For statistical analysis SpO<sub>2</sub>  
20 and HR measurements were averaged for each hour in the chamber and every two hours outside of  
21 the chamber. Typical error of measurement (TEM) calculations were carried out on duplicate [EPO],  
22 IL-6 and TNF $\alpha$  samples using a method previously described by (Hopkins 2000). All statistical tests  
23 were completed using SPSS Statistics 22 (International Business Machines Corp., Armonk, New York).  
24 Significance was accepted at  $P < 0.05$ . Values are reported as mean  $\pm$  SD unless otherwise indicated.  
25 Effect sizes for main effects and interactions are presented as partial eta squared ( $\eta_p^2$ ) in accordance  
26 with Lakens (2013).

## 1 Results

### 2 Physiological Measures

3 There was an effect on SpO<sub>2</sub> over time ( $F = 927.298$ ,  $P = 0.001$ ,  $\eta_p^2 = 0.99$ ), in hypoxia ( $F = 258.717$ ,  $P = 0.001$ ,  $\eta_p^2 = 0.97$ ) and an interaction effect between time\*hypoxia ( $F = 156.411$ ,  $P = 0.001$ ,  $\eta_p^2 = 0.96$ ). Bonferroni comparison identified significant differences between Pre and 1h/2h, respectively, at 3,600 m ( $P = 0.001$ ), 4,200 m ( $P = 0.001$ ) and 4,800 m ( $P = 0.001$ ). Between trials significant differences in SpO<sub>2</sub> were found at 1h ( $P = 0.001$ ) and 2h ( $P = 0.001$ ) between SL ( $98 \pm 0$  and  $98 \pm 1\%$ ), 3,600 m ( $87 \pm 2$  and  $87 \pm 3\%$ ), 4,200 m ( $83 \pm 1$  and  $83 \pm 1\%$ ) and 4,800 m ( $76 \pm 2$  and  $75 \pm 3\%$ ). No differences were found at any point at SL ( $P = 1.000$ ) and no differences were found between Pre and 4h, 6h or 8h at 3,600 m, 4,200 m or 4,800 m. Post-hoc data is presented in figure 3A.

11 There was an effect on HR over time ( $F = 21.294$ ,  $P = 0.001$ ,  $\eta_p^2 = 0.75$ ), in hypoxia ( $F = 11.739$ ,  $P = 0.001$ ,  $\eta_p^2 = 0.63$ ) and an interaction effect between time\*hypoxia ( $F = 9.837$ ,  $P = 0.001$ ,  $\eta_p^2 = 0.59$ ). Bonferroni comparison identified significant differences ( $P = 0.022$ ) between mean HR at SL ( $57 \pm 7$  b·min<sup>-1</sup>) and 4,800 ( $65 \pm 10$  b·min<sup>-1</sup>). At SL no differences ( $P > 0.05$ ) were observed over time. At 4,200 m and 4,800 m HR at was significantly higher ( $P < 0.05$ ) at 1h ( $66 \pm 5$  and  $75 \pm 8$  b·min<sup>-1</sup>) and 2h ( $63 \pm 6$  and  $72 \pm 10$  b·min<sup>-1</sup>), respectively, compared to 4h ( $58 \pm 4$  and  $61 \pm 9$  b·min<sup>-1</sup>), 6h ( $58 \pm 6$  and  $60 \pm 7$  b·min<sup>-1</sup>) and 8h during ( $55 \pm 8$  and  $58 \pm 9$  b·min<sup>-1</sup>). Post-hoc data is presented in figure 3B.

18 FIGURE 3A/B

### 19 Haematological Measures

20 An effect on [EPO] was observed over time ( $F = 9.959$ ,  $P = 0.001$ ,  $\eta_p^2 = 0.59$ ). Mean [EPO] peaked at 21 4h in 3,600 m, in 4,200 m and in 4,800 m. No differences were observed in [EPO] from pre-hypoxia 22 ( $5.36 \pm 1.61$  mU·mL<sup>-1</sup>) to 8h ( $6.4 \pm 1.45$  mU·mL<sup>-1</sup>) during the SL trial. Bonferroni comparison 23 identified a difference in [EPO] between Pre and 4h at 4,200 m ( $P = 0.009$ ) and at 4,800m ( $P = 0.037$ ), 24 but not at 3,600 m ( $P = 1.000$ ). There was no main effect for hypoxia ( $F = 0.359$ ,  $P = 0.704$ ,  $\eta_p^2 = 0.05$ ), nor an interaction effect between time\*hypoxia ( $F = 1.296$ ,  $P = 0.250$ ,  $\eta_p^2 = 0.16$ ). Figure 4 25 illustrates the response of plasma [EPO] during each hypoxic trial. 26

27 FIGURE 4

28 No effect on IL-6 was found over time ( $F = 0.683$   $P = 0.547$ ,  $\eta_p^2 = 0.09$ ), simulated hypoxia ( $F = 0.242$ , 29  $P = 0.789$ ,  $\eta_p^2 = 0.03$ ), or an interaction effect between time\*hypoxia ( $F = 0.465$ ,  $P = 0.907$ ,  $\eta_p^2 = 0.06$ ). Figure 5 illustrates the response of plasma IL-6 during each hypoxic trial. 30

1 FIGURE 5

2 There was also no effect on TNF $\alpha$  found over time ( $F = 1.748, P = 0.182, \eta_p^2 = 0.20$ ), hypoxia ( $F =$   
3  $0.945, P = 0.412, \eta_p^2 = 0.12$ ), or an interaction effect between time\*hypoxia ( $F = 1.545, P = 0.142, \eta_p^2$   
4  $= 0.18$ ). Figure 6 illustrates the response of plasma TNF $\alpha$  during each hypoxic trial.

5 FIGURE 6

6

7 TABLE 2

8 **Relationship between peak [EPO] and other measures**

9 No correlation ( $P > 0.05$ ) was found between desaturation during hypoxic exposure and peak  $\Delta$ [EPO]  
10 ( $r = -0.106$ ) across all three hypoxic trails (see Fig 7A). Further to this no correlation ( $P > 0.05$ ) was  
11 found between peak  $\Delta$ [EPO] and baseline IL-6 ( $r = 0.140$ ) (see Fig 7B), and also between peak  $\Delta$ [EPO]  
12 and baseline TNF $\alpha$  ( $r = 0.159$ ) (see Fig 7C).

13 FIGURE 7 A/B/C



## 1 Discussion

2 The novel findings of this study were that a normobaric hypoxic exposure of two hours at an  $\text{FiO}_2$  of  
3  $<0.125$  ( $>4,200$  m) are sufficient to increase EPO production, which peaked 2h post-exposure and are  
4 maintained up to 4h post-exposure. Despite observing a greater increase in [EPO] as a result of  
5 increased severity of hypoxia, large individual variability (see Table 2) between participants resulted  
6 in no main effect from hypoxia itself. All [EPO] returned to baseline levels 6h post-exposure. The  
7 present study also found that there was no relationship between baseline IL-6 and  $\text{TNF}\alpha$  and peak  
8  $\Delta[\text{EPO}]$ . There were also no differences in IL-6 and  $\text{TNF}\alpha$  production as a result of three different  
9 simulated altitudes.

### 10 EPO response to normobaric hypoxia

11 Figure 1 illustrates the results of the previous investigation into acute hypoxic exposures and EPO.  
12 The present investigation found that two hours of normobaric hypoxia at  $\text{FiO}_2$ :  $\sim 0.135$  (3,600 m),  
13  $\text{FiO}_2$ :  $\sim 0.125$  (4,200 m) and  $\text{FiO}_2$ :  $\sim 0.115$  (4,800 m) caused an increase in [EPO] of 22% (range: -  
14 16—53%), 43% (range: 14—100%) and 52% (range: 16—113%), respectively, peaking two hours  
15 post-exposure, maintaining until four hours and returning to baseline after six hours. Knaupp et al.  
16 (1992) revealed that two hours of normobaric hypoxia at  $\sim 5,500$  m elicited a  $\sim 50\%$  increase in [EPO]  
17 and Ge et al. (2002) also found an increase of  $\sim 50\%$  in [EPO] after 24 hours of hypobaric hypoxia at  
18  $\sim 2,500$ - $2,800$  m. For the application of a hypoxic exposure on return to sea level post-altitude  
19 training camp, normobaric hypoxia is the most accesible option, at a moderate to high-altitude  
20 ( $<4,000$  m), and at a short enough duration that it would fit into an athletes daily training schedule  
21 ( $<2$  h).

### 22 FIGURE 8

23 Chronically increased EPO synthesis leads to a progressive increase in tHbmass (Lundby et al. 2007),  
24 however, hypoxia-induced changes in EPO release seem to be subject to a marked inter-individual  
25 variability (Chapman et al. 1998). This may explain why there is a varied athlete response in tHbmass  
26 to altitude training camps (McClean et al. 2013). In our study despite a greater reduction in  $\text{SpO}_2$  from  
27 an increased severity of hypoxia causing a greater production in [EPO], individuals who were more  
28  $\text{O}_2$  desaturated did not always produce a greater [EPO] (see Fig 7). As such, the finding are in  
29 agreement with previous literature (Ge et al. 2002; Friedmann et al. 2005; MacKenzie et al. 2008)  
30 who also found a marked individual variability in EPO release at different altitudes. Although the  
31 participants in the present study were well-trained, similar value and variations of [EPO] have been  
32 reported in elite athletes (Clark et al. 2009; Garvican et al. 2012; Pottgiesser et al. 2012).

1 The exact mechanisms for individual variability in EPO response to altitude are not well determined  
2 (Fudge et al. 2012). Chapman et al. (2010) found that there was no correlation between changes in  
3 EPO at altitude and hypoxic ventilatory response measured at sea level; suggesting that peripheral  
4 chemoresponsiveness may not be responsible for the variability in EPO response, and the likely  
5 mechanisms may be downstream from the lung. Ge et al. (2002), however, believed EPO production  
6 at altitude is governed by “upstream” factors related to renal parenchymal  $PO_2$ , as well as other  
7 undetermined mechanisms, possibly related to transcriptional regulation of EPO by renal tissue  
8 hypoxia. Alternatively, pro-inflammatory cytokines have been shown to trigger the suppression of  
9 renal EPO production and therefore erythropoiesis (Morceau et al. 2009), with the inhibition of EPO  
10 production shown in vitro and in vivo to potentially involve IL-1, IL-6, and  $TNF\alpha$  (Morceau et al.  
11 2009). However, the present study found that baseline IL-6 and  $TNF\alpha$  did not correlate with the peak  
12  $\Delta[EPO]$  and there were no differences in IL-6 and  $TNF\alpha$  production as a result of three different  
13 levels of hypoxia.

14 The present investigation found no relationship between  $\Delta SpO_2$  and peak  $\Delta[EPO]$  percentage.  
15 MacKenzie et al. (2008) suggested EPO production is noticeably augmented by the depression of  
16 arterial  $O_2$  content ( $CaO_2$ ), as a result of decreases in  $SpO_2$ . Therefore, a greater reduction in  $SpO_2$   
17 combined with an inability to increase HVR could facilitate a greater secretion of EPO (Jelkmann  
18 1992). Ge et al. (2002) also suggested that the mechanism of an individual response to altitude is  
19 likely to include the greater oxyhemoglobin desaturation. This occurs as the  $PO_2$  falls to the steep  
20 portion of the oxyhemoglobin dissociation curve and, therefore changes in  $SpO_2$  are mirrored by EPO  
21 levels at all altitudes. The investigations by Ge et al. (2002) and MacKenzie et al. (2008) only found a  
22 moderate relationship between  $\Delta CaO_2/\Delta SpO_2$  and changes in EPO.

### 23 **Inflammatory response to normobaric hypoxia**

24 Hypoxia and inflammation are interrelated at molecular, cellular, and clinical levels (Eltzschig and  
25 Carmeliet 2011). Oxidative stress and the release of pro-inflammatory cytokines (e.g., IL-1, IL-6,  
26  $TNF\alpha$ ), which are systemic inflammatory markers, are associated with acute hypoxia closely and are  
27 proportional to the severity of hypoxia (He et al. 2014). Previously, Klausen et al. (1997) found that  
28 after 1 day at 4,350 m, there was a non-significant change in serum IL-6 by 56% and by day 4 had  
29 significantly increased by 86%, however, there were no changes in IL-1 or  $TNF\alpha$ . The increase in IL-6  
30 was significantly correlated ( $r = -0.45$ ) with hypoxemia (mean  $SpO_2$ : 79-83%), but not HR or  
31 symptoms of AMS. The authors suggested that the increase of serum IL-6 was not secondary to  
32 increased sympathetic nervous activity or general distress during altitude acclimatisation due to the  
33 lack of relation between serum IL-6 and heart rates or AMS scores.

1 The present investigation found that baseline levels of IL-6 and TNF $\alpha$  did not inhibit the production  
2 of EPO (See Fig. 7B/C). Furthermore, there were no difference in IL-6 and TNF $\alpha$  at three levels of  
3 normobaric hypoxia, despite increases in HR and decreases in SpO $_2$ . Jelkmann et al. (1992) found  
4 that the addition of IL-1 and TNF $\alpha$  inhibited the production of EPO in hypoxic human hepatoma cell  
5 cultures, however, inhibition did not occur with introduction of IL-6, thus believing that IL-1 and  
6 TNF $\alpha$  have been shown to affect gene expression in human hepatoma cultures at the transcriptional  
7 level (Jelkmann et al. 1994). The authors reported that IL-6 does not affect EPO production *in vitro*;  
8 moreover, IL-6 appears to inhibit renal EPO formation. Inflammatory responses to hypoxia are  
9 complex with various cytokines both inhibiting and preserving the production of EPO. At present it is  
10 not clear what pro-inflammatory cytokines regulate the production of EPO, but in this investigation  
11 inflammation did compromise endogenous [EPO].

## 12 **Future Directions**

13 Additional normobaric hypoxic exposures of two hours, with the aim of increasing the production of  
14 EPO, have not been implemented on return to sea-level after an altitude training camp. Rodríguez et  
15 al. (2000) and Casas et al. (2000) exposed trained volunteers to hypobaric hypoxia at simulated  
16 altitudes of ~4,000-5,000 m for 90 minutes, three times a week for three weeks. This stimulus led to  
17 an effective stimulation of erythropoietic adaptations, such as, significant increases in red blood cell  
18 (RBC) count, [Hb] and reticulocytes. Katayama et al. (2003), however, utilised a similar protocol  
19 (4,500 m for two hours, three times a week for three weeks) with endurance runners and found no  
20 changes in haematological parameters, including [Hb], Hct, RBC count, reticulocytes or EPO. The  
21 large individual variation and differing populations used could account for these contradictory  
22 findings; nevertheless, the protocol should be tested post-altitude training camp alongside  
23 measurement of EPO and tHbmass.

24 Further to this, tighter controls surrounding exhaustive exercise pre-hypoxic exposure should be  
25 considered, as different types of exercise (concentric, eccentric, submaximal, maximal) are known to  
26 cause increases in pro-inflammatory cytokines (Pedersen et al. 1998; Nieman et al. 2001; Jürimäe et  
27 al. 2011) and intense exercise provides a physiological stimulus to increase EPO production (Roberts  
28 and Smith 1999). Additional modifications to the protocol could include blood samples 12, 14 and 48  
29 hours post-hypoxic exposure to determine if there is a delayed increased in EPO or pro-inflammatory  
30 cytokines as has previously been suggested (Pedersen et al. 1998; Ge et al. 2002). The present  
31 investigation did control for diurnal variations in haematological markers by ensuring that all trials  
32 were started between 07:30 and 09:30 as previous research has shown that EPO is subject to distinct  
33 diurnal variation in trained and untrained individuals (Klausen et al. 1993), as well as in both

1 normoxia and hypoxia (Klausen et al. 1996). Keramidas et al. (2011) observed diurnal variation of a  
2 nadir in values of EPO in the morning hours, and zenith levels during the evening and night hours.  
3 Additional haematological measurements prior to the experimental period would provide a more  
4 accurate baseline interpretation.

## 5 **Practical Applications**

6 A decrease in EPO and tHbmass on return to sea level has been observed in athletes after altitude  
7 training camps lasting 3-4 weeks (Heinicke et al. 2005; Clark et al. 2009; Garvican et al. 2012;  
8 Pottgiesser et al. 2012). When red cell mass exceeds the physiological requirement at the altitude  
9 resided in, EPO secretion is suppressed (Rice and Alfrey 2005) and a destruction in red cells, or  
10 neocytolysis, occurs. Athletes who are acclimated after an altitude training camp, who then descend  
11 to sea level to compete would have red cell mass that is higher than necessary for homeostasis their  
12 new environment. The rapid destruction of reticulocytes and the decline in production of new ones  
13 may depend on a drop in EPO levels (Alfrey et al. 1997). Additionally, EPO not only regulates red cell  
14 mass but also prolongs its survival (Rice et al. 2001). For an athlete with a faster than normal decline  
15 in tHbmass upon return to sea level competing as soon as possible may be the most beneficial  
16 strategy but this is not always logistically achievable. Additional hypoxic exposures have been  
17 suggested as strategy to prevent the sudden drop off in EPO (Pottgiesser et al. 2012).

18 An athlete's busy travel schedule, external commitments and competition programme, make it  
19 difficult to time competing at sea level after an altitude training camp. Chapman et al. (2013b),  
20 suggested that if an athlete completes a 4 week altitude training camp, followed by a short time (~7-  
21 14 days) at sea level to compete, returning to altitude even for a short time may mitigate or delay  
22 the effects of neocytolysis by re-establishing EPO levels, although this has not been proven. This is  
23 not for added erythropoiesis, as is typically done with altitude residence, but more to delay the  
24 selective destruction of reticulocytes due to lower than baseline EPO concentrations, as a result of  
25 the athlete just remaining at sea level. By keeping EPO elevated, in addition to preventing  
26 neocytolysis, exercise performance might be improved (Schuler et al. 2012; Durussel et al. 2013).

## 27 **Perspectives**

28 In Caucasian populations erythropoiesis is a key acclimatization response that increases the O<sub>2</sub>-  
29 carrying capacity of the blood, i.e. tHbmass, as a result of chronic exposure to altitude (Chapman et  
30 al. 2013). A change in tHbmass by 1 g causes a change in  $\dot{V}O_{2max}$  by approximately 4 ml·min<sup>-1</sup>  
31 (Schmidt and Prommer 2010), for an athlete increased blood gas storage capacity is very important,  
32 therefore, the maintenance of tHbmass should be considered. Chapman et al. (2014) stated that

1 brief, short-term periods of normobaric hypoxia may provide a sufficient stimulus to significantly  
2 increase EPO, despite this not being enough to accelerate erythropoiesis itself. These exposures  
3 could take place during the day, around the athlete training schedule, to preserve the hematologic  
4 acclimatization response for a longer time, thereby expanding the window for optimal competition.  
5 The increases in [EPO] found in the current investigation indicate that a normobaric hypoxic 'dose'  
6 (i.e.,  $FiO_2 \sim 0.125-0.115$ , equivalent to 4,200 m and above, for two hours) may prevent the sudden  
7 drop in EPO that has been shown post-altitude and, therefore, maintain any enhancements in  
8 tHbmass. The release of EPO is subject to a distinct inter-individual variation that can only be  
9 partially explained by reductions in oxyhaemoglobin saturation but is not effected by systemic  
10 markers of inflammation.

1 **Acknowledgements**

2 The authors would like to thank the voluntary participants for the considerable time given to this  
3 study. Funding and support was obtained from University of Brighton and the English Institute of  
4 Sport.

5

## 1 **References**

- 2   Alfrey CP, Rice L, Udden MM, Driscoll TB (1997) Neocytolysis: physiological down-regulator of red-  
3       cell mass. *Lancet* 349:1389–90.
- 4   Brugniaux J V, Schmitt L, Robach P, et al (2006) Eighteen days of “living high , training low” stimulate  
5       erythropoiesis and enhance aerobic performance in elite middle-distance runners. *J Appl*  
6       *Physiol* 100:203–211. doi: 10.1152/jappphysiol.00808.2005
- 7   Casas H, Casas M, Ricart A, et al (2000) Effectiveness Of Three Short Intermittent Hypobaric Hypoxia  
8       Protocols: Hematological Responses. *J Exerc Physiol* 3:38–45.
- 9   Chapman RF (2013) The individual response to training and competition at altitude. *Br J Sports Med*  
10       47 Suppl 1:i40–44. doi: 10.1136/bjsports-2013-092837
- 11   Chapman RF, Laymon AS, Levine BD (2013) Timing of Arrival and Pre-acclimatization Strategies for  
12       the Endurance Athlete Competing at Moderate to High Altitudes. *High Alt Med Biol* 14:319–24.  
13       doi: 10.1089/ham.2013.1022
- 14   Chapman RF, Laymon Stickford AS, Lundby C, Levine BD (2014) Timing of return from altitude  
15       training for optimal sea level performance. *J Appl Physiol* 116:837–43.
- 16   Chapman RF, Stray-Gundersen J, Levine BD (1998) Individual variation in response to altitude  
17       training endurance athletes. *J Appl Physiol* 85:1448–1456.
- 18   Chapman RF, Stray-Gundersen J, Levine BD (2010) Epo production at altitude in elite endurance  
19       athletes is not associated with the sea level hypoxic ventilatory response. *J Sci Med Sport*  
20       13:624–629.
- 21   Clark SA, Quod MJ, Clark MA, et al (2009) Time course of haemoglobin mass during 21 days live  
22       high:train low simulated altitude. *Eur J Appl Physiol* 106:399–406.
- 23   Dick FW (1992) Training at altitude in practice. *Int J Sport Med* 13:S203–6.
- 24   Durnin J, Womersley J (1974) Body fat assessed from total body density and its estimation from  
25       skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *Br J Nutr*  
26       32:77–97.
- 27   Durussel J, Daskalaki E, Anderson M, et al (2013) Haemoglobin mass and running time trial  
28       performance after recombinant human erythropoietin administration in trained men. *PLoS One*  
29       8:e56151.
- 30   Eckardt KU, Boutellier U, Kurtz A, et al (1989) Rate of erythropoietin formation in humans in  
31       response to acute hypobaric hypoxia. *J Appl Physiol* 66:1785–1788.
- 32   Eltzschig HK, Carmeliet P (2011) Mechanisms of Disease: Hypoxia and Inflammation. *New Engl J Med*  
33       364:656–665.
- 34   Faquin BWC, Schneider TJ, Goldberg MA (1992) Effect of Inflammatory Cytokines on Hypoxia-  
35       Induced Erythropoietin Production. *Blood* 79:1987–1994.

- 1 Friedmann B, Frese F, Menold E, et al (2005) Individual variation in the erythropoietic response to  
2 altitude training in elite junior swimmers. *Br J Sports Med* 39:148–154. doi:  
3 10.1136/bjism.2003.011387
- 4 Fudge BW, Pringle JSM, Maxwell NS, et al (2012) Altitude training for elite endurance performance: a  
5 2012 update. *Curr Sport Med Rep* 11:148–54.
- 6 Garvican LA, Martin DT, Quod MJ, et al (2012) Time course of the hemoglobin mass response to  
7 natural altitude training in elite endurance cyclists. *Scand J Med Sci Sport* 22:95–103.
- 8 Ge R, Witkowski S, Zhang Y, et al (2002) Determinants of erythropoietin release in response to short-  
9 term hypobaric hypoxia. *J Appl Physiol* 92:2361–2367.
- 10 Gibson OR, Turner G, Tuttle JA, et al (2015) Heat Acclimation attenuates physiological strain and the  
11 Hsp72, but not Hsp90 $\alpha$  mRNA response to acute normobaric hypoxia. *J Appl Physiol*  
12 jap.00332.2015. doi: 10.1152/jappphysiol.00332.2015
- 13 Hartmann G, Tschöp M, Fischer R, et al (2000) High altitude increases circulating interleukin-6,  
14 interleukin-1 receptor antagonist and C-reactive protein. *Cytokine* 12:246–52.
- 15 He Q, Yang Q-C, Zhou Q, et al (2014) Effects of Varying Degrees of Intermittent Hypoxia on  
16 Proinflammatory Cytokines and Adipokines in Rats and 3T3-L1 Adipocytes. *PLoS One* 9:e86326.
- 17 Heinicke K, Heinicke I, Schmidt W, Wolfarth B (2005) A three-week traditional altitude training  
18 increases hemoglobin mass and red cell volume in elite biathlon athletes. *Int J Sport Med*  
19 26:350–355.
- 20 Hopkins WG (2000) Measures of reliability in sports medicine and science. *Sport Med* 30:1–15.
- 21 Jelkmann W (1992) Erythropoietin: structure, control of production, and function. *Physiol Rev*  
22 72:449–489.
- 23 Jelkmann W, Pagel H, Wolff M, Fandrey J (1992) Monokines inhibiting erythropoietin production in  
24 human hepatoma cultures and in isolated perfused rat kidneys. *Life Sci* 50:301–308.
- 25 Jelkmann WE, Fandrey J, Frede S, Pagel H (1994) Inhibition of erythropoietin production by  
26 cytokines. Implications for the anemia involved in inflammatory states. *Ann N Y Acad Sci*  
27 718:300–309; discussion 309–311.
- 28 Jürimäe J, Mäestu J, Jürimäe T, et al (2011) Peripheral signals of energy homeostasis as possible  
29 markers of training stress in athletes: a review. *Metabolism* 60:335–50.
- 30 Katayama K, Matsuo H, Ishida K, et al (2003) Intermittent Hypoxia Improves Endurance Performance  
31 and Submaximal Exercise Efficiency. *High Alt Med Biol* 4:291–304.
- 32 Keramidis ME, Kounalakis SN, Debevec T, et al (2011) Acute normobaric hyperoxia transiently  
33 attenuates plasma erythropoietin concentration in healthy males: evidence against the  
34 “normobaric oxygen paradox” theory. *Acta Physiol* 202:91–8.
- 35 Klausen T, Dela F, Hippe E, Galbo H (1993) Diurnal variations of serum erythropoietin in trained and  
36 untrained subjects. *Eur J Appl Physiol* 67:545–548.



- 1 Klausen T, Olsen NV, Poulsen TD, et al (1997) Hypoxemia increases serum interleukin-6 in humans.  
2 Eur J Appl Physiol Occup Physiol 76:480–482. doi: 10.1007/s004210050278
- 3 Klausen T, Poulsen TD, Fogh-Andersen N, et al (1996) Diurnal variations of serum erythropoietin at  
4 sea level and altitude. Eur J Appl Physiol 72:297–302.
- 5 Knaupp W, Khilnani S, Sherwood J, et al (1992) Erythropoietin response to acute normobaric hypoxia  
6 in humans. J Appl Physiol 73:837–840.
- 7 Lakens D (2013) Calculating and reporting effect sizes to facilitate cumulative science: a practical  
8 primer for t -tests and ANOVAs. Front Psychol 4:1–12. doi: 10.3389/fpsyg.2013.00863
- 9 Lundby C, Thomsen JJ, Boushel R, et al (2007) Erythropoietin treatment elevates haemoglobin  
10 concentration by increasing red cell volume and depressing plasma volume. J Physiol 578:309–  
11 14.
- 12 MacKenzie RW, Watt PW, Maxwell NS (2008) Acute normobaric hypoxia stimulates erythropoietin  
13 release. High Alt Med Biol 9:28–37.
- 14 Mazzeo RS, Donovan D, Fleshner M, et al (2013) Interleukin-6 response to exercise and high-altitude  
15 exposure: influence of  $\alpha$  -adrenergic blockade. J Appl Physiol 91:2143–2149.
- 16 Mclean BD, Buttifant D, Gore CJ, et al (2013) Year-to-year variability in haemoglobin mass response  
17 to two altitude training camps. Br J Sports Med. doi: 10.1136/bjsports-2013-092744
- 18 Morceau F, Dicato M, Diederich M (2009) Pro-inflammatory cytokine-mediated anemia: regarding  
19 molecular mechanisms of erythropoiesis. Mediat Inflamm 2009:405016.
- 20 Nieman DC, Henson DA, Smith LL, et al (2001) Cytokine changes after a marathon race. J Appl Physiol  
21 91:109–114.
- 22 Niess a M, Fehrenbach E, Lorenz I, et al (2004) Antioxidant intervention does not affect the response  
23 of plasma erythropoietin to short-term normobaric hypoxia in humans. J Appl Physiol 96:1231–  
24 1235; discussion 1196. doi: 10.1152/jappphysiol.00803.2003
- 25 Pedersen BK, Ostrowski K, Rohde T, Bruunsgaard H (1998) The cytokine response to strenuous  
26 exercise. Can J Physiol Pharm 76:505–511.
- 27 Pottgiesser T, Garvican LA, Martin DT, et al (2012) Short-term hematological effects upon  
28 completion of a four-week simulated altitude camp. Int J Sport Physiol Perform 7:79–83.
- 29 Prommer N, Thoma S, Quecke L, et al (2009) Oxygen transport in Kenyan runners. Med Sci Sport  
30 Exerc 41:2127.
- 31 Prommer N, Thoma S, Quecke L, et al (2010) Total hemoglobin mass and blood volume of elite  
32 Kenyan runners. Med Sci Sport Exerc 42:791–797. doi: 10.1249/MSS.0b013e3181badd67
- 33 Rice L, Alfrey CP (2005) The Negative Regulation of Red Cell Mass by Neocytolysis: Physiologic and  
34 Pathophysiologic Manifestations. Cell Physiol Biochem 15:245–250.

- 1 Rice L, Ruiz W, Driscoll T, et al (2001) Neocytolysis on descent from altitude: a newly recognized  
2 mechanism for the control of red cell mass. *Ann Intern Med* 134:652– 656.
- 3 Roberts D, Smith DJ (1999) Erythropoietin concentration and arterial haemoglobin saturation with  
4 supramaximal exercise. *J Sport Sci* 17:485–493.
- 5 Rodríguez FA, Ventura JL, Casas M, et al (2000) Erythropoietin acute reaction and haematological  
6 adaptations to short, intermittent hypobaric hypoxia. *Eur J Appl Physiol* 82:170–177.
- 7 Sawka MN, Burke LM, Eichner ER, et al (2007) American College of Sports Medicine position stand.  
8 Exercise and fluid replacement. *Med Sci Sport Exerc* 39:377–90.
- 9 Schmidt W, Prommer N (2010) Impact of alterations in total hemoglobin mass on VO<sub>2</sub>max. *Exerc  
10 Sport Sci Rev* 38:68–75.
- 11 Schobersberger W, Hoffmann G, Fries D, et al (2004) AMAS (Austrian Moderate Altitude Study)-  
12 2000: Effects of Hiking Holidays at Moderate Altitude on Immune System Markers in Persons  
13 with Metabolic Syndrome. *Pteridines* 15:149 – 154.
- 14 Schuler B, Vogel J, Grenacher B, et al (2012) Acute and chronic elevation of erythropoietin in the  
15 brain improves exercise performance in mice without inducing erythropoiesis. *FASEB J* 1–7.
- 16 Shaw AJ, Ingham S a., Folland JP (2014) The valid measurement of running economy in runners. *Med  
17 Sci Sports Exerc* 1:1968–1973. doi: 10.1249/MSS.0000000000000311
- 18 Stray-Gundersen J, Chapman RF, Levine BD (2001) “ Living high-training low ” altitude training  
19 improves sea level performance in male and female elite runners. *J Appl Physiol* 91:1113–1120.
- 20 Wahl P, Schmidt A, Demarees M, et al (2013) Responses of angiogenic growth factors to exercise, to  
21 hypoxia and to exercise under hypoxic conditions. *Int J Sport Med* 34:95–100.
- 22

**1 Tables****2 Table 1: Participant anthropometric characteristics and baseline haematological values**

Characteristics	
Age (years)	27 ± 4
Stature (cm)	179 ± 6
Body Mass (kg)	77.5 ± 9.0
Body Fat (%)	13.1 ± 2.5
VO <sub>2</sub> max (ml·kg·min <sup>-1</sup> )	50.9 ± 8.2
[EPO] (mU·ml <sup>-1</sup> )	5.95 ± 1.51
IL-6 (pg·ml <sup>-1</sup> )	8.86 ± 7.17
TNFα (pg·ml <sup>-1</sup> )	3095.6 ± 3934.9

3

4

1 Table 2: Haematological data measured pre-hypoxia, during hypoxia (1h and 2h) and post-hypoxia (4h, 6h and 8h).

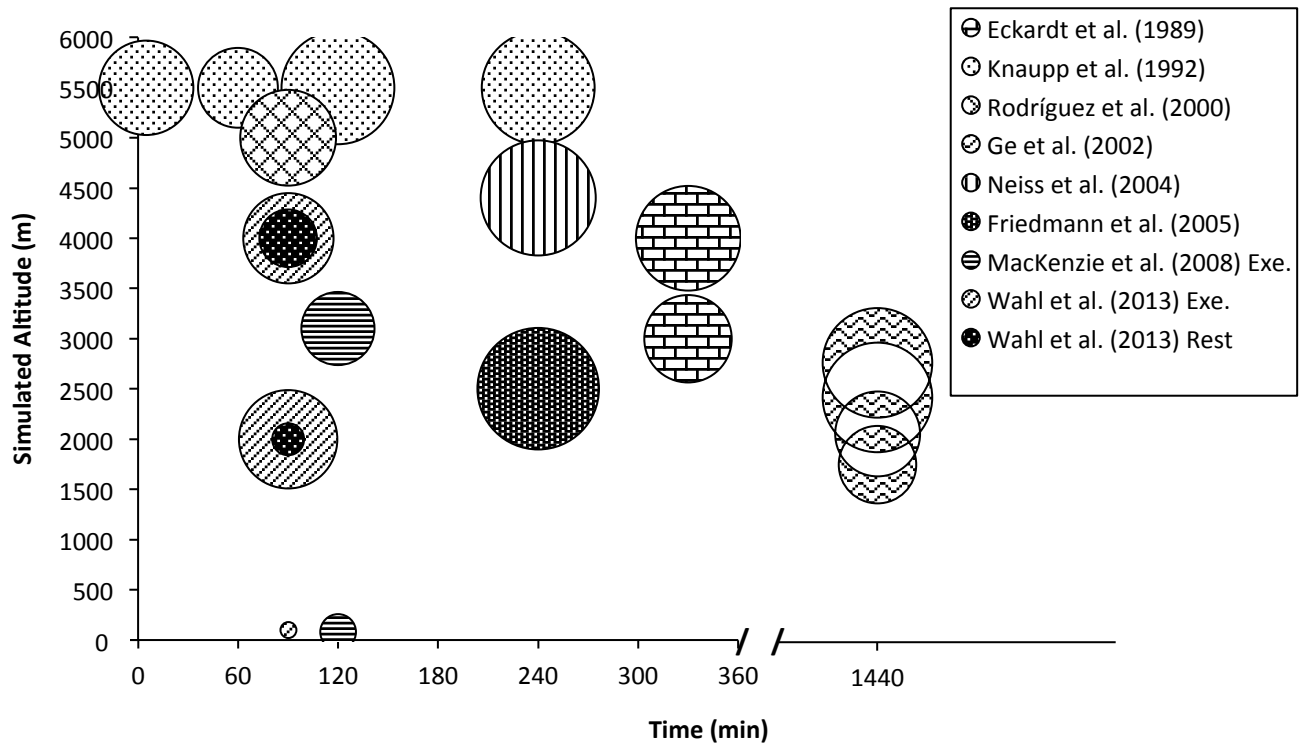
		Pre	1h	2h	4h	6h	8h
[EPO] (mU·ml <sup>-1</sup> )	3,600 m	6.54 ± 3.54	6.48 ± 1.33	6.34 ± 1.17	7.61 ± 1.45	7.52 ± 1.63	7.31 ± 1.96
	4,200 m	5.86 ± 1.48	5.93 ± 1.48	7.29 ± 1.84	8.06 ± 1.47	7.96 ± 3.06	6.81 ± 2.78
	4,800 m	6.04 ± 1.40	6.07 ± 1.57	6.69 ± 1.40	8.94 ± 2.01	8.85 ± 2.75	7.50 ± 3.11
IL-6 (pg·ml <sup>-1</sup> )	3,600 m	8.91 ± 8.12	9.38 ± 7.84	9.52 ± 9.16	9.27 ± 8.86	9.64 ± 7.36	10.44 ± 8.41
	4,200 m	9.82 ± 8.14	9.11 ± 9.07	10.42 ± 11.36	9.20 ± 8.56	8.68 ± 7.55	9.03 ± 7.63
	4,800 m	8.99 ± 7.37	8.50 ± 6.11	9.34 ± 7.89	7.85 ± 5.68	8.70 ± 6.59	9.79 ± 7.57
TNFα (pg·ml <sup>-1</sup> )	3,600 m	2766.3 ± 3625.8	3113.2 ± 4276.7	3739.8 ± 5145.9	3397.7 ± 4539.1	3105.4 ± 4227.8	3189.9 ± 4198.0
	4,200 m	3372.2 ± 4701.6	3211.4 ± 4528.6	3088.1 ± 4379.5	2905.3 ± 3838.5	2992.5 ± 4090.2	3317.3 ± 4443.0
	4,800 m	3132.6 ± 4144.4	3213.8 ± 4110.6	3165.2 ± 3916.5	3399.0 ± 4646.5	3278.6 ± 4650.5	3154.6 ± 4553.2

2

3

1 **Figures**

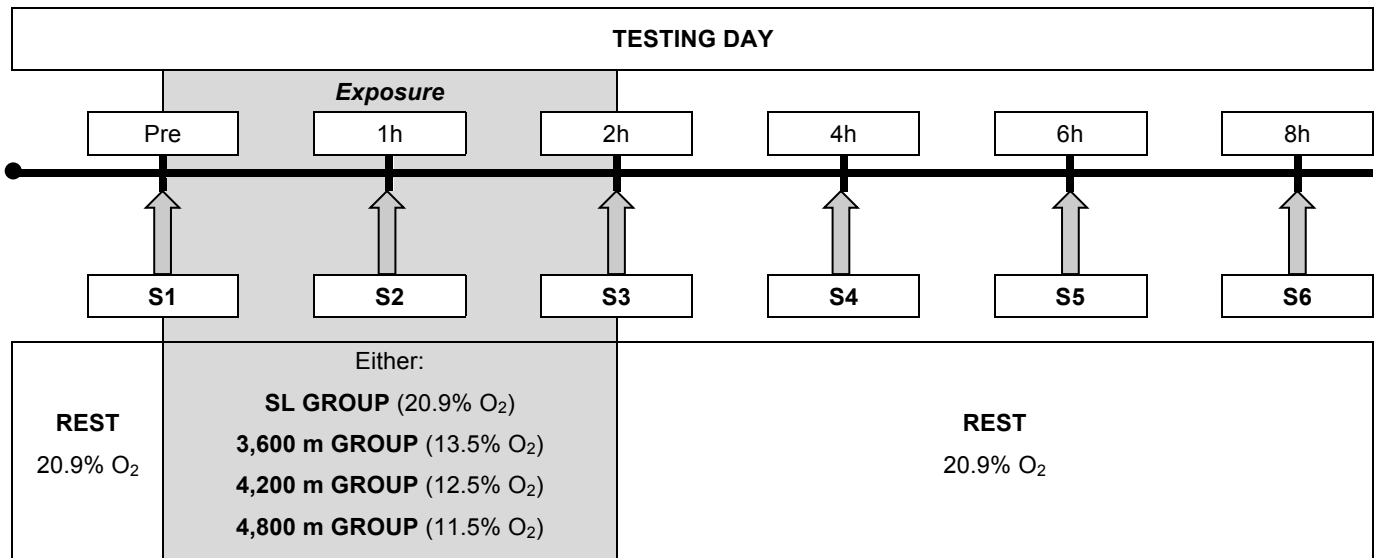
2



3

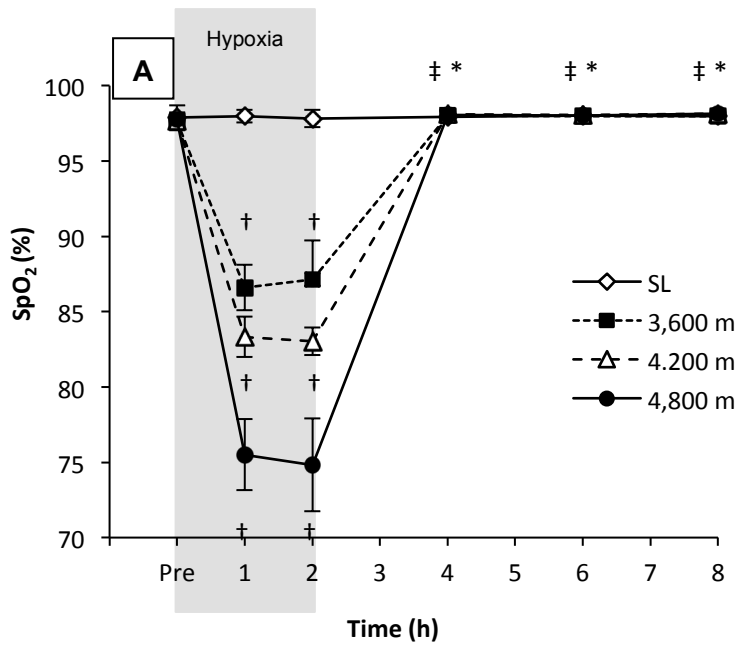
4 Figure 1: EPO response to hypoxic exposure duration and simulated altitude. The different bubbles  
 5 represent the magnitude of increase in EPO as a result of hypoxic exposures. Different patterns  
 6 within bubble indicate different investigations

1

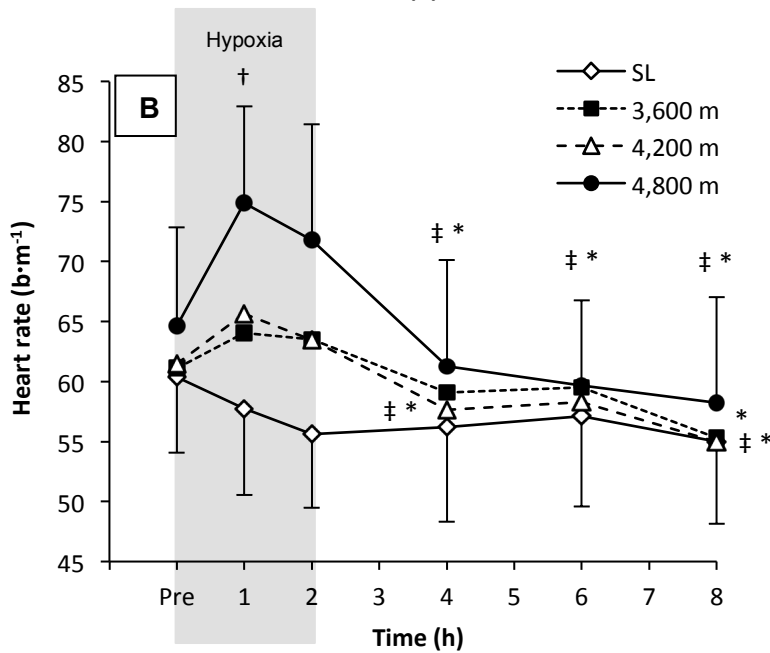


2

3 Figure 2: Schematic representation of the study outline. (S = blood sample)

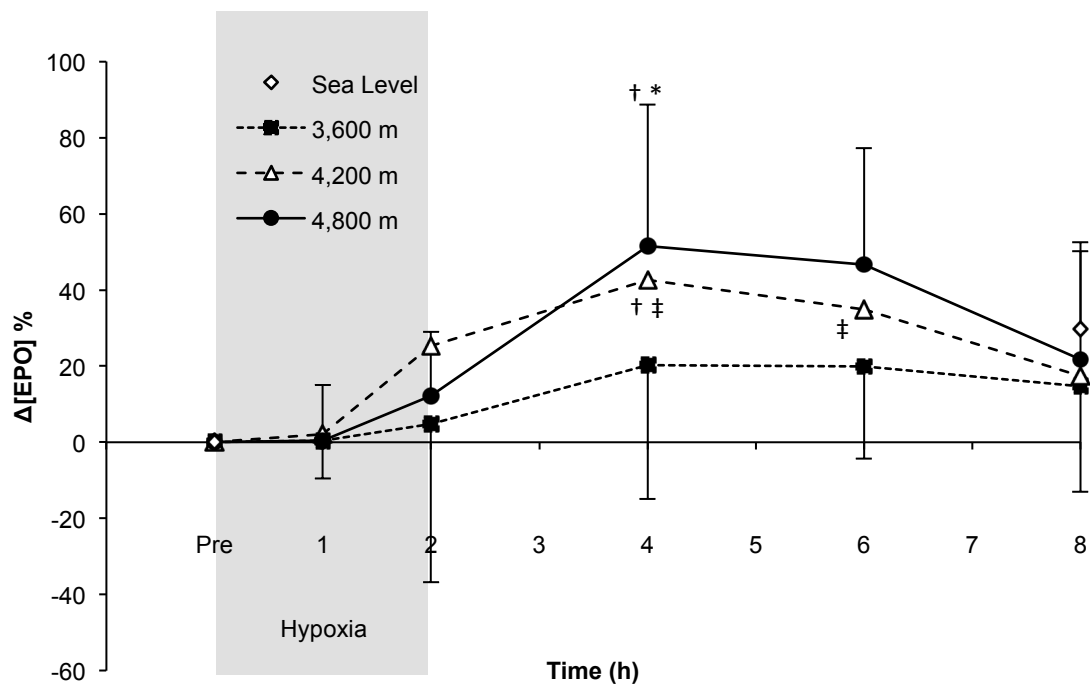


1



2

3 Figure 3: Difference in arterial oxyhaemoglobin saturation (SpO<sub>2</sub>; A) and heart rate (HR; B) after two  
 4 hours simulated hypoxia at SL, 3,600 m, 4,200 m and 4,800 m. Values are means  $\pm$  SD. ( $\dagger P \leq 0.05$   
 5 denotes differences from Pre,  $\ddagger P \leq 0.05$  denotes differences from 1h and  $* P \leq 0.05$  denotes  
 6 differences from 2 h). Note 3,600 m and 4,200 m error bars removed for clarity.

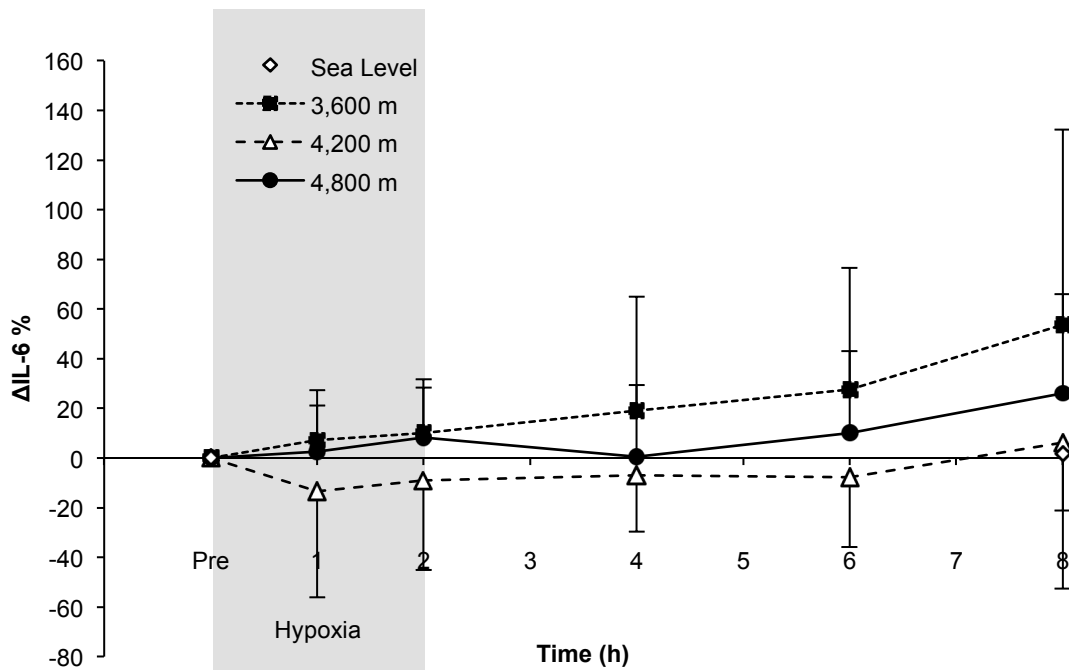


1

2 Figure 4: Percentage differences from baseline in erythropoietin concentration ( $\Delta [EPO]$ ) of blood  
 3 plasma after two hours at simulated altitudes of sea level, 3,600 m, 4,200 m and 4,800 m. Values are  
 4 means  $\pm$  SD. ( $\dagger P \leq 0.05$  denotes differences from Pre,  $\ddagger P \leq 0.05$  denotes differences from 1h and \*  
 5  $P \leq 0.05$  denotes differences from 2 h). Note 4,200 m error bars removed for clarity.



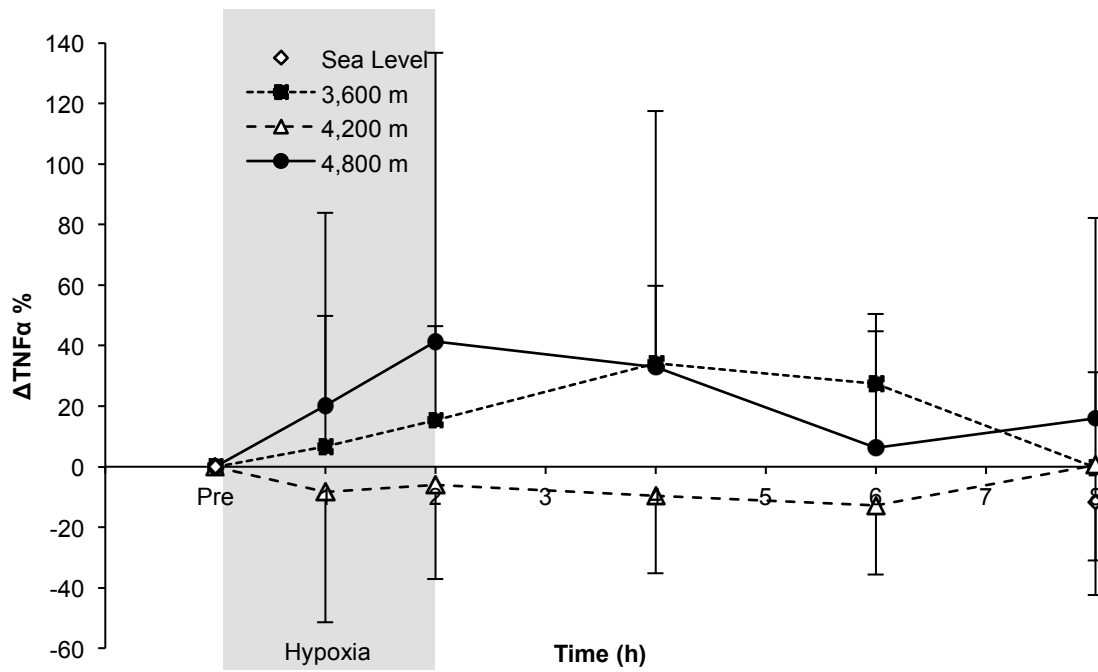
1



2

3 Figure 5: Percentage differences from baseline in interleukin-6 ( $\Delta$  IL-6) of blood plasma after two  
4 hours at simulated altitudes of sea level, 3,600 m, 4,200 m and 4,800 m.

1



2

3 Figure 6: Percentage differences from baseline in tumor necrosis factor alpha ( $\Delta TNF\alpha$ ) of blood  
4 plasma after two hours at simulated altitudes of sea level, 3,600 m, 4,200 m and 4,800 m.

1

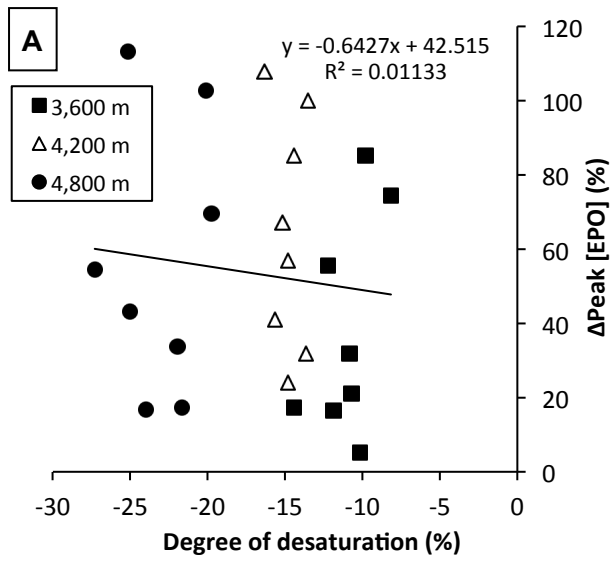


Figure 7: Relationship between the degree of desaturation averaged over the 2 hours of simulated hypoxia (A); baseline IL-6 (B); baseline TNF $\alpha$  (C) and the percentage difference of the peak in [EPO] during each hypoxic condition. As a group, there was no correlation between any of the variables and Peak  $\Delta$  [EPO].

11

