The time-course response of endogenous erythropoietin, IL-6 and TNFα in response to acute hypoxic exposures

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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 addition stress is required. Eight physically active males (age 27 ± 4 yrs, body mass 77.5 ± 9.0 kg, height 179 ± 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: ~0.135 (3,600 m), FiO_2: ~0.125 (4,200 m) and FiO_2: ~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 (TNFα). During 4,200 m and 4,800 m [EPO] increased from 5.9 ± 1.5 to 8.1 ± 1.5 mU·mL^{-1} (P = 0.009) and 6.0 ± 1.4 to 8.9 ± 2.0 mU·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 TNFα 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,

Introduction

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

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

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

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

Stray-Gundersen et al. (2001) believed that when athletes attend altitude training camps it is possible that the presence of injury or infection, and therefore pro-inflammatory cytokines (such as IL-6 and TNFα), could impair the erythropoietic response to altitude (Faquin et al. 1992; Jelkmann et al. 1992). Hypoxia is a stressor that alters homeostasis (subsequent to a reduction in arterial oxygen (O_2) saturation) and may result in an inflammatory response (Mazzeo et al. 2013). Previous research has found that in persons with acute mountain sickness, levels of interleukin-6 (IL-6) and C-reactive protein (CRP) were increased after 3 nights at elevations higher than 3400 m (Hartmann et al. 2000) and IL-6 was elevated after 12 nights at 4,300 m (Mazzeo et al. 2013). Conversely, Schobersberger et al. (2004) found in clinical trials of individuals with metabolic syndrome, there were no differences in IL-6 or tumor necrosis factor-α (TNFα) after a 3-week sojourn (living and hiking) at moderate altitude (1,700 – 2,500 m). Consequently, if additional hypoxic exposures are to be considered post-altitude training then the role of pro-inflammatory cytokines and EPO production should be investigated.

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

Method

Participants

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

TABLE 1

Preliminary Testing

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

Experimental design

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

FIGURE 2

Hypoxic Exposures

Following a 15 min resting period at sea level, during which baseline measures were recorded, participants spent two hours resting in a normobaric hypoxic chamber achieved using a purposed built nitrogen-enriched chamber (Altitude Centre; London, UK), at four different simulated altitudes [FiO_2: 0.209, (SL), FiO_2: 0.135, (3,600 m), FiO_2: 0.125, (4,200 m) and FiO_2: 0.115, (4,800 m)]. Oxygen concentration was monitored and adjusted continually by automated computer feedback. Participants remained seated during the two hours exposure. The laboratory environmental conditions [temperature = 22.8 ± 0.7°C, relative humidity = 38.4 ± 1.8%, pressure = 760 ± 2mmHg, $FiCO_2$ (range) = 0.05 – 0.1%] were maintained stable throughout. For the remaining six hours, participants rested in normoxic conditions in temperate laboratory conditions.

FiO_2 was measured in the chamber every 15 minutes. SpO_2 was measured every 15 minutes, should a participant remain below 70% they were removed from the chamber (zero incidences). The Lake Louise Questionnaire was also completed every 30 mins to assess for symptoms off acute mountain sickness (AMS), should a participant remain at 6 or above. They were removed from the chamber (zero incidences).

Preliminary Measures

Upon arrival participants weighed themselves (SECA 778; SECA UK, Birmingham, UK) and provided a urine sample. Urine specific gravity (Uspg) was assessed using a refractometer (Atago, USA) and urine osmolality (Uosm) was measured using an osmometer (Osmocheck; Vitech Scientific, West Sussex, UK). Euhydration was achieved when urine osmolality and urine specific gravity were below 700 $mOsmol.kg^{-1}$ H_2O and 1.020, respectively (Sawka et al. 2007). Participants were required to consume 500 ml of water and wait 30 min before they entered the chamber if they were above the criteria (MacKenzie et al. 2008). Participants then sat and rested for 15 minutes for a resting heart rate (HR) and O_2 saturation (SpO_2) to be measured prior to entering the chamber.

Physiological measures

Heart rate (Polar 810i heart rate monitor; Kempele, Finland) and SpO_2 (Nonin 2500; Nonin Medical Inc, Minesota, USA) were measured every 15 min whilst in the hypoxic chamber and every 30 min outside of the chamber. The measurement was recorded after 30 seconds.

Haematological measures

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

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

[EPO], IL-6 and TNFα concentrations were measured in plasma for all four trials (SL, 3,600 m, 4,200 m, and 4.800 m). Enzyme-linked immunosorbent assays were used in accordance with manufacturer instructions to determine concentrations for [EPO] (Roche Diagnostics Ltd., Lewes, UK) and for IL-6 and TNFα (DuoSet ELISA Development System; R&D Systems Inc., Abingdon, UK). The technical error of measurement (TEM) between duplicate samples for [EPO] was 3.8%, with a unit error value of 0.7 $mU \cdot mL^{-1}$, for IL-6 it was 7.1%, with a unit error value of 2.76 $pg \cdot mL^{-1}$ and for TNFα it was 4.1%, with a unit error value of 518.7 $pg \cdot mL^{-1}$.

Statistical Analysis

Data was assessed for normality and sphericity and adjusted where necessary using the Huynh-Feldt method. Differences in [EPO], IL-6 and TNFα at each time point (e.g. Pre, 1h, 2h, 4h,6h and 8h.), HR and SpO_2 were analysed with a two-way repeated measures ANOVA (hypoxia x time), with Bonferroni correction used to determine differences between groups. For statistical analysis SpO_2 and HR measurements were averaged for each hour in the chamber and every two hours outside of the chamber. Typical error of measurement (TEM) calculations were carried out on duplicate [EPO], IL-6 and TNFα samples using a method previously described by (Hopkins 2000). All statistical tests were completed using SPSS Statistics 22 (International Business Machines Corp., Armonk, New York). Significance was accepted at $P < 0.05$. Values are reported as mean ± SD unless otherwise indicated. Effect sizes for main effects and interactions are presented as partial eta squared (η_p^2) in accordance with Lakens (2013).

Results

Physiological Measures

There was an effect on SpO_2 over time (F = 927.298, P = 0.001, η_p^2 = 0.99), in hypoxia (F = 258.717, P = 0.001, η_p^2 = 0.97) and an interaction effect between time*hypoxia (F = 156.411, P = 0.001, η_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_2 were found at 1h (P = 0.001) and 2h (P = 0.001) between SL (98 ± 0 and 98 ± 1%), 3,600 m (87 ± 2 and 87 ± 3%), 4,200 m (83 ± 1 and 83 ± 1%) and 4,800 m (76 ± 2 and 75 ± 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.

There was an effect on HR over time (F = 21.294, P = 0.001, η_p^2 = 0.75), in hypoxia (F = 11.739, P = 0.001, η_p^2 = 0.63) and an interaction effect between time*hypoxia (F = 9.837, P = 0.001, η_p^2 = 0.59). Bonferroni comparison identified significant differences (P = 0.022) between mean HR at SL (57 ± 7 b·min^{-1}) and 4,800 (65 ± 10 b·min^{-1}). 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 ± 5 and 75 ± 8 b·min^{-1}) and 2h (63 ± 6 and 72 ± 10 b·min^{-1}), respectively, compared to 4h (58 ± 4 and 61 ± 9 b·min^{-1}), 6h (58 ± 6 and 60 ± 7 b·min^{-1}) and 8h during (55 ± 8 and 58 ± 9 b·min^{-1}). Post-hoc data is presented in figure 3B.

FIGURE 3A/B

Haematological Measures

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

FIGURE 4

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

FIGURE 5

There was also no effect on TNFα found over time (F = 1.748, P = 0.1.82, η_p^2 = 0.20), hypoxia (F = 0.945, P = 0.412, η_p^2 = 0.12), or an interaction effect between time*hypoxia (F = 1.545, P = 0.142, η_p^2 = 0.18). Figure 6 illustrates the response of plasma TNFα during each hypoxic trial.

FIGURE 6

TABLE 2

Relationship between peak [EPO] and other measures

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

FIGURE 7 A/B/C

Discussion

The novel findings of this study were that a normobaric hypoxic exposure of two hours at an FiO_2 of <0.125 (>4,200 m) are sufficient to increase EPO production, which peaked 2h post-exposure and are maintained up to 4h post-exposure. Despite observing a greater increase in [EPO] as a result of increased severity of hypoxia, large individual variability (see Table 2) between participants resulted in no main effect from hypoxia itself. All [EPO] returned to baseline levels 6h post-exposure. The present study also found that there was no relationship between baseline IL-6 and TNFα and peak Δ[EPO]. There were also no differences in IL-6 and TNFα production as a result of three different simulated altitudes.

EPO response to normobaric hypoxia

Figure 1 illustrates the results of the previous investigation into acute hypoxic exposures and EPO. The present invesigation found that two hours of normobaric hypoxia at FiO_2: ~0.135 (3,600 m), FiO_2: ~0.125 (4,200 m) and FiO_2: ~0.115 (4,800 m) caused an increase in [EPO] of 22% (range: -16—53%), 43% (range: 14—100%) and 52% (range: 16—113%), respectively, peaking two hours post-exposure, maintaining until four hours and returning to baseline after six hours. Knaupp et al. (1992) revealed that two hours of normobaric hypoxia at ~5,500 m elicited a ~50% increase in [EPO] and Ge et al. (2002) also found an increase of ~50% in [EPO] after 24 hours of hypobaric hypoxia at ~2,500-2,800 m. For the application of a hypoxic exposure on return to sea level post-altitude training camp, normobaric hypoxia is the most accesible option, at a moderate to high-altitude (<4,000 m), and at a short enough duration that it would fit into an athletes daily training schedule (<2 h).

FIGURE 8

Chronically increased EPO synthesis leads to a progressive increase in tHbmass (Lundby et al. 2007), however, hypoxia-induced changes in EPO release seem to be subject to a marked inter-individual variability (Chapman et al. 1998). This may explain why there is a varied athlete response in tHbmass to altitude training camps (Mclean et al. 2013). In our study despite a greater reduction in SpO_2 from an increased severity of hypoxia causing a greater production in [EPO], individuals who were more O_2 desaturated did not always produce a greater [EPO] (see Fig 7). As such, the finding are in agreement with previous literature (Ge et al. 2002; Friedmann et al. 2005; MacKenzie et al. 2008) who also found a marked individual variability in EPO release at different altitudes. Although the participants in the present study were well-trained, similar value and variations of [EPO] have been reported in elite athletes (Clark et al. 2009; Garvican et al. 2012; Pottgiesser et al. 2012).

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

The present investigation found no relationship between ΔSpO_2 and peak Δ[EPO] percentage. MacKenzie et al. (2008) suggested EPO production is noticeably augmented by the depression of arterial O_2 content (CaO_2), as a result of decreases in SpO_2. Therefore, a greater reduction in SpO_2 combined with an inability to increase HVR could facilitate a greater secretion of EPO (Jelkmann 1992). Ge et al. (2002) also suggested that the mechanism of an individual response to altitude is likely to include the greater oxyhemoglobin desaturation. This occurs as the PO_2 falls to the steep portion of the oxyhemoglobin dissociation curve and, therefore changes in SpO_2 are mirrored by EPO levels at all altitudes. The investigations by Ge et al. (2002) and MacKenzie et al. (2008) only found a moderate relationship between $\Delta CaO_2/\Delta SpO_2$ and changes in EPO.

Inflammatory response to normobaric hypoxia

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

The present investigation found that baseline levels of IL-6 and TNFα did not inhibit the production of EPO (See Fig. 7B/C). Furthermore, there were no difference in IL-6 and TNFα at three levels of normobaric hypoxia, despite increases in HR and decreases in SpO_2. Jelkmann et al. (1992) found that the addition of IL-1 and TNFα inhibited the production of EPO in hypoxic human hepatoma cell cultures, however, inhibition did not occur with introduction of IL-6, thus believing that IL-1 and TNFα have been shown to affect gene expression in human hepatoma cultures at the transcriptional level (Jelkmann et al. 1994). The authors reported that IL-6 does not affect EPO production *in vitro*; moreover, IL-6 appears to inhibit renal EPO formation. Inflammatory responses to hypoxia are complex with various cytokines both inhibiting and preserving the production of EPO. At present it is not clear what pro-inflammatory cytokines regulate the production of EPO, but in this investigation inflammation did compromise endogenous [EPO].

Future Directions

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

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

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

Practical Applications

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

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

Perspectives

In Caucasian populations erythropoiesis is a key acclimatization response that increases the O_2-carrying capacity of the blood, i.e. tHbmass, as a result of chronic exposure to altitude (Chapman et al. 2013). A change in tHbmass by 1 g causes a change in $\dot{V}O_{2max}$ by approximately 4 ml·min^{-1} (Schmidt and Prommer 2010), for an athlete increased blood gas storage capacity is very important, therefore, the maintenance of tHbmass should be considered. Chapman et al. (2014) stated that

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

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Tables

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
VO2max ($ml \cdot kg \cdot min^{-1}$)	50.9 ± 8.2
[EPO] ($mU \cdot ml^{-1}$)	5.95 ± 1.51
IL-6 ($pg \cdot ml^{-1}$)	8.86 ± 7.17
TNFα ($pg \cdot ml^{-1}$)	3095.6 ± 3934.9

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] ($mU \cdot ml^{-1}$)	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 ($pg \cdot ml^{-1}$)	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 ($pg \cdot ml^{-1}$)	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

Figures

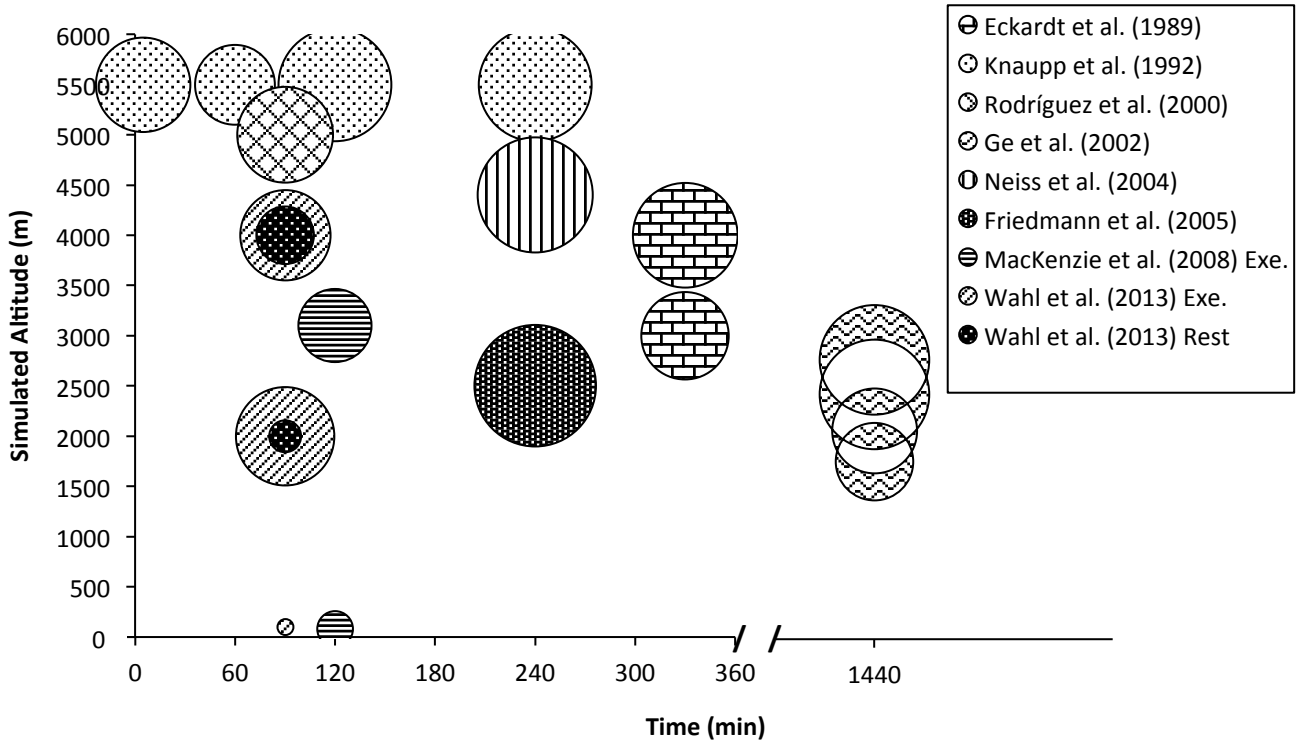
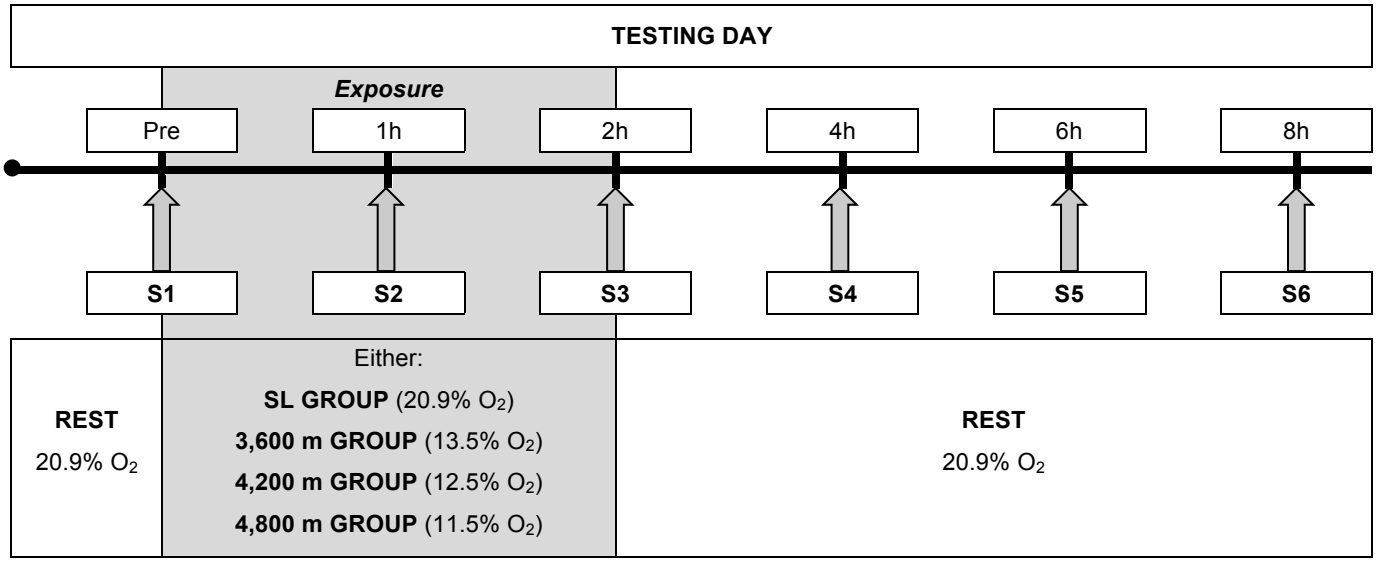


Figure 1: EPO response to hypoxic exposure duration and simulated altitude. The different bubbles represent the magnitude of increase in EPO as a result of hypoxic exposures. Different patterns within bubble indicate different investigations

1



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

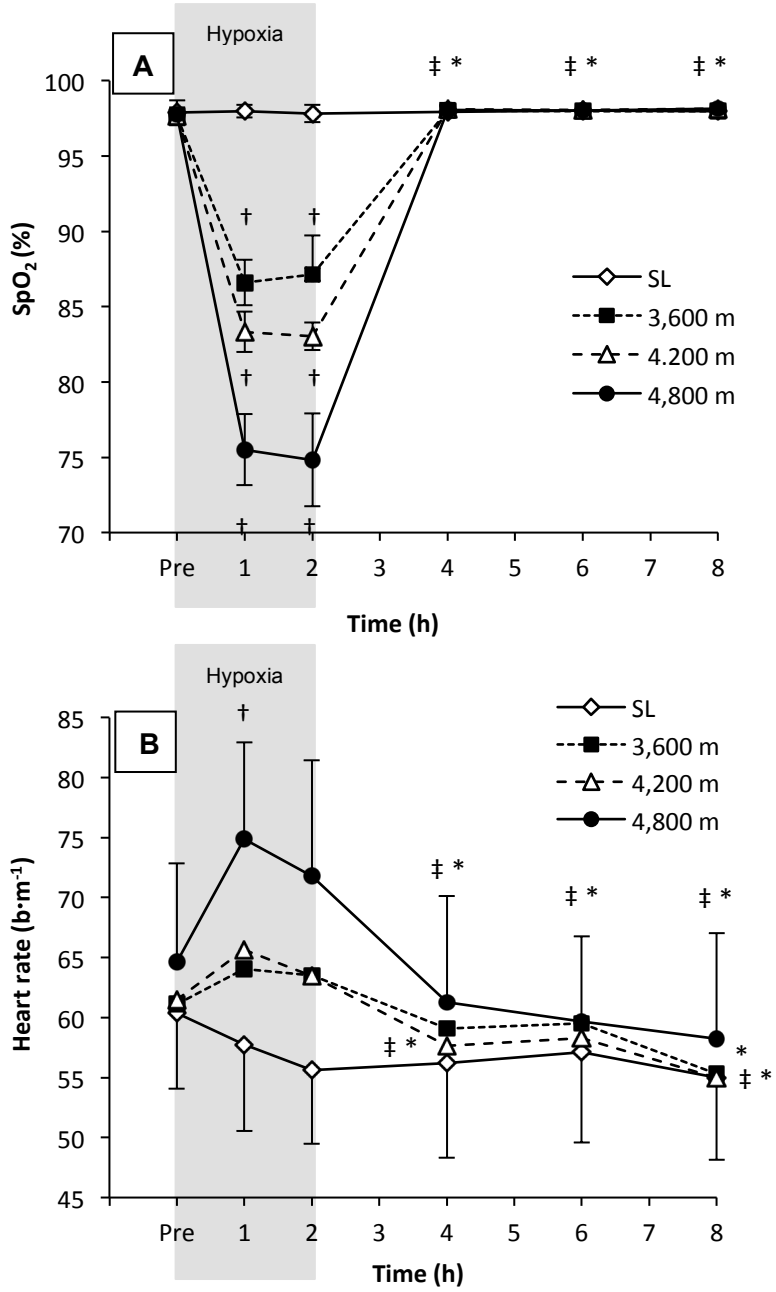

Figure 3: Difference in arterial oxyhaemoglobin saturation (SpO_2; A) and heart rate (HR; B) after two hours simulated hypoxia at SL, 3,600 m, 4,200 m and 4,800 m. Values are means ± SD. († $P \leq 0.05$ denotes differences from Pre, ‡ $P \leq 0.05$ denotes differences from 1h and * $P \leq 0.05$ denotes differences from 2 h). Note 3,600 m and 4,200 m error bars removed for clarity.

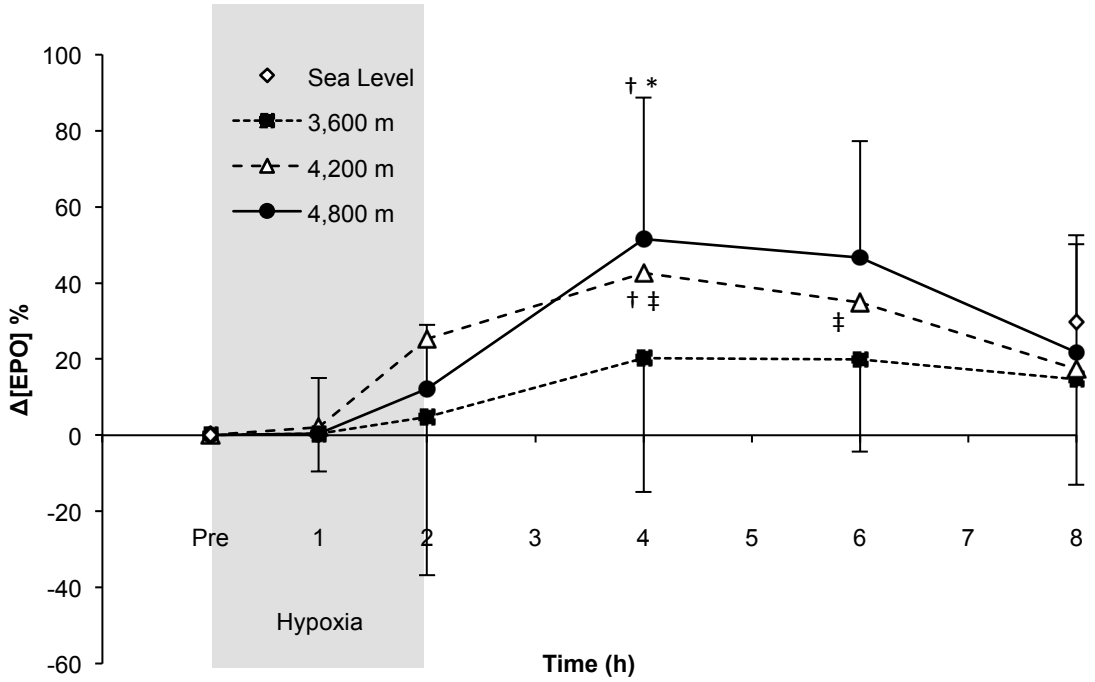

Figure 4: Percentage differences from baseline in erythropoietin concentration (Δ [EPO]) of blood plasma after two hours at simulated altitudes of sea level, 3,600 m, 4,200 m and 4,800 m. Values are means ± SD. († $P \leq 0.05$ denotes differences from Pre, ‡ $P \leq 0.05$ denotes differences from 1h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

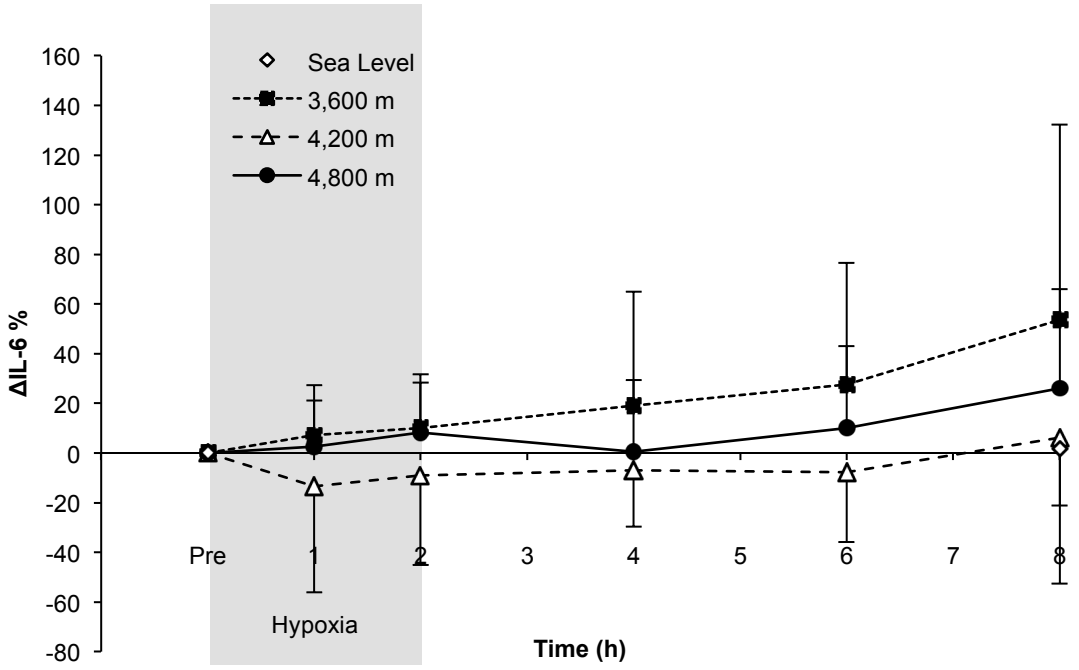

Figure 5: Percentage differences from baseline in interleukin-6 (Δ IL-6) of blood plasma after two hours at simulated altitudes of sea level, 3,600 m, 4,200 m and 4,800 m.

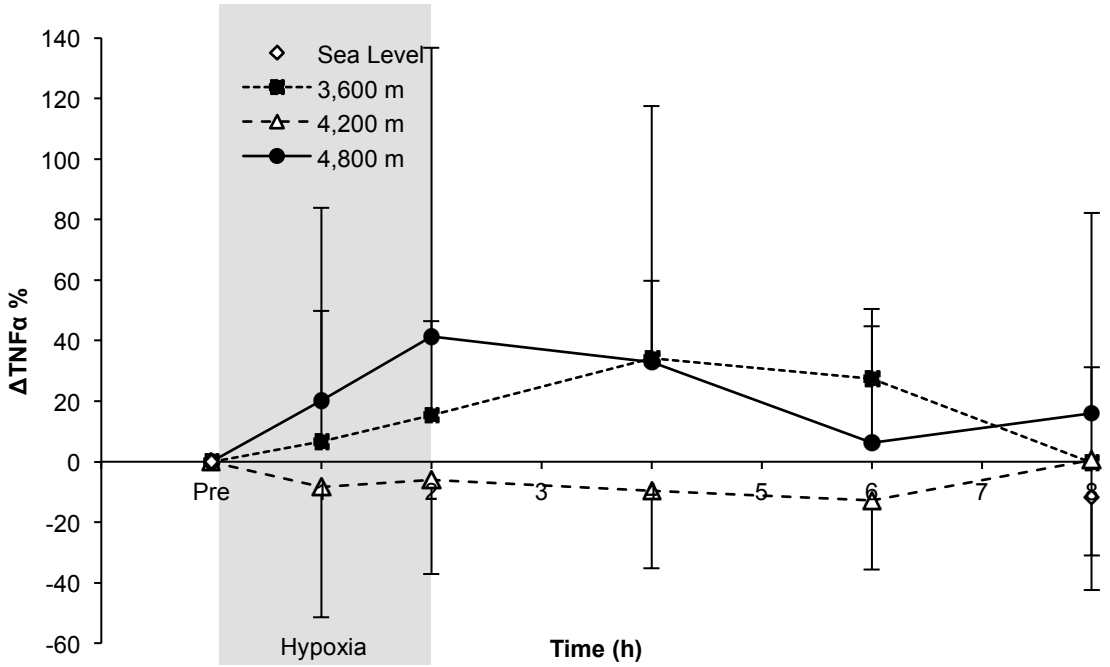

Figure 6: Percentage differences from baseline in tumor necrosis factor alpha (ΔTNFα) of blood plasma after two hours at simulated altitudes of sea level, 3,600 m, 4,200 m and 4,800 m.

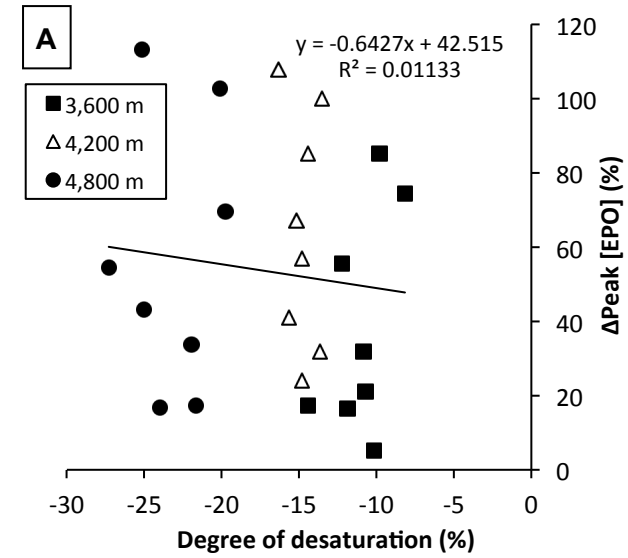

Figure 7: Relationship between the degree of desaturation averaged over the 2 hours of simulated hypoxia (**A**); baseline IL-6 (**B**); baseline TNFα (**C**) and the percentage difference of the peak in [EPO] during each hypoxic condition. As a group, there was no correlation between any of the variables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