Acute normobaric hypoxia stimulates erythropoietin release

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ABSTRACT

MacKenzie, Richard W. A., Peter W. Watt, and Neil S. Maxwell. Acute normobaric hypoxia stimulates erythropoietin release. *High Alt. Med. Biol.* 9:28–37, 2008.—Investigations studying the secretion of EPO (erythropoietin) in response to acute hypoxia have produced mixed results. Further, the errors associated with the various methods used to determine EPO are not well documented. The purpose of the current study was to determine the EPO response of 17 trained male subjects to either an acute bout of normobaric hypoxia (Hy; $n = 10$) or normoxia (Con; $n = 7$). A secondary aim was to determine the error associated with the measurement of EPO. After baseline tests, the treatment group (Hy) underwent a single bout of hypoxic exposure ($F_{I_{O_2}} \sim 0.148$; 3100 m) consisting of a 90-min rest period followed by a 30-min exercise phase (50%$\dot{V}_{O_{2max}}$). Venous blood samples were drawn pre (0 min) and post (120 min) each test to assess changes in plasma EPO (ΔEPO). The control (Con) group was subjected to the same general experimental design, but placed in a normoxic environment ($F_{I_{O_2}} \sim 0.2093$). The Hy group demonstrated a mean increase in EPO [19.3 (4.4) vs. 24.1 (5.1) mU/mL], $p < 0.04$, post 120 min of normobaric hypoxia. The calculated technical error of measurement for EPO was 2.1 mU/mL (9.8%). It was concluded that an acute bout of hypoxia, has the capacity to elevate plasma EPO. This study also demonstrates that the increase in EPO accumulation was 2 times greater than the calculated measurement of error.

Key Words: erythropoietin; hypoxia; altitude training

INTRODUCTION

Exposure to altitude is commonly incorporated into many athletes' training programes. The rationale for such an inclusion is that the reduction in inspired oxygen (O_2) will aggravate hematological, hormonal, metabolic, and structural adjustments, facilitating improvements in O_2 delivery and extraction that may lead to improvements in sea-level exercise performance. Erythropoietin (EPO) is largely produced by the cells of the kidney and is responsible for the regulation of red blood cell production (see review: Wang and Semenza, 1996). The drop in tissue partial pressure of O_2 (P_{O_2}), resulting from both environmental hypoxia and altitude, has the ability to encourage EPO release (see reviews: Wilber, 2001; Levine, 2002). Despite the depth of research supporting hypoxic-induced increases in EPO, there is

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still evidence contradicting such findings (Table 1).

To understand the inconsistency in these findings, it seems necessary to consider that hypoxic-induced increases in EPO are ultimately governed by upstream physiological characteristics involved in the delivery of O_2 to renal tissue (Ge et al., 2002) and the transcriptional factors associated to the O_2-sensitive hypoxic-inducible factor-1 (HIF-1) (Jedlickova et al., 2003). However, a dose–response relationship seems to exist between the magnitude of EPO release and the degree of hypoxia, which comprise of two factors that are relatively simple to manipulate [i.e., intensity ($F_{I_{O_2}}$) and duration of exposure].

Ge et al. (2002) concluded that an optimal threshold for hypoxic-induced increases in EPO lies at altitudes of ≥2100 to 2500 m (equivalent to $F_{I_{O_2}} \sim 0.169$ to 0.157, respectively). This is in agreement with previous work. The summary of data provided in Table 1 shows clearly that when the $F_{I_{O_2}}$ is equivalent to ~0.14 to 0.16, EPO concentrations are significantly increased. Indeed, only one of the studies analyzed in Table 1, which used a hypoxic stress within the suggested threshold, offered no EPO response (Karlsen et al., 2002).

Erythropoietin concentrations have been repeatedly shown to increase as a result of ~70 to 120 min of acute and continuous hypoxic exposure (Eckardt et al., 1989; Rodriguez et al., 2000). Knaupp et al. (1992) demonstrated that at least 120 min of continuous hypoxia ($F_{I_{O_2}} \sim 0.105$) is required to augment kidney-induced EPO production. Further, earlier work advocated that continuous exposure to both 3000 m for 114 min and 4000 m for 84 min results in elevated EPO concentrations from 16.0 to 22.5 and 16.7 to 28.0 mU/mL, respectively (Ab-

Table 1. Summary of Studies Measuring Erythropoietin Response to Hypoxia

	Positive EPO response	*No EPO change*
Altitude (actual and simulated)		
<2000 m (F_{IO2} ~0.167)	Dehnert et al. (2002)	
≥2000 m (F_{IO2} ~0.167)	Eckardt et al. (1989); Laitinen et al. (1995); Rusko et al. (1995); Mattila and Rusko, (1996); Chapman et al. (1998); Piehl-Aulin et al. (1998); Rusko et al. (1999); Ashenden et al. (2000); Koistinen et al. (2000); Stray-Gundersen et al. (2001); Ge et al. (2002); Friedmann et al. (2005)	Karlsen et al. (2002)
≥3800 m (F_{IO2} ~0.135)	Eckardt et al. (1989); Knaupp et al. (1992); Savourey et al. (1996); Rodriguez et al. (2000); Niess et al. (2004)	Vallier et al. (1996); Garcia et al. (2000); Katayama et al. (2003); Julian et al. (2004); Lundby et al. (2005)
Duration (h/day)		
≤2	Knaupp et al. (1992); Rodriguez et al. (2000); Niess et al. (2004)	Garcia et al. (2000); Karlsen et al. (2002); Katayama et al. (2003); Julian et al. (2004); Lundby et al. (2005)
>2	Knaupp et al. (1992); Friedmann et al. (2005)	N/A
≥5	Eckardt et al. (1989); Laitinen et al. (1995); Rusko et al. (1995); Mattila and Rusko (1996); Savourey et al. (1996); Chapman et al. (1998); Piehl-Aulin et al. (1998a); Piehl-Aulin et al. (1998b); Rusko et al. (1999); Ashenden et al. (2000); Koistinen et al. (2000); Stray-Gundersen et al. (2001); Dehnert et al. (2002); Ge et al. (2002)	N/A

brecht and Littell, 1972; Eckardt et al., 1989). However, most studies have used exposure times greater than 2 h (Table 1).

The recent study by Julian et al. (2004) suggested that 70 min of 5:5-min hypoxia ($F_{I_{O_2}} \sim 0.10$ to 0.12) to normoxia ratio initiated no erythropoietic response. The authors hypothesized that the timings of blood collection could have prevented the detection of possible changes in EPO (Katayama et al., 2003, Julian et al., 2004) and that a hypoxic–normoxic ratio of 5:5 is an insufficient stress. Garcia et al. (2000) failed to witness any change in EPO following an exposure ($F_{I_{O_2}} \sim 0.13$; 3500 m) above what Ge et al. (2002) termed the optimal threshold, and the period of exposure was continuous (120 min). The authors suggested, however, that erythropoiesis may have indeed taken place, because reticulocyte count increased significantly after day 5 of continuous intermittent hypoxia.

The majority of investigations examining the effects of acute or intermittent hypoxia on EPO response have used either rest or exercise protocols. Consequently, few studies have combined inactive hypoxic exposure with a period of exercise. Recently, Vogt and colleagues (2001) demonstrated that mRNAs coding for hypoxic-inducible factor-1α (HIF-1α), a major hypoxic transcriptional activator, are significantly increased when exercise is combined with hypoxia. This may suggest that HIF-1α stabilization is greater or even additive when environmental hypoxia is combined with metabolic stress, such as exercise (Vogt et al., 2001).

Three key factors could be used to explain some of the lack in hypoxic-induced EPO increases during acute (single) or intermittent (repeated) exposure: (1) inadequate hypoxic stimulus in both $F_{I_{O_2}}$ and time of exposures, (2) limitations in study designs, such as timing of blood collection, and (3) maybe associated with the error in EPO measurement. Indeed, only 8 of the 23 studies summarized in Table 1 provided data for coefficient variations or technical error of measurement.

Thus, the purpose of the current study was to determine plasma EPO response and its associated error in measurement to a total of 120 min of normobaric hypoxic ($F_{I_{O_2}} \sim 0.148$; 3100 m) exposure (rest phase, 90 min, and exercise phase, 30 min, at 50% $\dot{V}_{O_{2max}}$). We hypothesized that this duration and intensity of hypoxia (i.e., $F_{I_{O_2}}$), combined with moderate-intensity exercise (Vogt et al., 2001) would induce an EPO response.

MATERIALS AND METHODS

Subjects

Seventeen physically trained males were recruited for this study, which was approved by the University of Brighton ethics committee. Subjects gave their informed consent prior to be being accepted to take part. Subjects were healthy nonsmokers with no history of respiratory diseases. Volunteers were also free from any hematological infections or renal diseases. Individuals were also excluded if they had visited altitudes above 1600 m or traveled by commercial aircraft in the 8 weeks leading up to data collection (Niess et al., 2004). Table 2 shows means (SD) physical characteristics for both groups.

Study design

The experimental designed was based on two laboratory visits. The first visit was used to familiarize subjects and collect preliminary data, and the second acted as the main test pro-

Table 2. Subject Characteristics

	Age, (yr)	*Ht (cm)*	*Body mass (kg)*	*Body fat (%)*	$\dot{V}_{O_{2max}}$ *(mL/min/kg^{-1})*	*Hb (g/dL)*
Control group ($n = 7$)	23.3 (2.9)	181.0 (2.6)	74.6 (1.6)	10.1 (1.9)	60.8 (8.3)	15.5 (1.2)
Hypoxic group ($n = 7$)	24.0 (3.4)	178.2 (5.2)	76.0 (5.1)	11.7 (2.5)	57.4 (4.9)	15.2 (1.3)

Values are mean (SD).

tocol. The first test visit provided values of the subject's lactate threshold and maximal oxygen consumption ($\dot{V}_{O_{2max}}$). Lactate threshold (LT) tests began with a treadmill speed of 9 km/h^{-1} and consisted of 5 to 8 submaximal stages that increased by 1 km/h^{-1} every 3 min. Oxygen uptake was measured throughout using an online system (Oxycon Pro, Jaeger, Germany) shown to be both a valid and reliable means of measuring online respiratory parameters (Carter et al., 2002). The $\dot{V}_{O_{2max}}$ protocol was adapted from Costill et al. (1969). Post the LT phase, treadmill velocity was set at intensities relative to individuals, using the velocity that matched 150 (8) beats/min^{-1} of heart rate obtained during the LT phase. Treadmill gradient started at 1% and increased by 1% every minute until the end of the test.

Main protocol

On arrival to the laboratory, subjects provided a urine sample from which hydration status was determined (Micro Osmometer; Vitech 3300, Massachusetts, USA). If subject's hydration values exceeded 400 mOsmol/kg H_2O (Maresh et al., 2004) they were required to consume 500 mL of water and wait 30 min before being retested. Body mass, subject's height, and the percentage of body fat, using the four-site method (bicep, triceps, subscapula, and suprailiac), as described by Durnin and Womersley (1974), were then recorded. The main test protocol required the subjects to return to the laboratory to sit at rest (90 min) prior to performing treadmill exercise (30 min) set at 50% relative $\dot{V}_{O_{2max}}$ in either a normobaric hypoxic environment (Hy; $F_{I_{O_2}} \sim$ 0148; 3100 m; $n = 10$) or in normoxia (Con; $F_{I_{O_2}} = 0.2093$; $n = 7$). To minimize the effects of variations in circadian rhythms (Reilly et al., 1984), all tests were carried out between 16:00 and 17:30 h.

Hemoglobin [Hb] (10 μL) and resting blood [La] (20–30 μl) samples were also taken before each condition (Hy and Con). [Hb] was measured from whole blood using a HemoCue device (HemoCue limited, Sheffield, UK). A mean value was recorded from 2 or 3 samples. If there was a difference of more than 3 g/dL between the first two samples, the test was repeated (Rechner et al., 2002). A separate fingertip puncture (warmed to ensure arterialized blood) was used to measure whole blood [La] (YSI 2300 STAT Plus lactate analyzer, Yellow Springs Instrument, Ohio, USA).

Normobaric hypoxia (Hy; $F_{I_{O_2}} \sim 0.148$) was generated using a modified Colorado Altitude Training Tent (Colorado Altitude Training, Louisville, USA). Temperature, humidity, carbon dioxide, and barometric pressure were monitored throughout. Minute ventilation ($\dot{V}_E$), O_2 uptake ($\dot{V}_{O_2}$) and carbon dioxide production ($\dot{V}_{C_{O_2}}$), respiratory exchange ratio (RER), heart rate (HR), oxyhemoglobin saturation (Sp_{O_2}; pulse oximeter Nonin 2500, Minneapolis, Minnesota, USA), and rate of perceived exertion (RPE) were recorded every 15 min during rest and every 5 min during the exercise phase of each condition (Con and Hy). Expired air was collected using the Douglas bag technique. During each Hy exposure, whole-blood [La] samples were taken from a fingertip puncture (capillary) site every 15 min during rest and 5 min during exercise. Arterial O_2 content (Ca_{O_2}) was indirectly estimated using the equation described by Ge et al. (2002), [C_aO_2 = Hb (g/dL) $\times$ Sp_{O_2} (%) $\times$ 1.36 (mL/O_2/dL^{-1})]. The equation assumes a hemoglobin–O_2 constant of 1.36 mL/O_2/dL^{-1}. Erythropoietin concentrations were measured in plasma, drawn from whole blood samples taken pre (normoxic environment; 0 min^{-1}) and post (120 min^{-1}) for both groups (Con and Hy). Concentrations were determined using an enzyme-linked immunosorbent assay kit (Roche Diagnostics Ltd., Lewes, UK). The within and between coefficient variations were 6.2% and 9.9%, respectively.

Statistics

Changes in plasma EPO concentrations were analyzed using dependent *t*-tests with statistical significance set at $p < 0.05$. Values are presented as means and standard deviation (SD) with Pearson product correlation coefficients calculated and shown where appropriate. Bland and Altman (1986) 95% limits of agreement (LOA) were calculated to show differences between duplicate EPO samples. Typical error of measurement (TEM) calculations were carried out on duplicate EPO samples using a

method previously described by Hopkins (2000). Briefly, the mean and SD of the differences between duplicate EPO samples were determined and the value then divided by $\sqrt{2}$ and back-transformed to obtain a coefficient variation (CV) (Gore and Hopkins, 2005). The resultant TEM between duplicate samples was 9.8%, with a unit error value of 2.1 mmole/mL. Statistics software SPSS (version 12.0) was used to analyze all data.

RESULTS

Mean (SD) arterial oxygen content (Ca_{O_2}) values are shown in Fig. 1. The hypoxic protocol utilized was found to be effective, as demonstrated by significantly lower values in the Hy condition when compared to the Con group during both the rest phase [Con, 20.3 (1.6); Hy, 19.8 (1.5) $mL/O_2/dL^{-1}$], $p = 0.003$, and the exercise phase [Con 19.2 (1.4); Hy, 18.0 (1.8) $mL/O_2/dL^{-1}$], $p = 0.003$.

Changes in plasma EPO (ΔEPO) concentrations for pre and post experimental trials are shown in Fig. 2 for both the Con and Hy group. The Hy group demonstrated a mean increase from 19.3 (4.4) to 24.1 (5.1) mU/mL post 120 min^{-1} of hypoxic exposure ($p = 0.04$), with a range from −5.6% to 61.8%. No significant difference was observed for the Con group [18.0 (2.1) vs. 19.1 (1.8) mU/mL] (Fig. 2.).

The degree of desaturation in Sp_{O_2} from baseline ($F_{I_{O_2}} = 0.2093$) to the point of hypoxic exposure was noted to be significant in the Hy group [normoxia: 98 (1); hypoxia: 91 (3)], $p = 0.001$. Correlation coefficients were calculated for ΔEPO against ΔSp_{O_2} and ΔCa_{O_2}. The degree of Sp_{O_2} desaturation correlated moderately with ΔEPO ($r = -0.54$), $p = 0.017$). Delta changes in EPO and Ca_{O2} generated a similar correlation coefficient of $r = -0.54$ ($P = 0.026$) (Fig. 3.).

The level of agreement calculated for the differences between duplicate EPO samples was −5.45 and 6.01 mmol/mL, with a mean difference of 0.28 (2.9), $p = 0.001$. Figure 4 shows that 4.7% of the data points fell outside of 95% limits of agreement. Technical error of measurement calculation for differences between the same duplicate samples offered an error value of 2.1 mmol/mL. Correlation coefficient for duplicate EPO samples was also carried out ($r = 0.94$).

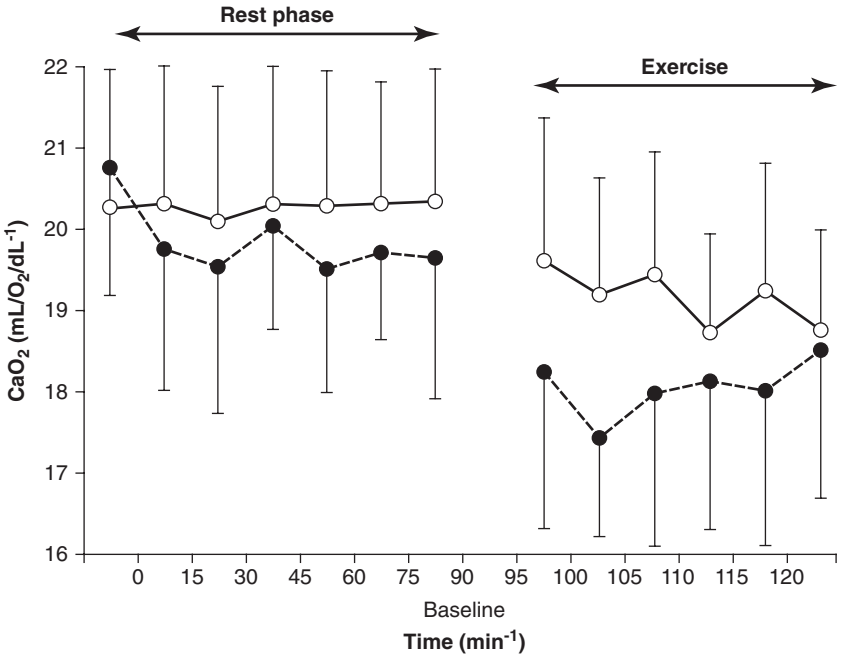

FIG. 1. Arterial O_2 content (Ca_{O_2}) for Con (open circles) and Hy group (black circles). Values are mean (SD). Statistical difference was noted between Hy and Con for both rest ($p = 0.003$) and exercise ($p = 0.003$).

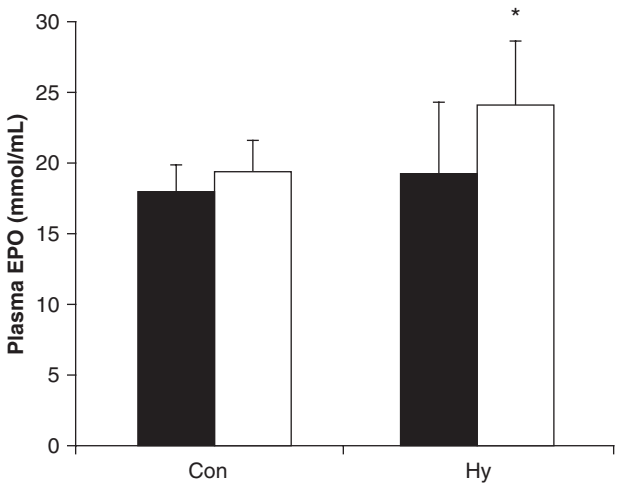

FIG. 2. Mean (SD) EPO response for baseline (closed bars) and postexperimental trial (open bars) for both control (Con) and hypoxic (Hy) groups. *Level of significance ($p = 0.04$)

DISCUSSION

The aim of the current investigation was to measure the response of plasma EPO to normobaric hypoxia. The hypoxic protocol utilized in the current study was concluded as an effective stress with exercise further exaggerating hypoxemia (Fig. 1.) (Garcia et al., 2000). Thus the salient observation was that an acute bout (120 min) of hypoxia ($F_{I_{O_2}} \sim 0.148$) combined with the metabolic stress of exercise statistically increased plasma EPO levels and that this increase was 2 times greater (4.8 mmol/mL) than the error associated with our measurement (2.1 mmole/mL).

Hypoxic-induced elevations in EPO and so possible increased erythropoiesis has clear tangible benefits for both athletic (Stray-Gundersen et al., 2001) and clinical (Silverberg et al., 2004; Corwin, 2004) populations. However, other investigations have failed to conclude the effectiveness of acute on intermittent hypoxia (Vallier et al., 1996; Karlsen et al., 2002; Katayama et al., 2003; Julian et al., 2004). For a review, see Wilber (2001). Further, investigations that have demonstrated EPO increases (Eckardt et al., 1989; Knaupp et al., 1992; Ashenden et al., 2000; Rodriguez et al., 2000; Ge et al., 2002) have failed to publish data on their error of measurement.

It is possible that the error in EPO measurement or reliability of this measure could prevent some investigations from detecting a true effect (Hopkins, 2000). As the current study aimed to measure the change in EPO, the within subjects and between tests (pre vs. post) variations were of critical importance. It is essential to point out that there was a magnitude of variation in the light absorbance detected during EPO measurement (TEM = 2.1 mmol/

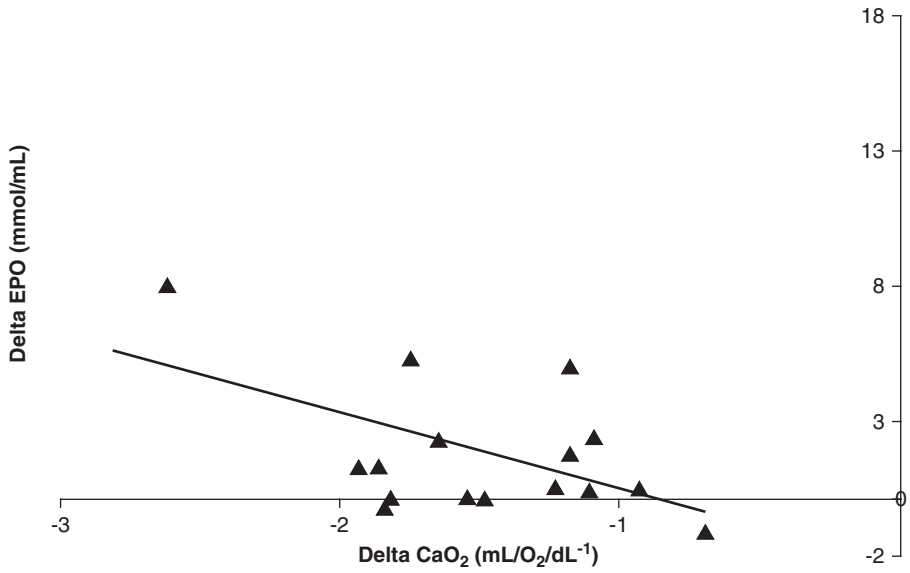

FIG. 3. Relationship between the degrees of change in EPO (ΔEPO) is correlated with the magnitude of desaturation in Ca_{O_2} ($p = 0.026$).

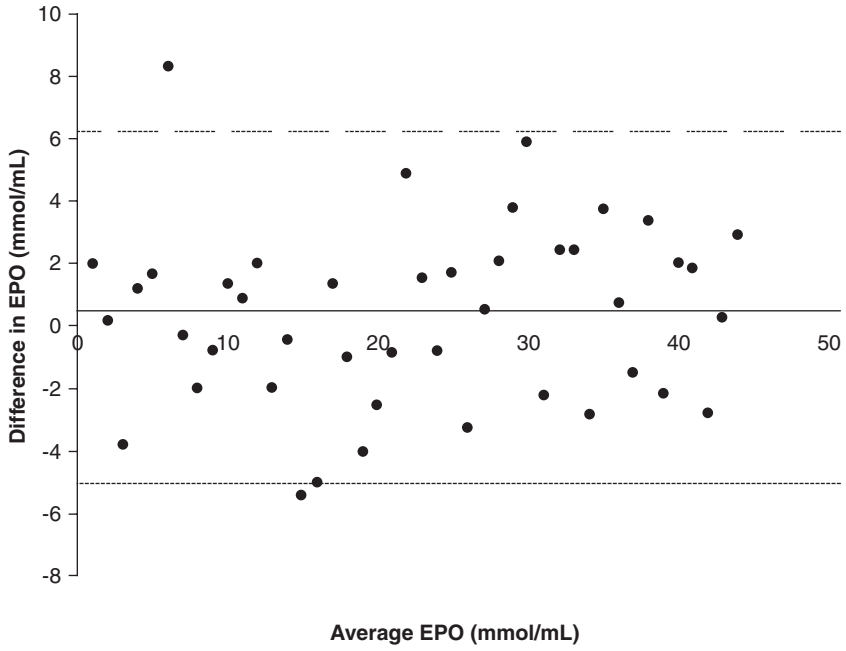

FIG. 4. Plot of differences between duplicate EPO samples using limits of agreement (Bland and Altman, 1986).

mL). This within-test variation was not detected through the more traditional methods of identifying differences, demonstrated by a correlation coefficient of $r = 0.94$, which suggests a high level of agreement, and is further highlighted by the limits of agreement plot (LOA; Fig. 4.). This plot is proposed to offer more validity when comparing two different samples measuring the same substance. Essentially, the plot suggests that 95% confidence can be assumed when using the mean value for each duplicated sample (Bland and Altman, 1986). However, the calculated bias (-5.45 ± 6.01) and LOA plot are considerable and therefore do not highlight differences that are technically important. More precisely, the LOA are approximately 3 times the size of the error of measurement (Hopkins, 2000).

However, it was concluded that there was a true effect in individual EPO response within the current study. Recently, Lundby and colleagues (2005) concluded that 120 min of hypobaric hypoxia was not great enough to cause a significant increase in EPO; yet their data produced a mean increase of 31.6%. It is at least plausible that the lack of a statistical change in EPO in the Lundby et al. (2005) study and other work (Katayama et al., 2003) may have been due to error of measurement (TEM). However, this is at best speculative, as the authors did not publish these data. In fact, only 8 out of the 23 studies summarized in Table 1 provided values for coefficient variations (CV) or TEMs.

Klausen's work has shown that circulating EPO concentrations are subject to a distinct diurnal variation in both normoxia and hypoxia (Klausen et al., 1996). The present study controlled for differences in diurnal variation of EPO by ensuring that subjects were tested within a 1.5 h period. Further, it is entirely possible that discrepancies between investigations could also be attributed to differences in the assays kits used (Clanton and Klawitter, 2001).

Hypoxic-induced changes in EPO release seem to be subject to a marked interindividual variability (Chapman et al., 1998), which may further explain some of the inconsistencies in the literature. It is clear from the data in the current study that EPO release is subject to a distinct difference in the level of response, with values ranging from −5.6% to 61.8% (mean, Δ21%). The production of EPO is noticeably augmented by the depression of Ca_{O_2}, resulting from decreases in oxyhemoglobin saturation (Sp_{O_2}), (Eckardt et al., 1993; Maxwell et al., 1990). Thus, a greater reduction in Sp_{O_2} com-

bined with an inability to increase HVR could facilitate a greater secretion of EPO (Jelkmann, 1992), which could, in part, explain some of the individual EPO variation. However, the magnitude of oxyhemoglobin desaturation (Sp_{O_2}) and reduction in Ca_{O_2} correlated only moderately with changes in EPO ($r = -0.54$ and $r = -0.54$, respectively), which agrees almost identically with the data of Ge et al. (2002) and suggests that ΔSp_{O_2} and ΔCa_{O_2} during hypoxic exposure can only partly explain the differences in EPO secretion (Cohen, 1988).

Finally, the use of recurrent episodes of hypoxia, which are indicative of the live-high, train-low (LHTL) method of hypoxia training, have produced mixed results on both hematological adjustments and sea-level exercise performance variables. Recently, Gore et al. (2005) suggested that intermittent hypoxic exposure did not stimulate erythropoiesis despite elevations in serum EPO. In contrast, Basset et al. (2006) clearly demonstrated that short-term hypoxia increased levels of EPO, Hb, hematocrit, and red blood cells without offering an expected sea-level exercise performance. These authors proposed that differences in individual adaptive responses to intermittent hypoxia may affect exercise performance and that insensitive physiological measurers may prevent the detection of possible changes in exercise performance.

In conclusion, it is clear from the findings of the present study that EPO release is moderately increased as a result of 120 min of acute normobaric hypoxic (rest = 90 min; exercise [$50\%\dot{V}_{O_{2max}}$] = 30 min), equivalent to 3100 m. Further, the change in EPO accumulation can be described as a true effect because the increase was greater than the TEM. The increases noted in the current investigation indicate that the intensity (i.e., $F_{I_{O_2}} \sim 0.148$), duration (120 min) and type of exposure (rest combine with an exercise phase) may have the ability to stimulate erythropoiesis, if the exposure is part of a continued programe of hypoxic treatments, that is, the live-high, train-low method of altitude training (Levine and Stray-Gunderson, 2005). However, this EPO release seems to be subject to a distinct interindividual variation that can only partly be explained by a reduction in oxyhemoglobin saturation and maybe affected to a larger extent by factors related to the stabilization of HIF-1α.

REFERENCE

Abbrecht P.H., and Littell J.K. (1972). Plasma erythropoietin in men and mice during acclimatization to different altitudes. J. Appl. Physiol. 32:54–58.

Ashenden M.J., Gore C.J., Dobson G.P., Boston T.T., Parisotto R., Emslie J.R., Trout G.J., and Hahn A.G. (2000). Simulated moderate altitude elevates serum erythropoietin but does not increase reticulocyte production in well-trained runners. Eur. J. Appl. Physiol. 81:428–435.

Basset F.A., Joanisse D.R., Boivin F., St-Onge J., Billaut F., Dore J., Chouinard R., Falgairette G., Richard D., and Boulay M.R. (2006). Effects of short-term normobaric hypoxia on haematology, muscle phenotypes and physical performance in highly trained athletes. Exp. Physiol. 91:391–402.

Bland J.M., and Altman D.G. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 8:307–310.

Carter J and Jeukendrup A. (2002). Validity and reliability of three commercially available breath-by-breath respiratory systems. Eur. J. Appl. Physiol. 86:435.

Chapman R.F., Stray-Gundersen J., and Levine B.D. (1998). Individual variation in response to altitude training. J. Appl. Physiol. 85:1448–1456.

Clanton T.L., and Klawitter P.F. (2001). Physiological and genomic consequences of intermittent hypoxia. Invited review: adaptive responses of skeletal muscle to intermittent hypoxia: the known and the unknown. J. Appl. Physiol. 90:2476–2487.

Cohen J. (1988). Statistical Power Analysis for the Behavioural Sciences, 2nd ed., Erlbaum, Mahwah, NJ.

Corwin H.L. (2004). Erythropoietin in the critically ill—is it more than just blood? Critical Care 8:325–326.

Dehnert C., Hütler M., Liu Y., Menold E., Netzer C., Schick R., Kubanek B., Lehmann M., Böning D., and Steinacker J.M. (2002). Erythropoiesis and performance after two weeks of living high and training low in well trained triathletes. Int. J. Sport Med. 8:561–566.

Eckardt K.U., Boutellier U., Kurtz A., Schopen M., Koller E.A., and Bauer C. (1989). Rate of erythropoietin formation in humans in response to acute hypobaric hypoxia. J. Appl. Physiol. 66:1785–1788.

Friedmann B., Frese F., Menold E., Kauper F., Jost J., and Bartsch P. (2005). Individual variation in the erythropoietic response to altitude training in elite junior swimmers. Br. J. Sports Med. 39(3):148–153.

Garcia N., Hopkins S.R., and Powell F.L. (2000). Intermittent vs. continuous hypoxia: effects on ventilation and erythropoiesis in humans. Wilderness Environ. Med. 11:172–179.

Ge R.L., Witkowski S., Zhang Y., Alfrey C., Sivieri M.,

Karlsen T., Resaland G.K., Harber M., Stray-Gundersen J., and Levine B.D. (2002). Determinants of erythropoietin release in response to short-term hypobaric hypoxia. J. Appl. Physiol. 92:2361–2367.

Gore C.J., Hopkins W.G., and Burge C.M. (2005). Errors of measurement for blood volume parameters: a meta-analysis. J. Appl. Physiol. 99:1745–1758.

Gore C.J., and Hopkins W.G. (2005). Counterpoint: positive effects of intermittent hypoxia (live high:train low) on exercise performance are not mediated primarily by augmented red cell volume. J. Appl. Physiol. 99: 2055–2057.

Hopkins W.G. (2000). Measures of reliability in sports medicine and science. Sport Med. 30:1–15.

Jedlickova K., Stockton W.D., Chen Hua., Stray-Gundersen J., Witkowski S., Ge R.L., Jelinek J., Levine B.D., and Prchal J.T. (2003). Search for genetic determinants of individual variability of the erythropoietin response to high altitude. Blood Cell Molecules Dis. 31:175–182.

Jelkmann W. (1992). Erythropoietin: structure, control of production, and function. Physiol. Rev. 72:449–489.

Julian C.G., Gore C.J., Wilber R.L., Daniels J.T., Fredericson M., Stray-Gundersen J., Hahn A.G., Parisotto R., and Levine B.D. (2004). Intermittent normobaric hypoxia does not alter performance or erythropoietic markers in highly trained distance runners. J. Appl. Physiol. 96:1800–1807.

Karlsen T., Madsen O., Rolf S., and Stray-Gundersen J. (2002). Effects of 3 weeks hypoxic interval training on sea-level cycling performance and haematological parameters. Med. Sci. Sports Exerc. 34(5):S224.

Katayama K., Matsuo H., Ishida K., Mori S., and Miyamura M. (2003). Intermittent hypoxia improves endurance performance and submaximal exercise efficiency. High Alt. Med. Biol. 4(3):291–304.

Klausen T., Poulsen D.T., Fogh-Andersen N., Richalet J.P., Nielsen O.J., and Olsen N.V. (1996). Diurnal variations of serum erythropoietin at sea level and altitude. Eur. J. Appl. Physiol. Occup. Physiol. 72:297–302.

Knaupp W., Khilnani S., Sherwood J., Scharf S., and Steinberg H. (1992). Erythropoietin response to acute normobaric hypoxia in humans. J. Appl. Physiol. 73:837–840.

Koistinen P.O., Rusko H., Irjala K., Rajamaki A., Penttinen K., Sarparanta V.P., Karpakka J., and Leppaluot J. (2000). EPO, red cells, and serum transferring receptor in continuous and intermittent hypoxia. Med. Sci. Sports Exerc. 32:800–804.

Laitinen H., Alopaeus K., Heikkinen R., Hietanen H., Mikkelsson L., Tikkanen H., and Rusko H.K. (1995). Acclimatization to living in normobaric hypoxia and training in normoxia at sea level in runners. Med. Sci. Sports Exerc. 27:S109.

Levine B.D. (2002). Intermittent hypoxic training: fact and fancy. High Alt. Med. Biol. 3(2):177–193.

Levine B.D. and Stray-Gundersen J. (2005). Point: Positive effects of intermittent hypoxia (live high:train low) on exercise performance are mediated primarily by augmented red cell volume. J. Appl. Physiol. 99:2053–2055.

Lopez-Barneo J., del Toro R., Levitsky K.L., Chiara M.D., and Ortega-Saenz P. (2004). Regulation of oxygen sensing by ion channels. J. Appl. Physiol. 96: 1187–1195.

Lundby C., Nielsen T.K., Dela F., and Damsgaard R. (2005). The influence of intermittent altitude exposure to 4100 m on exercise capacity and blood variables. Scand. J. Med. Sci. Sport 15(3):82–187.

Maresh C.M., Gabaree-Boulant C.L., Armstrong L.E., Judelson D.A., Hoffman J.R., Castellani J.W., Kenefick R.W., Bergeron M.F. and Casa D.J. (2004). Effect of hydration status on thirst, drinking, and related hormonal responses during low-intensity exercise in the heat. J. Appl. Physiol. 97:39–44.

Mattila V., and Rusko H.K. (1996). Effect of living high and training low on sea level performance in cyclists. Med. Sci. Sports Exerc. 28:S156.

Maxwell A.P., Johnston L.T., Bridges C.F., and McGeown M.G. (1990). Erythropoietin production in kidney tubular cells. Br. J. Haematol. 74(4):535–539.

Niess A.M., Fehrenbach E., Lorenz I., Muller A., Northoff H., Dickhuth H.H., and Schneider E.M. (2004). Antioxidant intervention does not affect the response of plasma erythropoietin to short-term normobaric hypoxia in humans. J. Appl. Physiol. 96:1231–1235.

Piehl Aulin K., Svedenhag J., Wide L., Berglund B., and Saltin B. (1998). Short-term intermittent normobaric hypoxia—haematological, physiological and mental effects. Scand. J. Med. Sci. Sports 8:32–137.

Rechner I.J., Twigg A., Davies A.F. and Imong S. (2002). Evaluation of the HemoCue compared with the Coulter STKS for measurement of neonatal haemoglobin. Arch. Dis. Child Fetal Neonatal Ed 86:F188–189.

Rodriguez F.A., Ventura J.L., Casas M., Casas H., Pages T., Rama R., Ricart A., Palacios L., and Viscor G. (2000). Erythropoietin acute reaction and haematological adaptations to short, intermittent hypobaric hypoxia. Eur. J. Appl. Physiol. 82:170.

Rusko H.K., Leppavuori A., Makela P., and Leppaluoto J. (1995). Living high, training low: a new approach to altitude training at sea level in athletes. Med. Sci. Sports Exerc. 27(5):S6.

Rusko H.K., Tikkanen H., Paavolainen L., Hämäläinen I., Kalliokoski K., and Puranen A. (1999). Effect of living in hypoxia and training in normoxia on sea level $V O_{2max}$ and red cell mass. Med. Sci. Sports Exerc. 31:S86.

Savourey G., Garcia N., Besnard Y., Guinet A., Hanniquet A.M., and Bittel J. (1996). Pre-adaptation, adaptation and de-adaptation to high altitude in humans: cardioventilatory and haematological changes. Eur. J. Appl. Physiol. 73:529–535.

Silverberg D.S., Wexler D., Blum M., Keren G., Sheps D., Leibovitch E., Brosh D., Laniado S., Schwartz D., Yachnin T., et al. (2004). The use of subcutaneous erythropoietin intravenous iron for the treatment of the anemia of severe, resistant congestive heart failure improves cardiac and renal function and functional cardiac class and markedly reduces hospitalization. J. Am. Coll. Cardiol. 35:1737–1744.

Stray-Gundersen J., Chapman R.F., and Levine B.D. (2001). Living high-training low altitude training im-

proves sea level performance in male and female elite runners. J. Appl. Physiol. 91:1113–1120.
Vallier J.M., Chateau P., and Guezennec C.Y. (1996). Effects on physical training in a hypobaric chamber on the physical performance of competitive triathletes. Eur. J. Appl. Physiol. 73:471–478.
Vogt M., Puntschart A., Geiser J., Zuleger C., Billeter R., and Hoppeler H. (2001) Molecular adaptations in human skeletal muscle to endurance training under simulated hypoxic conditions. J. Appl. Physiol. 91:173–182.
Wang G.L., and Semenza G.L. (1996). Molecular basis of hypoxia-induced erythropoietin expression. Curr. Opinions Hematol. 3:156–162.
Wilber R.L. (2001). Current trends in altitude training. sports medicine 31(4):249–265.

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