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Determinants of erythropoietin release in response to short-term hypobaric hypoxia

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Ge, Ri-Li, S. Witkowski, Y. Zhang, C. Alfrey, M. Sivieri, T. Karlsen, G. K. Resaland, M. Harber, J. Stray-Gundersen, and B. D. Levine. Determinants of erythropoietin release in response to short-term hypobaric hypoxia. *J Appl Physiol* 92: 2361–2367, 2002. First published November 30, 2001; 10.1152/japplphysiol.00684.2001.—We measured blood erythropoietin (EPO) concentration, arterial O_2 saturation (Sa_{O_2}), and urine P_{O_2} in 48 subjects (32 men and 16 women) at sea level and after 6 and 24 h at simulated altitudes of 1,780, 2,085, 2,454, and 2,800 m. Renal blood flow (Doppler) and Hb were determined at sea level and after 6 h at each altitude (n = 24) to calculate renal O_2 delivery. EPO increased significantly after 6 h at all altitudes and continued to increase after 24 h at 2,454 and 2,800 m, although not at 1,780 or 2,085 m. The increase in EPO varied markedly among individuals, ranging from −41 to 400% after 24 h at 2,800 m. Similar to EPO, urine P_{O_2} decreased after 6 h at all altitudes and returned to baseline by 24 h at the two lowest altitudes but remained decreased at the two highest altitudes. Urine P_{O_2} was closely related to EPO via a curvilinear relationship ($r^2 = 0.99$), although also with prominent individual variability. Renal blood flow remained unchanged at all altitudes. Sa_{O_2} decreased slightly after 6 h at the lowest altitudes but decreased more prominently at the highest altitudes. There were only modest, albeit statistically significant, relationships between EPO and Sa_{O_2} ($r = 0.41$, $P < 0.05$) and no significant relationship with renal O_2 delivery. These data suggest that *1*) the altitude-induced increase in EPO is "dose" dependent: altitudes ≥2,100–2,500 m appear to be a threshold for stimulating sustained EPO release in most subjects; *2*) short-term acclimatization may restore renal tissue oxygenation and restrain the rise in EPO at the lowest altitudes; and *3*) there is marked individual variability in the erythropoietic response to altitude that is only partially explained by "upstream" physiological factors such as those reflecting O_2 delivery to EPO-producing tissues.

high altitude; ventilatory acclimatization; urine P_{O_2}; renal blood flow

WHEN MAMMALS ARE EXPOSED to high-altitude hypoxia, they exhibit certain physiological responses, such as the production of erythropoietin (EPO), which triggers an increase in red cell mass and Hb concentration (7, 10, 11, 35). This hematologic acclimatization response facilitates the restoration of normal blood O_2 content and improves tissue oxygenation, despite lowered arterial P_{O_2} (Pa_{O_2}). The concentration of EPO in blood increases ~90–120 min after reduction of the inspiratory P_{O_2} (5), rises progressively during the first 24–48 h, and then declines toward baseline over days to weeks (1, 28).

The role of EPO in the polycythemia induced by field and simulated (hypobaric chamber) altitude has been studied extensively in humans (1, 28, 29) and animals (12, 15). However, most studies were conducted at high altitudes (>4,000–6,000 m) (15, 21, 28, 29). Cross-sectional studies show a curvilinear relationship between Pa_{O_2} and red cell mass, with no increase until Pa_{O_2} decreases below 70 Torr, and arterial O_2 saturation (Sa_{O_2}) begins to decrease prominently (35). However, whether sea level natives will experience stimulated erythropoiesis in response to lower altitudes is unknown. This question is particularly important to certain populations such as endurance athletes who travel to moderate altitude for training purposes (17, 31), as well as the millions of people exposed to moderate altitude during occupational and/or recreational activities (23).

The EPO-producing site in response to hypoxia is primarily the kidney (20), although extrarenal sensing mechanisms also have been postulated in experimental animals (30). It is assumed that a change in the delivery of O_2 to renal tissue is a key factor for stimulating EPO production at altitude. However, this assumption has not been demonstrated directly in human studies. Renal O_2 delivery is regulated not only by renal blood flow (RBF) and arterial O_2 content (Ca_{O_2}), but also by hemodynamics (such as cardiac output) and the affinity of Hb for O_2 (35). Ultimately, it is the difference between renal O_2 delivery and renal O_2 utilization that determines O_2 content of the kidney cells, which is not necessarily linearly related to arterial oxyhemoglobin saturation.

In the present study, we hypothesized that there would be a threshold "dose" of altitude exposure that would be required for sustained EPO release and that

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the magnitude of the response would be based on variability in known physiological parameters. To test this hypothesis, we measured blood EPO, urine P_{O_2} as an index of renal tissue P_{O_2}, RBF, and Sa_{O_2} in 48 subjects at sea level and at four levels of simulated altitude.

METHODS

Subjects. Forty-eight young, healthy subjects (32 men and 16 women, 21 ± 2.5 yr, 61.3 ± 10.4 kg, 170 ± 12 cm) volunteered to take part in the study. All subjects received written and verbal explanations of the experiment before giving consent. The Institutional Review Boards of the University of Texas Southwestern Medical Center and Presbyterian Hospital of Dallas approved the study.

Protocol. Each week, for a total of 4 wk, the subjects spent 24 h in a decompression chamber at simulated altitudes of 1,780, 2,085, 2,454, and 2,800 m (612, 590, 564, and 538 Torr barometric pressure, respectively) in pseudo-random and balanced order (ultimately, order was 2,454, 2,800, 2,085, and 1,780 m). The last altitude was fixed a priori at 1,780 m to minimize any effect of the chamber exposures on subsequent experiments conducted in the field. For comfort, only 12 subjects were in the chamber during any 24-h exposure. The temperature (25 ± 0.5°C), humidity (28 ± 1%), and CO_2 concentration (0.07 ± 0.02%) in the chamber were carefully controlled.

EPO concentration. EPO concentration was measured at sea level (before decompression) and after 6 and 24 h at each simulated altitude. For logistical reasons, in half of the subjects, EPO was measured in plasma by radioimmunoassy (Ramco, Houston, TX), and in the other half, it was determined in serum with an enzyme-linked immunosorbent assay kit (Human EPO Quantikine IVD, catalog no. DEP00, R & D Systems) (2). On one set of 72 samples, EPO concentration was assayed in plasma and serum with these respective kits to determine the relationship between the two methods. The plasma and serum EPO values were tightly correlated with a regression coefficient of 0.96, a slope of 1.0, and an intercept of 2.7 (i.e., plasma values were 2.7 mU/ml higher than serum values in a systematic fashion but varied together in a 1:1 relationship). To minimize any influence of this difference between the two methods for calculating percent changes in EPO, all serum values were adjusted by this offset (2.7) to make them equivalent to the plasma measurements. Hb was measured by a CO-oximeter (Instrumentation Laboratories).

Urinary P_{O_2}. Urine samples were collected anaerobically at sea level and after 6 and 24 h at each simulated altitude with the use of the following procedure. *1*) Each subject voided to empty his/her bladder. *2*) After the first void, each subject drank 500 ml of a hypotonic sports drink (Gatorade). *3*) About 30 min after drinking the Gatorade, subjects voided through an external catheter to a vinyl bag, with care taken to avoid exposure to air. For men, this was achieved with a standard "condom"-style catheter (C. R. Bard). For women, an external collection device developed for use in space was employed (Medpoint, Chicago, IL). This procedure produced a urine flow rate of 5–10 ml/min. *4*) Immediately after collection, the urine samples were drawn into a 2-ml syringe and placed immediately on ice. The urine P_{O_2} was analyzed with a blood-gas analyzer (Instrumentation Laboratories).

RBF. RBF was measured in 24 subjects (16 men and 8 women) at sea level and after 6 h of each altitude exposure by using Doppler ultrasonography (22, 36). A color Doppler ultrasound scanner (model HDI 5000, Advanced Technology Laboratory, Bothel, WA) was used for examination. After the flow in internal vessels was identified with color Doppler ultrasonography, a sample volume was positioned in the renal artery or its first branch. The sample volume was placed in exactly the same position for each determination (based on a scout image during the first measurement), and the Doppler velocity was corrected for the angle of insonation. Volumetric flow was calculated by multiplying the area of the insonated blood vessel by the time-averaged mean velocity over at least three consecutive beats. Renal O_2 delivery was calculated from the product of RBF and Ca_{O_2} [Ca_{O_2} = Hb (g/dl) × Sa_{O_2} (%) × 1.36 (ml O_2/g)].

Sa_{O_2}. Sa_{O_2} was estimated by pulse oximetry (model 3700, Ohmeda) at sea level and after 6 and 24 h at each simulated altitude.

Statistical analysis. Values are means ± SE. Sea level values (baseline) were taken from the mean values of four measurements, which were made immediately before each simulated altitude exposure. The EPO, urine P_{O_2}, and Sa_{O_2} data at different altitudes and times were analyzed by means of a two-way (repeated-measure) analysis of variance. The Newman-Keuls post hoc test was used for multiple comparisons between variables. Linear regression analysis and correlation coefficients were used to assess the relationships between variables. Comparison and correlation were considered significant when $P < 0.05$.

RESULTS

EPO. EPO was 14.3 ± 0.8 mU/ml at sea level; it increased significantly after 6 h at all four simulated altitudes and then declined slightly after 24 h at 1,780 and 2,085 m. In contrast to the two lowest altitudes, mean EPO concentration continued to increase significantly after 24 h at 2,454 and 2,800 m (Fig. 1*A*). There was marked individual variability in EPO release at all altitudes (Fig. 2) but generally consistent responses among individuals (i.e., those individuals with the greatest responses to the lowest altitudes had the greatest responses to the highest altitudes). For example, the coefficient of variation (standard deviation/mean) for the percent change from baseline after 24 h at 2,800 m was 0.83.

Urine P_{O_2}. Changes in urine P_{O_2} are shown in Fig. 1*C*. The mean value of urine P_{O_2} at sea level was 84.7 ± 1.7 Torr; similar to the pattern for EPO, it decreased after 6 h at all altitudes and returned rapidly to near baseline by 24 h at 1,780 and 2,085 m but remained lower at 2,454 and 2,800 m. There was a highly significant curvilinear (2nd-order regression) relationship between the mean values for urine P_{O_2} and EPO by 24 h at all four simulated altitudes (r^2 = 0.992; Fig. 3). When examined on an individual basis, the median r^2 for this relationship was 0.87 (range 0.11–1.00).

Sa_{O_2}. Sa_{O_2} at sea level was 98.1 ± 0.4%, decreased slightly but significantly after 6 h at 1,780 and 2,085 m (1.1 and 1.6%, respectively), and decreased to a greater extent at 2,454 and 2,800 m (4.8 and 5.4%, respectively). It returned to baseline by 24 h at the two lowest altitudes but remained significantly reduced at the two highest altitudes (Fig. 1*B*). The magnitude of desaturation from sea level after 24 h at each simulated altitude (ΔSa_{O_2}) was correlated to the degree of the increase in EPO (ΔEPO) rise (r^2 = 0.17, $P < 0.05$; Fig.

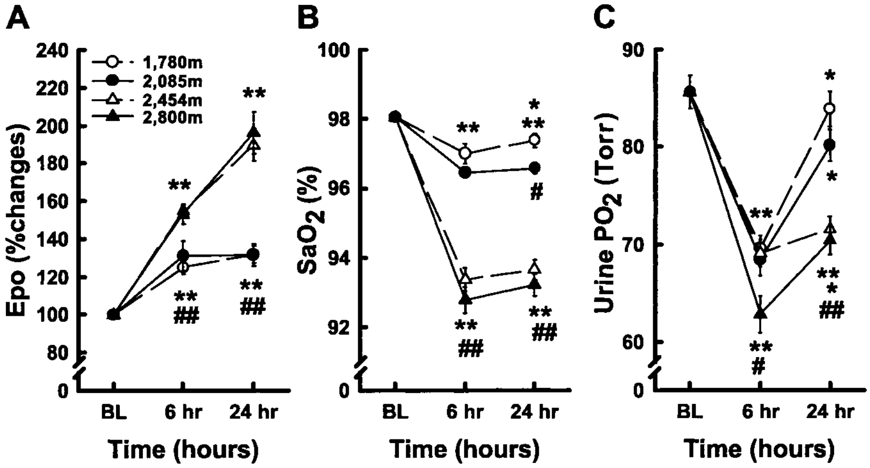

Fig. 1. Changes in erythropoietin (EPO), arterial oxyhemoglobin saturation (Sa_{O_2}), and Po_2 in urine from sea level (baseline, BL) and after 6 and 24 h at simulated altitudes of 1,780, 2,085, 2,454, and 2,800 m in 48 subjects. Values are means ± SE. Because data for 1,780 and 2,085 m and 2,454 and 2,800 m behave similarly, double symbols for significance mean that both groups achieved the same level of significance: $^{**}P <$ 0.05 compared with baseline for 6 and 24 h; $^{\#\#}P < 0.05$ compared with the lower altitudes. In *B* and *C*, double symbols represent the same comparison as in *A*; single symbols represent comparisons that are valid only for that specific altitude and are placed directly over the data symbol: $^{*}P < 0.05$ compared with the previous time point; $^{\#}P < 0.05$ compared with the lower altitude.

4). Thus the change in Sa_{O_2} accounted for <20% of the variability in EPO concentration.

RBF. RBF values are shown in Table 1. There was no significant difference in RBF at 6 h compared with sea level values at all simulated altitudes. When Sa_{O_2} and Hb were factored in (i.e., renal O_2 delivery), there was no significant influence of renal O_2 delivery on EPO release at these moderate altitudes.

DISCUSSION

The principal findings of this study include the observation that short-term, sustained EPO production occurs at moderate altitude once a threshold of 2,100–2,500 m is crossed. Below this altitude, changes in EPO are modest and not sustained after 24 h of exposure in the majority of individuals. Moreover, EPO production at altitude is marked by substantial interindividual variability, governed by "upstream" factors related to renal parenchymal Po_2, as well as other undetermined mechanisms, presumably related to transcriptional regulation of EPO by renal tissue hypoxia.

Optimal threshold altitude for EPO release. EPO is a glycoprotein hormone that regulates proliferation and differentiation of erythroid cells. Its production is markedly enhanced by reductions in Ca_{O_2} mediated by anemia or hypoxia (6, 20) and certain metals such as cobalt and nickel (37). A number of physiological studies demonstrated that the concentration of EPO is substantially increased after high-altitude exposure and then gradually declines toward baseline within the first few days to weeks of exposure (21, 28, 29).

However, most studies examining the erythropoietic effect of altitude have used much higher altitudes (and, therefore, more severe hypoxia) than reported in the present study, generally >4,000 m. In contrast, the majority of occupational and recreational exposures occur at more moderate altitudes, between 2,000 and 3,000 m (23). Some populations, such as endurance athletes, routinely travel to moderate altitudes with a goal to improve sea level performance via altitude-induced erythrocytosis (17). However, cross-sectional studies by Weil et al. (35) demonstrated that an eryth-

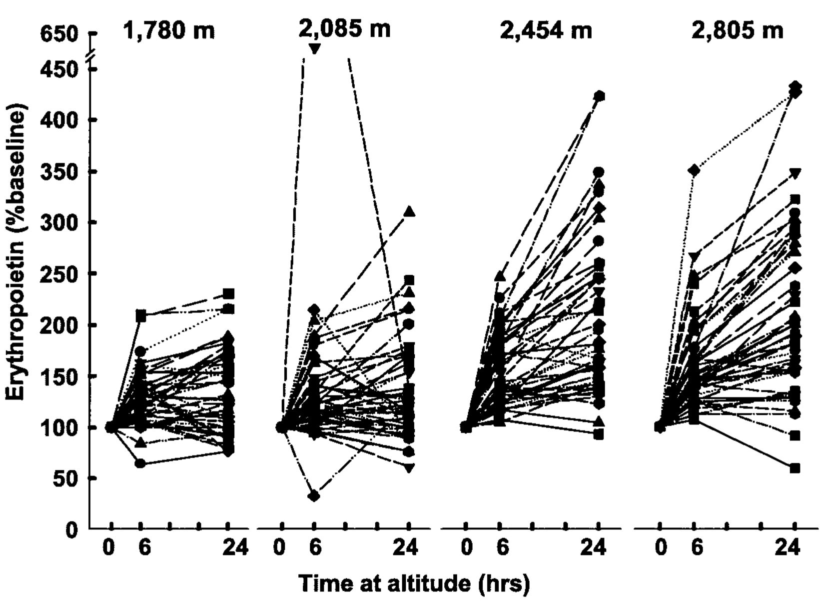

Fig. 2. Individual values for percent change in EPO from baseline in all subjects. Note marked individual variability that increases with increasing altitude.

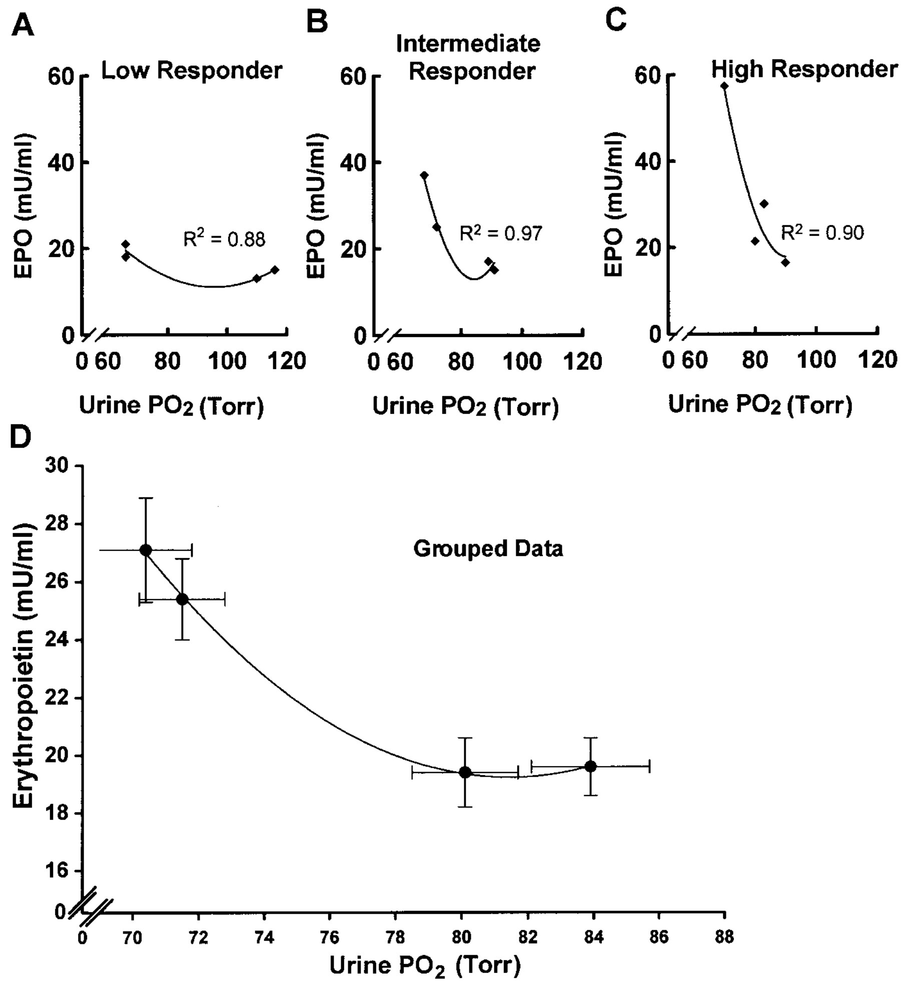

Fig. 3. *A–C*: EPO-urine P_{O_2} relationship in a subject with a relatively minimal increase in EPO, despite decreases in urine P_{O_2} (*A*), a subject with a brisk response (*C*), and a subject with an intermediate response (*B*). *D*: blood EPO levels plotted as a function of urine P_{O_2} at simulated altitudes of 1,780, 2,085, 2,454, and 2,800 m. Data were obtained from the mean group value for each altitude after 24 h (n = 48). Mean urine P_{O_2} was significantly correlated to blood EPO levels as a 2nd-order regression ($r^2 = 0.992$, $P < 0.01$).

ropoietic response to hypoxia is not achieved until Pa_{O_2} decreases to ~65–70 Torr (corresponding to an altitude of 2,500–3,000 m in sedentary individuals); red cell mass and Pa_{O_2} are linearly correlated when altitude-induced hypoxemia becomes more severe. Whether such a threshold is present in sea level natives ascending to moderate altitude is uncertain.

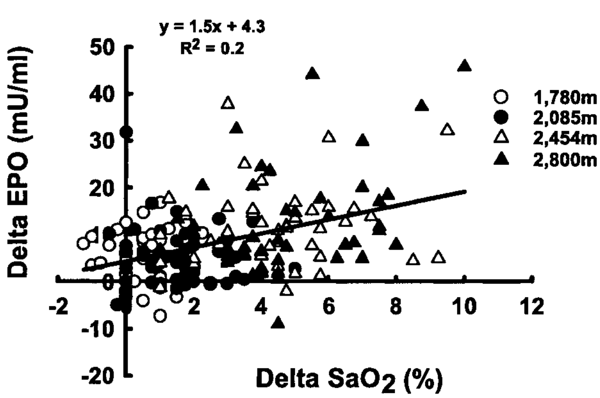

Fig. 4. Magnitude of desaturation (ΔSa_{O_2}) from baseline to simulated altitude is correlated to the degree of increase in EPO (ΔEPO). As a group, there was a modest correlation between ΔSa_{O_2} and ΔEPO by 24 h at 4 simulated altitudes (n = 192, r = 0.41, $P < 0.05$).

Previous studies at moderate altitudes have consistently demonstrated an increase in EPO of a magnitude similar to that observed in the present study in response to real or simulated altitude (normobaric or hypobaric hypoxia) of ~2,500 m (2, 4, 5, 9, 14, 17). One study (5) compared the acute (5.5 h) effect of 3,000 vs. 4,000 m in a hypobaric chamber and demonstrated a more rapid rate of rise and a proportionately greater increase in EPO at the higher altitude. The present study extends these previous observations by demonstrating what appears to be a threshold, rather than a continuous linear relationship, at lower altitudes. Specifically, the EPO levels at the two lowest altitudes increased modestly (24–30%), peaking at 6 h after exposure; in contrast, EPO increased more prominently (77–92%) at the two highest altitudes, with a continued increase after 24 h at 2,500 and 2,800 m (Fig. 1*A*). These observations suggest that altitudes >2,100–2,500 m appear to be required for stimulating sustained EPO release.

The mechanism of this differential response is likely to include the greater oxyhemoglobin desaturation that occurs as the P_{O_2} falls to the steep portion of the oxyhemoglobin dissociation curve. Thus the EPO levels at all altitudes paralleled the changes in Sa_{O_2}; the

Table 1. *Comparisons of various parameters at sea level and simulated altitude exposure*

		1,780 m		2,085 m		2,454 m		2,800 m	
	SL	6 h	24 h	6 h	24 h	6 h	24 h	6 h	24 h
EPO, μm/l	14.3 ± 5.3	18.6 ± 6.2	19.6 ± 6.9	18.9 ± 7.0	19.4 ± 8.2	19.8 ± 7.3	25.4 ± 9.8	20.9 ± 12.6	27.2 ± 5.3
uP_{O_2}, Torr	85.1 ± 11	69.6 ± 8.8	83.9 ± 12	68.4 ± 11	80.1 ± 11	69.1 ± 9.7	71.6 ± 8.7	62.9 ± 13.4	70.4 ± 9.8
Sa_{O_2}, %	98.1 ± 0.4	97.1 ± 0.9	97.4 ± 0.8	96.5 ± 1.0	96.6 ± 1.5	93.4 ± 2.3	93.7 ± 2.0	92.8 ± 2.5	93.2 ± 2.5
RBF, ml/min	326 ± 154	398 ± 102		396 ± 92		342 ± 121		362 ± 103	

Values are means ± SD. EPO, erythropoietin; uP_{O_2}, urine P_{O_2}, Sa_{O_2}, arterial O_2 saturation; RBF, renal blood flow.

magnitude of desaturation at altitude was correlated to the degree of the increase in EPO, suggesting that there may be an inadequate hypoxic stimulus for the production of EPO at <2,100 m. However, the strength of this relationship was relatively weak, with a correlation coefficient, albeit statistically significant, of only 0.42. Part of this weak relationship may be explained by the fact that the changes in Sa_{O_2}, among the different altitudes and at each altitude, compared with control were very small. For example, mean Sa_{O_2} values fell by <1% at 1,780 m, which was statistically significant as a group effect but within the limits of precision of the pulse oximeter for any individual measurement. In this regard, it is remarkable that such clear differences in EPO levels occurred, despite relatively small differences in Sa_{O_2}. The relationship between Sa_{O_2} and EPO, particularly at the earliest time point, may be further complicated by the fact that the rate of EPO production in response to altitude-induced hypoxia is likely to be variable depending on the absolute degree of hypoxia achieved (5).

One additional confounding factor may be that the renovascular bed autoregulates sufficiently to maintain adequate renal O_2 delivery, despite systemic hypoxia. In the present study, RBF after 6 h of exposure to simulated high altitude was not significantly different from RBF at sea level. Moreover, EPO levels at all simulated altitudes were not correlated with renal O_2 delivery, incorporating measures of RBF and Ca_{O_2}. These data suggest that, within the limits of our methodology to detect changes in RBF, renal O_2 delivery, by itself, is not a strong determinant of EPO production in humans, as has been demonstrated in animal studies (25).

Urine P_{O_2} as an estimate of renal tissue P_{O_2}. However, renal O_2 delivery reflects only one side of the equation: ultimately, renal parenchymal P_{O_2}, a function of renal O_2 delivery minus renal O_2 utilization, appears to be the essential determinant of EPO production (11, 37). Thus renal vein or urine P_{O_2} as an estimate of renal tissue P_{O_2} may be a better marker of the stimulus for EPO release than Sa_{O_2} or renal O_2 delivery (24). To our knowledge, this is the first study to measure urine P_{O_2} in humans at different altitude exposures, although other investigators have demonstrated increases in urine P_{O_2} during increases in P_{O_2} mediated by hyperoxia (13, 18, 27). Moreover, graded reductions in RBF lead to similarly graded reductions in urinary P_{O_2}, confirming the dependence of urine P_{O_2} on renal O_2 availability (13). According to the concept of countercurrent gas diffusion (27), urine P_{O_2} in the collecting ducts is lower than the venous P_{O_2}. The estimated renal venous P_{O_2} at sea level is ~70–80 Torr in humans (27) and 50–60 Torr in rats (24). Renal tissue EPO and plasma EPO in rats have been correlated to renal venous P_{O_2} during exposure to chronic hypoxia in a curvilinear relationship similar to that observed in humans in the present study (24).

A number of technical issues must be considered in the interpretation of measurements of urinary P_{O_2}. First, it should be recognized that the kidney is not uniform in its oxygenation, with gradations of P_{O_2} from the outer cortex to the inner medulla (16). Moreover, urine P_{O_2} is sensitive to hydration state, with most (16, 26), but not all (18), studies showing an increase in urine P_{O_2} with increasing degrees of hydration and/or urine flow rates. Conversely, if urine flow rates are too low, i.e., <3 ml/min, there is significant uptake of O_2 in the walls of the ureters and bladder, reducing bladder P_{O_2} compared with renal pelvic P_{O_2} (27); flow rates of ≥5 ml/min are necessary to ensure that urethral collections reflect the P_{O_2} from the renal pelvis (27). Finally, a prominent water diuresis (26) or saline loading (18) can influence the results by increasing or decreasing urine P_{O_2}, respectively. Fortunately, when hydration status is carefully controlled and urine flow rates are neither too low nor too high, urine P_{O_2} is very reproducible in any given subject from day to day and is a reasonable reflection of renal medullary oxygenation (8, 18, 26). In the present study, baseline sea level measurements of urine P_{O_2} were reproducible from week to week and similar to data reported by other investigators (8).

In our study, the urine P_{O_2} was significantly decreased by 6 h at all altitudes and then rapidly returned to near baseline by 24 h at the two lowest altitudes and remained significantly low at the two highest altitudes (Fig. 1*C*), closely paralleling the changes in arterial oxyhemoglobin saturation. The threshold above and below 2,100 m that was clearly evident in EPO and Sa_{O_2}, even at the first time point (6 h), was not manifest until 24 h for the urine P_{O_2} data. Although we cannot determine with certainty the mechanism for this differential response, we speculate that the different time courses of ventilatory acclimatization, neurohumoral activation, and renal metabolic compensation may be at least partly responsible for this inconsistency. The very small differences among the altitudes studied and the resultant experimental noise may also be playing a role at this early stage, before the overall response becomes more complete after a full 24 h of exposure.

We suspect, but cannot prove, that the short-term acclimatization response (primarily ventilatory acclimatization) appears to restore renal tissue oxygenation rapidly and restrain the rise in EPO at the lowest altitudes. For example, we did not measure ventilation or end-tidal CO_2 concentrations and thus have only indirect evidence that ventilatory acclimatization occurred at these low altitudes. However, we can say that although the magnitude of the hypoxia (as estimated from Sa_{O_2}) experienced at the lowest altitudes was modest, the consistent decrease in urine P_{O_2} and subsequent rise in EPO provide compelling evidence that this hypoxia was physiologically significant at all altitudes and likely to stimulate peripheral chemoreceptors. Most dramatically, on average, changes in urine P_{O_2} were strictly proportional to EPO levels at all four altitudes ($r^2 = 0.992$). This strong, possibly deterministic, relationship supports the hypothesis that renal tissue P_{O_2} is an essential factor for determining EPO production in humans at high altitude.

Individual variability of EPO production. Despite this tight relationship for the mean group data, the individual relationships between urine P_{O_2} and EPO were highly variable (Fig. 3). Moreover, EPO levels in response to short-term simulated altitude exposure demonstrated marked interindividual variability, ranging from −41 to 400% after 24 h of exposure to 2,800 m (Fig. 2). Intriguingly, the EPO response was generally consistent among individuals; i.e., those individuals with the greatest response to the lowest altitudes had the greatest response to the highest altitudes. Some insight into possible mechanisms for this variability may be found in a recent animal study in which blood EPO and renal tissue EPO in response to acute or chronic hypoxic exposure were dramatically different in two rat strains, suggesting that genetic factors may be involved in the regulation of the EPO response (24). In this study, the curvilinear relationship between renal tissue P_{O_2} and EPO, as well as a marked difference between strains in the transcription of mRNA for EPO in response to a given renal tissue P_{O_2}, argued strongly for transcriptional regulation of EPO synthesis at altitude and was remarkably similar to the data found in the present study in humans.

There is increasing evidence that hypoxia-induced physiological changes depend on intracellular O_2 sensors that are present in most mammalian cells (34, 37). A specific O_2 sensor has been identified in the carotid body (19), neuroepithelial bodies (3), and other cells with O_2 sensitivity (30, 32), although it remains unclear how the kidney responds precisely to O_2 content. EPO gene expression is regulated primarily at the level of transcription in these O_2-sensitive cells. This O_2-sensing system (by hypoxic exposure) triggers production of hypoxia-inducible factor-1α (33), a major transcription factor that binds to the human EPO gene 3′-flanking region and initiates transcriptional activation of the EPO gene in the hypoxic cells of the kidneys (34). In light of these observations, we speculate that these transcriptional and posttranscriptional mechanisms for governing the regulation of EPO gene expression may be playing a substantial role in the observed individual variability.

In summary, we conclude that short-term, sustained EPO production in response to simulated altitude has a clear threshold: altitudes >2,100–2,500 m are required to stimulate a sustained increase in group EPO concentrations over 24 h. Despite this population effect, EPO production at altitude is marked by substantial interindividual variability, with some individuals exhibiting ~100% increases in EPO levels to exposures of 1,780 m, whereas others did not increase EPO levels in response to 2,805 m. This variability appears to be governed by upstream factors related to renal parenchymal P_{O_2}, as well as by other undetermined mechanisms, presumably related to transcriptional regulation of EPO by renal tissue hypoxia.

REFERENCES

1. **Abbrecht PH and Littell JK.** Plasma erythropoietin in men and mice during acclimatization to different altitudes. *J Appl Physiol* 32: 54–58, 1972.
2. **Ashenden MJ, Gore CJ, Dobson GP, Boston TT, Parisotto R, Emslie KR, Trout GJ, and Hahn AG.** Simulated moderate altitude elevates serum erythropoietin but does not increase reticulocyte production in well-trained runners. *Eur J Appl Physiol* 81: 428–435, 2000.
3. **Bunn HF and Poyton RO.** Oxygen sensing and molecular adaptation to hypoxia. *Physiol Rev* 76: 839–885, 1996.
4. **Chapman RF, Stray-Gundersen J, and Levine BD.** Individual variation in response to altitude training. *J Appl Physiol* 85: 1448–1456, 1998.
5. **Eckardt K, Boutellier U, Kurtz A, Schopen M, Koller E, and Bauer C.** Rate of erythropoietin formation in humans in response to acute hypobaric hypoxia. *J Appl Physiol* 66: 1785–1788, 1989.
6. **Eckardt KU, Koury ST, Tan CC, Schuster SJ, Kaissling B, Ratcliffe PJ, and Kurtz A.** Distribution of erythropoietin producing cells in rat kidneys during hypoxic hypoxia. *Kidney Int* 43: 815–823, 1993.
7. **Faura J, Ramos J, Reynafarje C, English E, Finne P, and Finch CA.** Effect of altitude on erythropoiesis. *Blood* 33: 668–676, 1969.
8. **Giannakopoulos X, Evangelou A, Kalfakakou V, Grammeniatis E, Papandropoulos I, and Charalambopoulos K.** Human bladder urine oxygen content: implications for urinary tract diseases. *Int Urol Nephrol* 29: 393–401, 1997.
9. **Gunga HC, Kirsch K, Rocker L, and Schobersberger W.** Time course of erythropoietin, triiodothyronine, thyroxine, and thyroid-stimulating hormone at 2,315 m. *J Appl Physiol* 76: 1068–1072, 1994.
10. **Hurtado A, Merino C, and Delgado E.** Influence of anoxemia on the hemopoietic activity. *Arch Intern Med* 75: 284–323, 1945.
11. **Jelkman W.** Erythropoietin: structure, control of production, and function. *Physiol Rev* 72: 449–489, 1992.
12. **Jelkmann W and Seidl J.** Dependence of erythropoietin production on blood oxygen affinity and hemoglobin concentration in rats. *Biomed Biochim Acta* 46: S304–S308, 1987.
13. **Kainuma M, Kimura N, and Shimada Y.** Effect of acute changes in renal arterial blood flow on urine oxygen tension in dogs. *Crit Care Med* 18: 309–312, 1990.
14. **Koistinen PO, Rusko H, Irjala K, Rajamaki A, Penttinen K, Sarparanta VP, Karpakka J, and Leppaluoto J.** EPO, red cells, and serum transferrin receptor in continuous and intermittent hypoxia. *Med Sci Sports Exerc* 32: 800–804, 2000.
15. **Lechermann B and Jelkmann W.** Erythropoietin production in normoxic and hypoxic rats with increased blood O_2 affinity. *Respir Physiol* 60: 1–8, 1985.

16. **Leonhardt KO and Landes RR.** Oxygen tension of the urine and renal structures: preliminary report of clinical findings. *N Engl J Med* 269: 115–121, 1963.
17. **Levine BD and Stray-Gundersen J.** "Living high-training low": effect of moderate-altitude acclimatization with low-altitude training on performance. *J Appl Physiol* 83: 102–112, 1997.
18. **Lindner A and Cutler RE.** Studies of urinary P_{O_2} in humans. *Investig Urol (Berl)* 11: 21–27, 1973.
19. **Lopez-Barneo J, Pardal R, Montoro RJ, Smani T, Garcia-Hirschfeld J, and Urena J.** K^+ and Ca^{2+} channel activity and cytosolic $[Ca^{2+}]$ in oxygen-sensing tissues. *Respir Physiol* 115: 215–227, 1999.
20. **Maxwell AP, Lappin TR, Johnston CF, Bridges JM, and McGeown MG.** Erythropoietin production in kidney tubular cells. *Br J Haematol* 74: 535–539, 1990.
21. **Milledge JS and Cotes PM.** Serum erythropoietin in humans at high altitude and its relation to plasma renin. *J Appl Physiol* 59: 360–364, 1985.
22. **Miyamoto T, Hagio M, Mwanza T, Kobayashi T, Okumura M, and Fujinaga T.** Quantitative measurement of canine renal arterial blood flow using Doppler ultrasonography. *J Vet Med Sci* 57: 785–788, 1995.
23. **Moore LG.** Altitude-aggravated illness: examples from pregnancy and prenatal life. *Ann Emerg Med* 16: 965–973, 1987.
24. **Ou LC, Salceda S, Schuster SJ, Dunnack LM, Brink-Johnsen T, Chen J, and Leiter JC.** Polycythemic responses to hypoxia: molecular and genetic mechanisms of chronic mountain sickness. *J Appl Physiol* 84: 1242–1251, 1998.
25. **Pagel H, Jelkmann W, and Weiss C.** O_2 supply to the kidneys and the production of erythropoietin. *Respir Physiol* 77: 111–118, 1989.
26. **Prasad PV and Epstein FH.** Changes in renal medullary P_{O_2} during water diuresis as evaluated by blood oxygenation level-dependent magnetic resonance imaging: effects of aging and cyclooxygenase inhibition. *Kidney Int* 55: 294–298, 1999.
27. **Rennie DW, Reeves RB, and Pappenheimer JR.** Oxygen pressure in the urine and its relation to intrarenal blood flow. *Am J Physiol* 195: 120–132, 1958.
28. **Richalet JP, Souberbielle JC, and Antezana AM.** Control of erythropoiesis in humans during prolonged exposure to the altitude of 6,542 m. *Am J Physiol Regulatory Integrative Comp Physiol* 266: R756–R764, 1994.
29. **Savourey G, Garcia N, Besnard Y, Guinet A, Hanniquet AM, and Bittel J.** Pre-adaptation, adaptation and de-adaptation to high altitude in humans: cardio-ventilatory and hematological changes. *Eur J Appl Physiol* 73: 529–535, 1996.
30. **Schuster SJ, Koury ST, Bohrer M, Salceda S, and Caro J.** Cellular sites of extrarenal and renal erythropoietin production in anaemic rats. *Br J Haematol* 81: 153–159, 1992.
31. **Stray-Gundersen J, Chapman RF, and Levine BD.** The "living high-training low" altitude training paradigm improves sea level performance in elite male and female runners. *J Appl Physiol* 91: 1113–1120, 2001.
32. **Suzuki T and Sasaki R.** Immunocytochemical demonstration of erythropoietin immunoreactivity in peritubular endothelial cells of the anemic mouse kidney. *Arch Histol Cytol* 53: 121–124, 1990.
33. **Wang GL, Jiang BH, Rue EA, and Semenza GL.** Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O_2 tension. *Proc Natl Acad Sci USA* 92: 5510–5514, 1995.
34. **Wang GL and Semenza GL.** Molecular basis of hypoxia-induced erythropoietin expression. *Curr Opin Hematol* 3: 156–162, 1996.
35. **Weil JV, Jamieson G, Brown DW, and Grover RF.** The red cell mass-arterial oxygen relationship in normal man. *J Clin Invest* 47: 1627–1639, 1968.
36. **Yura T, Yuasa S, Fukunaga M, Badr KF, and Matsuo H.** Role for Doppler ultrasound in the assessment of renal circulation: effects of dopamine and dobutamine on renal hemodynamics in humans. *Nephron* 71: 168–175, 1995.
37. **Zhu H and Bunn HF.** Oxygen sensing and signaling: impact on the regulation of physiologically important genes. *Respir Physiol* 115: 239–247, 1999.