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ERYTHROPOIESIS IN HUMANS EXPOSED TO SEVERE ALTITUDE HYPOXIA.

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ABSTRACT

Some of the factors involved in the control of altitude polycythemia were studied in ten subjects (4 women, 6 men) exposed for 3 weeks to extreme altitude (6542 m). Blood was withdrawn in normoxia (N), after one (H1), two (H2) and three (H3) weeks at 6542 m, for the measurement of serum erythropoietin (EPO, mU/ml), blood hemoglobin (Hb, g/dl), hematocrit (Ht, %), intra-erythrocyte folate (FOL, ug/l) and plasma ferritin (FER, ug/l) concentrations. Renal absolute proximal reabsorption rate (APR) were measured by the lithium clearance method, in N and H2 conditions. After an initial sharp increase in EPO (N: 8 ± 2, H1: 302 ± 282, mean ± S.D.), EPO decreased at H2 (161 ± 151) and H3 (174 ± 212). Ht and Hb increased from N (43 ± 3; 13.8 ± 0.7) to H1 (51 ± 6, 15.7 ± 2.3) and H2 (53 ± 7, 16.1 ± 2.3) and then decreased from H2 to H3 (49 ± 7, 15.0 ± 2.5). Increase in EPO at H1 varied from 3 to 134-fold among individuals. Two women showed a large increase in EPO without increase in Hb. FER showed a marked decrease in H1 (8.1 ± 4.9) and H3 (9.2 ± 3.8) as compared to N (28.0 ± 24.5). Hb was positively related to FER in hypoxia. Iron intake in food was markedly decreased during the 2 weeks of ascent, before arriving at 6542 m. EPO was inversely related to CaO_2 and positively related to APR. The increase in Hb at H1 may have restored the oxygen availability in the kidneys and reduced the formation of EPO. The decrease in Hb from H2 to H3, in spite of a high EPO, may be due to a chronically reduced substrate (iron) availability, as suggested by the decrease in FER favoured by a low iron in take. We conclude that there is a great interindividual variability in erythropoiesis response to EPO in hypoxia. Factors involved in the modulation of this response include nutritional and sex differences, iron stores and tubular function that determines O_2 supply to renal sensors responsible for EPO secretion. **(Acta Andina 1996, 5:35-40)**

Key words: hypoxia, erythropoietin, hemoglobin, folate, ferritin, renal blood flow, renal tubular function.

RESUMEN

La hipoxia de altura estimula la eritropoyesis. Se estudiaron algunos de los factores que intervienen en el control de la poliglobulia de altura en diez sujetos (4 mujeres y 6 varones) expuestos a 6.542m durante tres semanas. Se obtuvieron muestras sanguíneas en condiciones de normoxia (N), después de una (H1), dos (H2) y tres (H3) semanas a 6,542m para dosificación de hemoglobina (Hb), hematocrito (Ht), eritropoyetina sérica (EPO), ferritina plasmática (Fer). Se midió la tasa de reabsorción proximal absoluta (APR) mediante clearance de litio en las condiciones N y H2. EPO aumentó inicialmente y disminuyó en H2 y H3; este incremento varió de 3 a 134 veces según los individuos. La Hb y el Ht aumentaron de N hasta H2, y curiosamente disminuyeron de H2 a H3. La Fer disminuyó en H1 y H3. El aporte en hierro disminuyó substancialmente durante la segunda semana del ascenso. En las mujeres se observó: el mayor aumento de EPO, y el menor incremento de Hb y Ht. Se encontró una relación inversa entre EPO y CaO_2, asi como una relación positiva entre EPO y APR. El aumento de la Hb en H1 pudo haber restituído la disponibilidad del oxígeno a nivel renal, disminuyendo la síntesis de EPO. La disminución de la Hb de H2 a H3, a pesar de niveles altos de EPO, puede explicarse por la reducción crónica del substrato (hierro), como lo sugiere la disminución de la ferritina. Existe una gran variabilidad interindividual de la respuesta hematopoyética a la EPO en hipoxia. Los factores implicados en esta modulación incluyen diferencias según el sexo, el estado nutricional y la reserva en hierro, asi como la función tubular renal, que determina el aporte de oxígeno a nivel de las células renales productoras de EPO. **(Acta Andina 1996, 5:35-40)**

Palabras claves: hipoxia, eritropyetina, hemoglobina, folato, ferritina, flujo sanguíneo renal, función tubular renal.

The altitude-induced increase in the number of erythrocytes is considered as the main feature of human long term acclimatization to high altitude [12;15;16]. However, the optimal range for hemoglobin concentration at high altitude has been questionned, at least in high altitude natives [16;17]. The role of erythropoietin (EPO) in the polycythemia induced by high altitude has been studied in humans by various authors [1,2,5,8,14]. EPO concentration in serum increases as soon as 90 minutes after induction of hypoxia [2], rises progressively during the first 48 hours [1], then declines to a lower level, but still above basal normoxic values [3]. To our knowledge, no attempt has yet been made to better understand the various factors involved in the human erythropoietic response to EPO in high altitude conditions.

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Iron or vitamin stores were proved as essential for a normal response to EPO stimulation in other pathologic conditions associated with anaemia. Renal hemodynamics or proximal tubular function may also interfere with EPO secretion [4]. The sympathetic nervous system, activated by altitude hypoxia, interacts with EPO production [6]. Most studies have been performed in male subjects and no data are available on hematological response of women at high altitude. The purpose of the present study was to evaluate the respective role of the above factors (nutrition, kidney, sex), liable to control EPO secretion and erythrocyte production in severe altitude hypoxia. Results were published in details elsewhere [13].

Subjects and Methods

Ten healthy subjects (4 female and 6 male, aged 35 ± 6, 27-44 yrs) participated in the study during a scientific expedition to Mount Sajama (Bolivia) organized by the "Association pour la recherche en physiologie de l'environnement". Nine subjects were sea-level natives, one subject (woman) was native from La Paz (3600 m) and was resident at low altitude (Paris, France) for 6 years before the expedition. All subjects were moderately trained and had a previous experience of high altitude. They were nonsmoking or occasionally smoking subjects. Two subjects had vegetarian habits at sea level (#2 and #3). Subjects were flown from France to La Paz (3600 m) where they stayed 5 days , then reached by car the altitude of 4200m within 3 days. From there, they walked and climbed for 10 days to reach the summit at 6542 m, where they installed the laboratory and stayed for 3 weeks, without intense physical activity (Fig. 1).

Effectiveness of acclimatization was evaluated daily by a clinical score of acute mountain sickness (AMS) [13]. Blood was withdrawn from an antecubital vein after a 30-min rest in normoxia (N) and after one (H1), two (H2) and three (H3) weeks at 6542 m for the measurement of serum immunoreactive EPO (125 I-EPO COATRIA, BioMérieux, France). Hematocrit (Ht) and hemoglobin concentration (Hb) were measured by means of a microcentrifuge and a microphotometer respectively. Serum ferritin concentration was measured in H1 and H3 conditions, using an immunoradiometric assay (RIA-gnost Ferritin, Behring, Germany). Food intake was evaluated by means of a personal diary where subjects wrote down all their food and liquid intake during three consecutive days in four conditions : normoxia (N), during the ascent period between 4200 and 6542 m (HA), during the first (H1) and the third (H3) week at 6542 m; detailed results are presented elsewhere [13]. Renal function was explored by means of para-amino-hippurate, inulin and lithium clearances measured in N and H2 conditions. Methodology of clearance measurements is presented elsewhere; absolute proximal reabsorption rate (APR) is the difference between glomerular filtration rate and lithium clearance [13]. Arterial O_2 saturation (SaO_2) was evaluated by ear oximetry, allowing the calculation of arterial O_2 content (CaO_2). Values are means ± S.D. Statistical differences between normoxic and hypoxic conditions were assessed by an analysis of variance with repeated measures. Linear regressions between increase in EPO and various parameters were calculated. A p value of less than 0.05 was considered to be statistically significant.

Results

AMS was slightly felt by the subjects when arriving at La Paz and rapidly disappeared with acclimatization (Fig. 1).

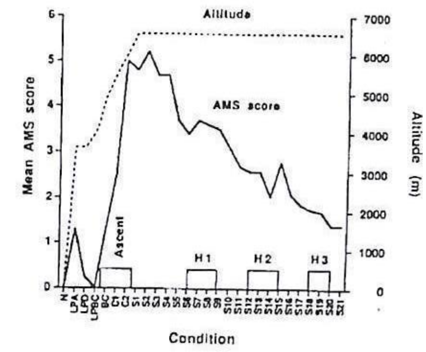

Fig. 1. Ascent profile (Altitude, broken line) and mean score of acute mountain sickness (AMS score, solid line). N: normoxia, LPA, LPD: La Paz, arrival and departure; LPBC: La Paz to base camp; BC: base camp; C1: camp 1 (5500m); C2: camp 2, (6000m); S1 to S21: days at the summit lab (6542m). Ascent period from 4200 to 6542m; H1, H2 and H3 : periods of experiments at 6542m [Modified from ref. 13].

Maximal scores were observed during the ascent, above 6000 m, and during the first 4 days at 6542 m. Scores progressively declined during the stay in the summit. Severe hypoxemia was evidenced by low values of SaO_2 in altitude conditions. After a sharp increase at H1, EPO decreased in most subjects from H1 to H2 and remained stable between H2 and H3. There was a great variability in individual EPO response to the hypoxia. In H1, EPO was 3 to 134-fold greater than in N condition depending of the subject. As expected, Ht and Hb increased from N to H1, then plateaued from H1 to H2 and unexpectedly decreased in all subjects from H2 to H3. Sex differences were evidenced in the hematological response to high altitude [13]. When comparing N and H2, women showed a smaller increase than men in Ht (+14% vs 32%) and Hb (+4% vs 24%). EPO decreased between H1 and H2 or H3 in male but remained high in female subjects during the whole stay at high altitude. Individual response to EPO is illustrated in Fig. 2, showing for each subject the increase in hemoglobin concentration at H1, as the physiologic response of bone marrow to EPO.

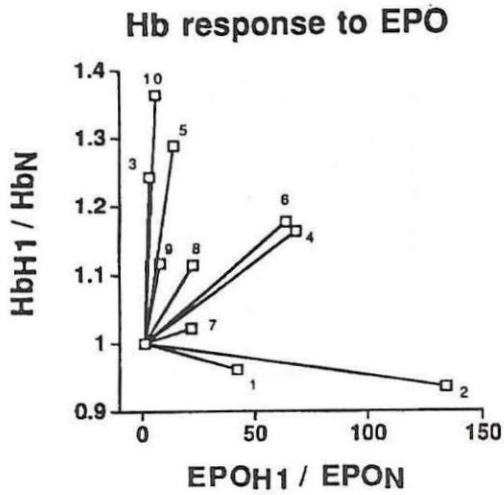

Fig. 2. Ratio of Hb concentration in H1 condition over Hb in normoxia, as a function of the same ratio for erythropoietin. The variation from N to H1, for each of the 10 subjects, is presented : female subjects are # 1, 2, 8 and 9. [Modified from ref. 13].

The slope of each line, illustrating the sensitivity of the erythropoietic response to EPO, is variable among subjects: subjects #3, 5, 10 and 9 are "brisk responders", subjects #8, 6, 4 and 7 are "moderate responders" and subjects #1 and 2 are "low responders". The four female subjects are #1, 2, 7 and 8. Serum ferritin concentrations decreased from normoxia to H1 and H3 by 71% and 67% respectively, reaching values far lower than normal range (Table 1).

Table 1. Hematological parameters and related hormones and substrates.

	Normoxia	H1	H2	H3
SaO_2	$97.2 \pm 1.2^{+++}$	$70.1 \pm 6.1^{+++}$	$74.4 \pm 6.8^{+++,*}$	$77.7 \pm 8.5^{+++}$
Ht	42.6 ± 2.9	$50.9 \pm 6.3^{+++}$	$53.2 \pm 6.7^{+++}$	$49.1 \pm 7.1^{++,\circ}$
Hb	13.8 ± 0.7	$15.7 \pm 2.3^{++}$	$16.0 \pm 2.3^{+++}$	$15.0 \pm 2.5^{\circ}$
EPO	8 ± 2	$302 \pm 282^{++}$	$161 \pm 151^{++,*}$	$174 \pm 212^{++}$
NEp	333 ± 129	1404 ± 525+++	/	$1165 \pm 653^{++}$
Ferritin	28.0 ± 24.5	$8.1 \pm 4.9^{++}$	/	$9.2 \pm 3.8^{++}$
Proteins	74 ± 5	75 ± 5	/	74 ± 4

SaO_2: arterial oxygen saturation (%), Ht: hematocrit (%), Hb: hemoglobin concentration (g/dl), EPO: serum erythropoietin concentration (mU/ml), NEp (pg/ml): norepinephrine, ferritin (µg/l), plasma protein concentration (g/l). Mean ± SD, n=10, H1, H2 or H3 vs normoxia : +: $p<0.05$, ++: $p<0.01$, +++: $p<0.001$. H2 vs H1 : *: $p<0.05$, H3 vs H2 : °: $p< 0.001$.

Iron intake in food was markedly decreased during the ascent period (HA), in parallel to the overall food intake [13]. There was a significant relationship between increase in EPO at high altitude and decrease in CaO_2; increase in EPO was related to the increase in APR (Fig. 3).

Discussion

The altitude of 6542m was the most severe hypoxic stress ever studied in humans, with respect to serum EPO concentrations. Very high values of EPO were observed, due to severe hypoxia evidenced by very low values of SaO_2. They were higher, in most subjects, than values obtained in alpinists after 2 to 4 weeks at 6300m on Mount Everest [8]. As shown by the decline in EPO during the stay at 6542m, the oxygen sensor adapts to the hypoxic stimulus by an unknown mechanism. Different hypotheses can be evoked to explain this decline in EPO: 1) a negative feedback involving an effect of the hormone itself on its production; 2) the existence of an inhibitory factor of erythropoiesis [9]; 3) modulation by factors progressively released in hypoxia (adenosine, prostaglandins, norepinephrine, cyclic AMP, free O_2 radicals). Stimulation of the adrenergic system seems to enhance hypoxia-induced EPO production by the kidney [6]. As expected, norepinephrine concentrations were elevated in hypoxic conditions, probably enhancing hypoxia-induced EPO production [6]. Thus, a decrease in adrenergic activation cannot be put forward to explain the decrease in EPO from H1 to H3. A down regulation of adenosine and adrenergic receptors on the membrane of renal peritubular cells could be responsible for a decrease in EPO production with prolonged hypoxia, as previously shown in rat heart [7;10]. The inverse relationship between the increase in serum EPO and CaO_2 is an evidence of hypoxemia as a main determinant of EPO production (Fig. 3).

The improvement in CaO_2 due to acclimatization could account by itself for a lowered hypoxic signal to the O_2 sensitive cells producing EPO. High and low responders can be described. Subjects with the lowest Hb response to EPO were two female (#1 and #2). Altitude-induced modifications in plasma volume depend on the accomplishment of acclimatization processes: water retention at high altitude may interfere with our results and partly account from low Hb values in subjects

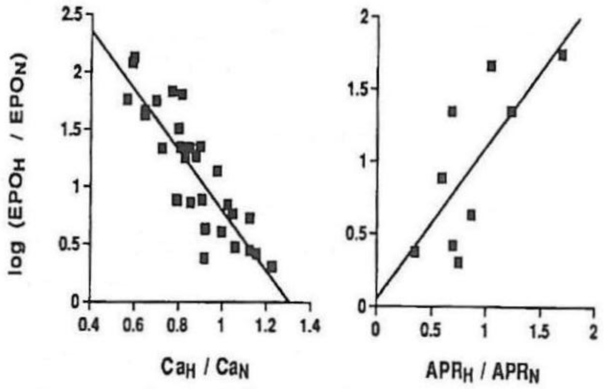

Fig. 3. Increase in EPO from normoxic value as a function of increase in arterial oxygen content (Ca, left: $y = 3.40-2.61.x$, $r = -0.86$, $p=0.0001$, $n=30$), absolute proximal reabsorption rate (APR, right: $y = 0.051 + 1.05.x$, $r = 0.74$, $p = 0.024$, $n=9$) (Ca=CaO2 for a simplified presentation). [Modified from ref. 13].

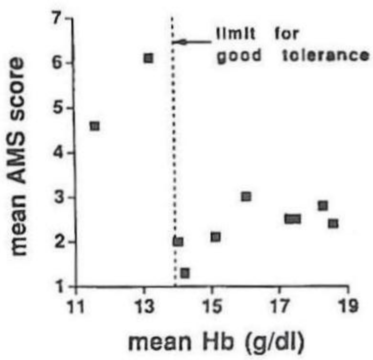

Fig. 4. Mean AMS score plotted versus mean value of hemoglobin concentration in H1, H2 and H3 conditions. [Modified from ref. 13].

suffering from AMS [11]. Dehydration is not likely to account for hemoconcentration since plasma protein concentration was not modified [13]. Decrease in Hb and Ht from H2 to H3 can be due to a decrease in bone marrow response to EPO: this resistance to EPO can be related to a decrease in substrate availability as shown by the strongly lowered ferritin content. The iron deficit in a period of high demand is not compensated by an increased iron intake in food and is parallel to the well-known decrease in overall food intake in hypoxia. The question of an optimal value for hemoglobin concentration is raised. Whereas anaemia is considered as a contra-indication to a stay at high altitude, excessive polycythemia (Hb > 23 g/dl) in high altitude dwellers is detrimental [16;17]. The optimum Hb at altitudes above 5000 m would be in the region of 18 g/dl [16]. However no systematic study is available whichcorrelates a wide range of Hb values with AMS or physical performance. In the present study, the two subjects with the lowest mean Hb values (#1 and #2) had the highest mean AMS score (Fig. 4).

In the range from 14 to 18.6 g/dl, no relation seems to exist between Hb and AMS. It can be concluded that a minimum value for Hb is necessary for a good acclimatization (> 14 g/dl) and that no beneficial or detrimental effect of Hb increase can be observed when Hb is lower than around 19 g/dl. Pre-expedition serum ferritin levels might be determinant for a normal erythropoiesis during altitude exposure. A control of this parameter should be done in subjects, especially women, preparing for a high

altitude expedition and an iron suppletive treatment proposed if necessary. A minimal prior serum ferritin value of 50 ug/dl could be proposed as a safety limit [13]. Gender differences here observed may be related to lower iron stores, classically observed in women. None of the four women suffered from excessive menstrual bleeding before or during the expedition. Two out of the 6 men showed low ferritin concentrations before the expedition (8 ug/l); one of them is a vegetarian. Whatever the sense of variation of APR with altitude exposure, this parameter is well related to EPO production (Fig. 3). This close relationship is in favour of the presence, inside or in the neighbourhood of the proximal tubule, of O_2 sensitive cells the stimulation of which determines EPO production [4]. Kidney function, which has been scarcely studied in altitude hypoxia, may thus play an important role in modulating one of the major components of acclimatization of humans to hypoxia. Extrarenal O_2 sensitive mechanisms involving nervous or humoral factors could also account for the good correlation between CaO_2 and with sub-chronic AMS, EPO would remain elevated, as an index of chronic tissue hypoxia. We propose that persistent high EPO can be considered as a good marker of unaccomplished acclimatization to high altitude.

Acknowledgments

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