Correlation between Erythropoietin and Lactate in Humans during Altitude Exposure

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Abstract: The plasma concentrations of both immunoreactive erythropoietin (EPO) and lactate were determined in four healthy untrained subjects at sea level and on the 2nd or 3rd day at altitudes (1,300 and 3,500 m). The mean plasma EPO (18.8 ± 1.6 mU/ml at sea level) increased significantly on the 3rd day at 1,300 m (25.5 ± 2.0 mU/ml, p < 0.05) and showed an almost threefold increase on the 2nd day at 3,500 m (53.5 ± 3.7 mU/ml, p < 0.001). Likewise, the mean plasma lactate at 3,500 m (3.98 ± 0.27 mmol/l) was 3.6 times as high as that at sea level (1.11 ± 0.05 mmol/l) (p < 0.001). The plasma EPO concentrations were found to correlate well with the lactate concentrations at sea level and altitudes (r = 0.86, p < 0.01). These results are consistent with the well-known EPO/lactate response to altitudes and suggest that the circulating EPO concentration as well as blood lactate concentration can be used as an index of anaerobic condition. [Japanese Journal of Physiology, 50, 285–288, 2000]

Key words: altitude, blood lactate, erythropoietin.

It is well established that renal glycoprotein hormone erythropoietin (EPO) is the predominant physiological regulator of the mass of circulating erythrocytes [1, 2]. Tissue hypoxia is the primary stimulus for the de novo production of EPO. Thus, the circulating EPO level was increased in humans during high-altitude exposure [3–6]. Another consequence of acute exposure to actual or simulated high-altitude is an enhanced production of lactate by hypoxic tissues [5, 7–9]. This is compatible with the well-known Pasteur effect, in which sufficient oxygen supply suppresses glucose utilization and deprivation of oxygen increases glycolysis due to the inhibition of mitochondrial respiration. Therefore, the blood lactate has been used as an index of anaerobic condition [10, 11]. In humans, however, the simultaneous measurement of both circulating EPO and lactate in response to different altitudes has not been reported as yet. This is the first paper reporting the changes in the plasma concentrations both of EPO and lactate in humans on sudden ascent to altitudes and also a relationship between the changes in these two substances.

The subjects participating in this study were four of us (two males and two females, nonsmoking untrained scientists), healthy sea-level residents with normal renal and hepatic function. Prior to the study, informed consent was obtained from all the participants after medical examination. The physical characteristics of the subjects (mean ± SE) were as follows: age, 44.8 ± 5.1 years; height, 164.3 ± 6.7 cm; body mass,
The blood components of the subjects were as follows: packed cell volume (PCV), 0.45±0.01 l/l; hemoglobin (Hb) concentration, 14.7±0.8 g/dl; reticulocyte count, 8.9±0.4%; total protein, 7.4±0.2 g/dl; albumin, 4.8±0.1 g/dl; albumin-globulin ratio, 1.89±0.06; blood urea nitrogen, 18.6±1.4 mg/dl; creatinine, 1.0±0.1 mg/dl; aspartate aminotransferase, 16.0±2.0 U/l.

The subjects left Osaka for Kathmandu by airplane. After a 1-week stay in Kathmandu (barometric pressure, 652 mmHg; altitude, 1,300 m), they flew to Jomsom via Pokhara by air. They spent one night in Jomsom (barometric pressure, 548 mmHg; altitude, 2,700 m) and started for Dzong on horseback. During ascent, one night was spent in Kagbeni (barometric pressure, 544 mmHg; altitude, 2,740 m). They stayed in Dzong (barometric pressure, 493 mmHg; altitude, 3,500 m) for 3 d, and then returned to Kathmandu by the same route on the 2nd day after leaving Dzong. No medical problems were encountered during this travel. The blood collection and measurements of HR and BP were carried out in Nara (barometric pressure, 761 mmHg; altitude 70 m) the day before leaving Japan, on the 2nd day after the first arrival in Kathmandu, the day after arrival in Dzong, and the day after return to Kathmandu. Three milliliters of blood was drawn from an antecubital vein of each subject in a sitting position at rest, and was expelled into tubes containing ethylenediaminetetraacetic acid. Part of the plasma samples and cyanmethemoglobin solutions, stored at 540°C for 50 d (data not shown). The mixture was stored at −20°C or −80°C until thawed for photometric measurement, for 2–3 weeks. The absorbance at 540 nm of such mixtures remained unchanged after storage at −20°C for 50 d (data not shown). The coefficients of variation (CV) for this method were in the range of 0.8–1.7% in five runs of three blood samples with different Hb concentrations. Reticulocytes were counted on air-dried blood smears after 5-min staining with brilliant cresyl blue. Plasma immunoreactive EPO was determined with a sandwich-type enzyme-linked immunosorbent assay, developed by us, using two anti-EPO monoclonal antibodies [12]. Recombinant human EPO Epogen (180,000 IU/mg; Chugai, Tokyo, Japan) was used as a working standard. Two different doses (1/2- and 1-fold concentrations) of plasma samples from each subject were assayed twice. Plasma lactate was measured by a commercially available kit (Boehringer Mannheim, Mannheim, Germany) based on enzymatic spectrometry at 340 nm, with a limit detection of 0.3 mmol/l. Mean values and standard errors (SE) were calculated. Data were analyzed using analysis of variance (ANOVA) for repeated measures (STATISTICA for the Macintosh, StatSoft, Tulsa, OK, USA). The means were compared using a post-hoc test with Newman-Keuls procedures when overall statistical differences were found. The level of significance was set at p<0.05.

The mean HR of four subjects at rest increased from 67±1.6 beats/min at sea level to 86±3.7 beats/min at 3,500 m (p<0.01). The mean systolic BP at 3,500 m (140.8±7.6 mmHg) was significantly higher than that at sea level (124.3±4.6 mmHg, p<0.05), though the mean diastolic BP remained unchanged (83.8±3.9 mmHg at sea level vs. 89.5±3.7 mmHg at 3,500 m, p>0.05).

The plasma EPO and lactate concentrations increased significantly as the altitude increased (Fig. 1). The mean plasma EPO (18.8±1.6 mU/ml at sea level) increased significantly on the 3rd day at 1,300 m (25.5±2.0 mU/ml, p<0.05) and showed an almost three-fold increase on the 2nd day at 3,500 m (53.5±3.7 mU/ml, p<0.001). Likewise, the mean plasma lactate at 3,500 m (3.98±0.27 mmol/l) was 3.6 times as high as that at sea level (1.11±0.05 mmol/l, p<0.001). The main finding of the present study is a good correlation between plasma lactate concentrations in plasma from the subjects at sea level and altitudes (Fig. 2, r=0.86, p<0.01). Such a correlation was also reported in cultures of fetal mouse liver cells under hypoxic conditions [13]. Of interest is the finding that EPO production in response to hypoxia may be attributable to renal cortical adenosine 3',5'-monophosphate (cyclic AMP) increased by lactate in rats under hypobaric hypoxia [9]. Since this finding, cyclic AMP has been reported by several investigators to be involved as a possible signal transduction pathway in in vivo and in vitro EPO production by a hypoxic stimulus [2]. In fetal sheep, on the other hand, lactate infusion was reported to cause no significant

change in plasma EPO levels [14]. Therefore, further studies would be required to obtain more detailed information on the physiological role of lactate in EPO response to hypoxia. In relation to the hypoxic regulation of EPO production, recent DNA binding studies have shown that oxygen-dependent expression of a variety of genes, such as EPO, glucose transporter-1 and several glycolytic enzymes, might be regulated via hypoxia-inducible factor-1, which is a ubiquitous universal transcription factor induced on hypoxic exposure of the cells [1, 15].

In the present study, a 3.6-fold increase in the lactate levels was found on the 2nd day at 3,500 m, while only a 1.3-fold lactate increase was observed on the 5th day at 4,559 m [5]. The two possible explanations for the difference in the rate of lactate increase are as follows. First, the subjects in this study were untrained scientists with relatively high percentages of body fat content. In the work of Mairbäurl et al. [5], on the other hand, the subjects were 11 male mountaineers. It is known that in untrained sedentary subjects, increased lactate production occurs at 50–60% of the maximal oxygen uptake ($\dot{V}O_{2\text{max}}$) during incremental exercise, whereas in endurance-trained athletes with higher $\dot{V}O_{2\text{max}}$, it occurs at 70–80% of $\dot{V}O_{2\text{max}}$ [11]. Second, our subjects were not completely acclimatized at the blood sampling time on the 2nd day at 3,500 m, probably due to the sudden ascent by air and on horseback. This is evidenced by the rise in both HR and systolic BP at rest. It seems reasonable, therefore, that our subjects produced a larger amount of lactate in response to acute hypoxia at 3,500 m.

The mean of reticulocyte counts (14.2 ± 0.5‰) was higher on the 3rd day after descent from 3,500 m of altitude as compared with sea-level counts (8.9 ± 0.4‰, $p < 0.001$), while the blood Hb concentration and PCV did not change significantly throughout the altitude-exposure; the mean Hb concentration ranged from 14.7 ± 0.8 to 15.0 ± 0.8 g/dl and the mean PCV from 0.43 ± 0.02 to 0.45 ± 0.02 l/l ($p > 0.05$). This increased reticulocyte count on the 3rd post-altitude day indicates that EPO, produced by the moderate-altitude exposure for 3 d, stimulated erythropoiesis in the bone marrow. However, the blood Hb concentration and PCV at that time did not increase significantly as compared with sea-level values. This is consistent with the observations that, after a 64 h-stay at 4,350 m [6] or a 10 d-stay at 4,359 m [3] and 3,500–4,500 m [4], the blood Hb and/or PCV were not significantly different from sea-level values, though there was a trend for the mean values to increase. Prolonged altitude exposure over 10 d appears to be necessary for a rise in the Hb and PCV because the PCV increased abruptly on the 15th day at 3,500–4,500 m [4].

In conclusion, the data in this report are consistent with the well-known EPO/lactate response to altitudes, and suggest that the circulating EPO concentration as well as blood lactate concentration can be used as an index of anaerobic condition.

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