Red Cell Disorders

Hematologic response to four weeks of intermittent hypobaric hypoxia in highly trained athletes

We investigated changes induced by four weeks of intermittent hypobaric hypoxia (IHH) at a simulated altitude of 4000-5500 m in highly trained athletes. Serum erythropoietin increased significantly (p<0.001) after the sessions of IHH, but reticulocyte and red cell parameters did not. Our IHH protocol stimulated endogenous erythropoietin secretion without producing the subsequent erythropoietic response.

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One of the main features of acclimatization to altitude is an increase in hemoglobin, which is responsible for improving work capacity by increasing the oxygen-carrying capacity of the blood. Short-term intermittent hypobaric hypoxia (IHH) protocols (1.5-3h/day for 2-3 weeks) have been shown to elicit acclimatization and hematologic adaptations in mountaineers and physically active individuals.[1,2]

Our aim was to test whether medium-term IHH stimulates erythropoiesis in highly trained athletes. In this regard, 16 male triathletes were randomly assigned to either the hypoxia (HYPO, n=8) or the control (n=8) group. The HYPO group combined training at sea level with exposure to IHH (3 h/day, 5 days a week, for 4 weeks). The simulated altitude increased from 4,000 up to 5,500 m or 614-504 hPa. Three venous blood samples were drawn: t1= before the first exposure to the hypobaric chamber, t2= before the last exposure, t3= 2 weeks after the last exposure. To study the acute effect of hypoxia, samples were also obtained 3 hours after the first (t1') and the last (t2') exposures. Blood cell counts and reticulocyte parameters were analyzed using a Sysmex XE-2100 autoanalyzer, including RBC-Y and RET-Y. Serum erythropoietin (Epo) was measured using an immunoassay (Quantikine, R&D Systems). Total plasma and red-cell volumes,[3] serum transferrin receptor, haptoglobin, and iron parameters were also evaluated.

In the HYPO group, a significant increase in serum Epo was observed after chamber exposure (100 and 440%, at t1' and t2', respectively). No changes were observed in the control group. However, no significant differences were found in any of the other parameters. During the IHH, the basal Epo level only decreased as compared with the initial situation in the HYPO group. Reticulocyte parameters, RBC-Y and RET-Y showed significant differences, but these differences occurred in both groups. Moreover, IHH significantly increased lymphocyte and monocyte counts (Table 1 and Figure 1).

The most relevant result was that IHH stimulated Epo secretion without an effective erythropoietic response. The Epo increase after a single exposure to hypoxia has been previously demonstrated.[2,4] This study adds two new observations: a) a brief (180 min) exposure to hypoxia increases Epo secretion; b) this response is not blunted over a four-week period, even if the baseline Epo level decreases. The use of IHH is based on the assump-

Table 1. Hematologic characteristics before (Pre) and after (Post 1, 2) four weeks of exposure to intermittent hypobaric hypoxia (3 h/d, 5 d/wk, 4000 - 5500 m) in the HYPO and control groups. Time of sampling is indicated: t_1: before the first chamber exposure, t_1': 3 hours after the first exposure. t_2: before the last exposure, t_2': 3 hours after the last exposure. t_3: 2 weeks after the last exposure.

	Pre			*Post 1*			*Post 2*
	CON t_1	*HYPO t_1*	*HYPO t_1'*	*CON t_2*	*HYPO t_2*	*HYPO t_2'*	*HYPO t_3*
Epo (U/L)	10.7±3.1	8.3±3.2	16.6±4.7*	10.8±2.8	4.6±1.4+	24.8±9.3*+	9.2±3.2+
Hemoglobin (g/L)	150±13	146±3	143±8	143±11+	139±4+	143±7*	135±5+
Reticulocytes (×10^9/L)	40.8±10.9	37.9±9.5	41.0±12.7	45.8±12.9	34.1±11.4	35.6±12.1	33.8±7.7
IRF (%)	9.1±3.0	9.7±3.3	10.7±4.3	2.9±1.4+	1.5±0.9+	2.1±1.1+	3.0±1.5+
RBC-Y (units)	162.8±3.4	163.2±3.0	162.9±2.7	171.2±3.0+	172.1±3.0+	171.3±3.8+	–
RET-Y (units)	1740±36	1750±35	1742±35	1803±44+	1833±36+	1818±35*	–
Plasma volume (mL/m^2)	2108±228	1993±261	–	2393±395	2191±749	–	2106±452
Red cell volume (mL/m^2)	1399±157	1285±173	–	1515±192	1290±379	–	1186±214
sTfr (mg/L)	3.5±0.3	3.5±0.4	3.3±0.3*	3.3±0.4	3.1±0.3	3.2±0.4*+	2.9±0.3+
Platelets (×10^9/L)	230.5±19.7	207.3±28.9	211.4±29.0	223.8±24.1	217.0±33.5	227.5±38.3	204.5±25.5
Leukocytes (×10^9/L)	7.5±1.9	7.7±2.3	8.2±1.2	6.5±1.5	7.1±1.1	8.6±1.2*	6.9±1.0
Lymphocytes (×10^9/L)	2.5±0.5	2.1±0.4	3.2±0.7*	2.1±0.4	2.0±0.3	2.7±0.5*	2.0±0.4
Monocytes (×10^9/L)	0.6±0.2	0.5±0.1	0.7±0.1*	0.5±0.2	0.5±0.1	0.7±0.2*	0.5±0.1

*Values are means ± SD. IRF: immature reticulocyte fraction. RBC-Y and RET-Y are expressed on arbitrary units provided by the Sysmex XE-2100. Erythropoietin (Epo), soluble transferrin receptor (sTfR), +p < 0.05 different from the initial sea level value (t1 vs t2) (Student's post-hoc test); *p < 0.05 different from the respective pre-chamber exposure value (Student's post-hoc test).*

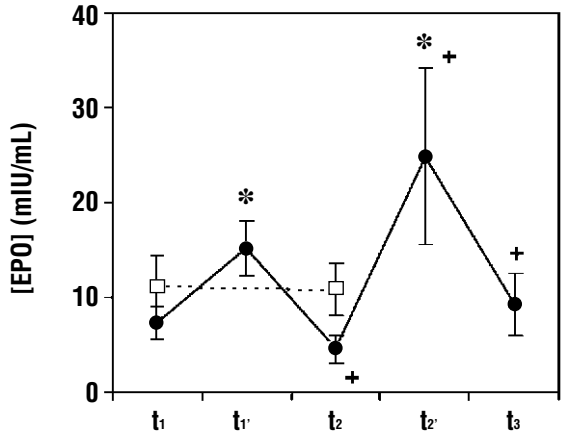

Figure 1. Changes in serum erythropoietin concentration of male triathlon athletes resting at a simulated altitude of 4000 and 5500 m for 3 hours, and along four weeks of exposure to intermittent hypobaric hypoxia. Filled circles represent the values of serum Epo in the HYPO group and open squares those of the control group at sea level. t_1: before the first chamber exposure, $t_{1'}$: 3 hours after the first exposure. t_2: before the last exposure, $t_{2'}$: 3 hours after the last exposure. t_3: 2 weeks after the last exposure. Values are mean ± SD. *+p<0.05 different from the initial sea level value (Student's t post hoc test); *p<0.05 different from the pre-chamber exposure value (Student's t post-hoc test).*

tion that when the rise in Epo secretion is maintained, erythropoiesis will be further stimulated and this will lead to an increase in hemoglobin and red cell mass. However, previous studies both support and refute this hypothesis. Repeated exposure to IHH significantly elevated Epo,[2,4] hemoglobin, and reticulocytes in some studies.[1,2] In contrast, in competitive athletes, neither training nor being passively exposed to normobaric hypoxia increased Epo levels,[5] or induced erythropoietic adaptation.[6]

Our study indicates that triggering Epo production does not always activate erythropoiesis.[6] When high-altitude natives or sojourners return to sea level, there is suppression of Epo production, a reduction of erythroid precursors, and a decrease in red cell survival. This phenomenon has been termed *neocytolysis*, and may be the consequence of rapid destruction of hypoxia-inducible factor 1α.[7] Neocytolysis may compromise the ability of short duration IHH to induce an increase in red cell mass.[7]

The anti-apoptotic effect of Epo is necessary for the differentiation of erythroid precursors into mature red cells.[8] The response to the increase in Epo seemed to be different in our athletes, in whom red cell and plasma volumes were increased,[9] from that in climbers and active subjects.[1,2] The lack of an erythropoietic effect may also suggest that the threshold of Epo could depend on the previous red cell mass. Furthermore, the duration of the hypoxia/normoxia cycle, the magnitude of adaptation to training, and neocytolysis may play integrated roles in the increase of the erythroid mass after IHH. Our IHH scheme was not able to increase the erythroid mass despite triggering Epo secretion. Our results also raise the question of whether this Epo stimulation could interfere with indirect Epo-abuse detection.[10] Direct distinction of Epo isoforms in urine (current anti-doping detection method) will probably not be affected. Ongoing research will clarify these aspects.

In summary, IHH (4000-5500 m, 3 h/day, 5 days/week over 4 weeks) stimulates endogenous Epo secretion without producing the subsequent erythropoietic response in highly trained athletes, in contrast to its effects in climbers and in active, healthy individuals.

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