

EFFECTS OF 'LIVING HIGH, TRAINING LOW' ON THE IMMUNE FUNCTION OF RED BLOOD CELLS AND ON ENDURANCE PERFORMANCE IN SOCCER PLAYERS

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Sixteen male soccer players were randomized and divided into two groups. One group, Living High, Training Low (HiLo) lived in normobaric hypoxic rooms (15.3% oxygen content, simulating an altitude of 2,500 m) for 10 hours per day for 4 weeks, had three soccer training lessons per week under normoxia, and exercised on the cycle ergometer for 30 minutes, with hypoxia, twice a week at 72% of maximal oxygen uptake ($\dot{V}O_{2max}$). The control group lived at sea level, had soccer training lessons three times per week, and twice a week had exercise at 80% $\dot{V}O_{2max}$ on the cycle ergometer for 30 minutes, all under normoxia. Both groups underwent the same training schedule. Blood samples were taken to investigate hematologic acclimatization and changes of the immune function of red blood cells (RBCs). The samples were collected before hypoxic exposure, after 10 hours, and after 2, 3, and 4 weeks of hypoxic exposure. The level of immune-adherent function of RBCs was evaluated by the RBC complement receptor type 1 (CR1) rosette test (RBC-CR1R, %) and by the RBC immune complex rosette test (RBC-ICR, %). $\dot{V}O_{2max}$ and Yo-Yo intermittent endurance were measured before and after the experiment under normoxia. The results indicated that a significant improvement in erythropoiesis, hemoglobin production, and $\dot{V}O_{2max}$. Yo-Yo intermittent endurance after the HiLo condition for 4 weeks confirmed this improvement, but in the HiLo group, the RBC-CR1R (%) was much lower, and the RBC-ICR (%) was much higher than in the control group. The immune function of RBCs at rest showed a clinical secondary descent. HiLo may affect RBC immunity significantly as a result of the two simultaneous stimuli of physical activity and exposure to hypoxia. Thus, changes in immune function may be critical during living high, training low conditions.

Keywords: hemoglobin, hypoxia, immunization, intermittent endurance

Introduction

The training concept of 'Living High, Training Low' (HiLo) was introduced by Levine and Stray-Gundersen (1991). When living at moderate altitude and training at low altitude or sea level, athletes theoretically should acquire

beneficial effects from altitude acclimatization, particularly in the adaptation of the oxygen delivery system, increase in total-body hemoglobin (Hb) content for maximizing oxygen transport and utilization, and training intensity. However, many components of such athletes' condition in HiLo were unclear, and there was a lack of basic research on HiLo, such as studies reporting the immune function of athletes during HiLo.

Siegel et al. (1981) demonstrated that red blood cells (RBCs) performed not only a respiratory function but also an immune function. One of the important immune

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functions of RBCs is immune adherence. RBCs bind to antigen-antibody-complement or antigen-complement complexes on their surface through complement receptor type 1 (CR1). The immune functions of RBCs and white blood cells interact in the body, thus complementing body defense functions against pathogens and immune surveillance against malignant cells (Guo 1990; Siegel et al. 1981). Some studies showed that different exercise programs induced an increase, decrease, or no change in CR1 activity of RBCs (Huang et al. 1999; Thomsen 1992). Both physical activity and exposure to environmental stressors, such as altitude training, modified various components of immune function. Altitude training could readily cause immune suppression and an increased risk of some diseases if the body was challenged by the two stimuli simultaneously (Chang et al. 2002; Shephard 1998). It was anticipated that the RBC immune system might play an important role in explaining the changes in immune function pertaining to altitude training, although there was a paucity of data relating to the qualities or functions of the RBCs in autoimmunity.

The purpose of this study was to investigate the changes in function of RBCs CR1 and hematologic acclimatization to HiLo conditions for 4 weeks, and its effect on sea-level performance in soccer players, as compared to similarly trained controls.

Methods

Subjects

We recruited 16 male soccer players from Beijing Sport University, who were free from medication, nonsmokers,

and had no history of cardiorespiratory disease. Subjects were asked to avoid altitude exposure for 6 weeks before the study began. Each subject gave informed written consent before commencing the study. Subject characteristics are presented in Table 1.

Experimental design and procedures

The subjects were divided into two groups in randomized order. One group (HiLo) lived in the normobaric hypoxic room's 15.3% oxygen content (a simulated altitude of 2,500 m), for 10 hours per day for 4 weeks. They had soccer training lessons three times per week under normoxia and exercised twice per week at 72% of maximal oxygen uptake ($\dot{V}O_{2max}$) on the cycle ergometer for 30 minutes with hypoxia. It has been suggested that there is a loss of 3.2% in $\dot{V}O_{2max}$ for every 305 m of ascent above 1,500 to 1,600 m. (Wolski et al. 1996). The control group lived at sea level and had soccer training lessons three times per week and exercised twice per week at 80% $\dot{V}O_{2max}$ on the cycle ergometer for 30 minutes, all under normoxia. The hypoxic instruments were the Hypoxic Tent System (Hypoxico Inc., New York, NY, USA). The conditions of the hypoxic room are given in Table 2.

On the morning before hypoxic exposure, 5 mL of blood was drawn from a vein in the forearm; blood was again drawn after 10 hours and after 2, 3, and 4 weeks of hypoxic exposure, to investigate hematologic acclimatization and changes in the immune function of RBCs. The level of immune-adherent function of RBCs was evaluated by the RBC CR1 rosette test (RBC-CR1R, %) and by the RBC immune complex rosette test (RBC-ICR, %). $\dot{V}O_{2max}$ and Yo-Yo intermittent endurance test were measured before and after the experiment under normoxia.

Table 1. Subject characteristics

Group	Number of subjects	Age (yrs)	Height (cm)	Weight (kg)
HiLo	8	21.19 ± 0.74	176.33 ± 3.45	69.92 ± 5.18
Control	8	21.19 ± 0.95	175.38 ± 4.75	69.13 ± 4.55

Table 2. Condition of hypoxic rooms

O ₂ content	CO ₂ content	Average temperature	Average air humidity	Room area
15.3%	0%	22° C	60%	30m ²

Immune-adherent function of red blood cells

The immune-adherent activity of RBCs CR1 was evaluated by the RBC-CR1R (%) test and by the RBC-ICR (%) test using Guo's method (Guo et al. 2002).

For these tests, 800 μL of blood was drawn into a clean citric acid-coated test tube and centrifuged at 2,000 rpm for 5 minutes. The supernatant was discarded and kept in another test tube; the remaining RBCs were drawn and washed with 0.9% sodium chloride (NaCl) solution. The cells were suspended by gently drawing them in and out of a Pasteur pipette and were centrifuged at 2,000 rpm for 5 minutes. RBCs were adjusted to a concentration of 1.25×10^7 cells mL^{-1} in 0.9% NaCl solution. Thereafter was this, known as the RBC suspension. A yeast suspension (obtained from Shanghai No. 2 Military Medical Academy, Shanghai, China) was adjusted to a concentration of 1×10^8 cells mL^{-1} in 0.9% NaCl solution. The procedures are outlined in Table 3.

RBCs and yeast cells (stained red and blue, respectively) were identified under the microscope; 200 RBCs were counted. Two or more yeast cells binding to an RBC were counted as one rosette. The number of rosettes formed by RBC CR1 rosette (RBC-CR1R, %) was calculated. The number of rosettes formed by the RBC immune complex rosette (RBC-ICR, %) was calculated in the same way.

Erythrocyte count and Hb concentration

Ethylenediamine tetraacetic acid-treated blood was used to determine the RBC count and Hb concentration with

the Beckman Coulter Hematology Analyzer (Beckman Coulter Inc., CA, USA).

$\dot{V}O_{2\text{max}}$ test

In normoxia in each subject before and after the experiment, $\dot{V}O_{2\text{max}}$ was determined by an incremental exercise on the Monark cycle ergometer with Medgraphics $\dot{V}O_{2000}$ portable metabolic testing system (Medical Graphics Corporation, St. Paul, MN, USA). The initial load was 60 W and was increased by 30 W every 3 minutes until volitional exhaustion occurred.

Yo-Yo intermittent endurance test

A soccer-specific running 20-m shuttle test paced by an audiotape was applied. A beep sounds as a signal to start running, another beep signals when to arrive and turn at the 20-m point, and then a final beep sounds when subjects are back at the starting line. The pace gradually increases; after each run there is a brief (5 seconds) recovery period. The beeps continue until the athlete fails to keep the pace set by the audiotape. The score consists of the total distance covered (number of runs \times 40 m).

Statistical analyses

Data are presented in Tables 4–6 (mean \pm SD). SPSS 11.5 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical calculations. $\dot{V}O_{2\text{max}}$ and Yo-Yo test were compared by paired *t* test. The independent-samples *t* test was used to analyze differences between the two groups. The general linear model (repeated measures)

Table 3. Experimental procedures

Procedure	RBC-CR1R	RBC-ICR	Remarks
Plasma (μl)	50	–	
Yeast suspension (μl)	50	50	
RBC suspension (μl)	50	50	
Mix and incubate in a water bath at 37° C (minutes)	30	30	Needs to be covered
0.9% sodium chloride solution (μl)	–	50	The temperature of 0.9% sodium chloride solution is 37° C
0.25% glutaraldehyde (μl)	25	25	Before and after adding, mix the cells gently
Smear	1/2 of the liquid was spread on a slide		The smear was allowed to air dry or a hair dryer was used to hasten the drying
Formaldehyde	A drop	A drop	Placed on the smear for fixation, then air dried
Staining	After the smear dried, the cells were stained with Wright's Stain Solution		

RBC-CR1R = Red blood cells complement receptor type 1 rosette test; RBC-ICR = Red blood cells immune complex rosette test.

Table 4. Time course of erythrocyte count and Hb concentration in both groups

	Group	Before	After 10 hours	After 2 weeks	After 3 weeks	After 4 weeks
Erythrocyte count ($\times 10^{12}/L$)	HiLo	4.79 \pm 0.52	4.74 \pm 0.44	5.05 \pm 0.55*	5.05 \pm 0.54*	5.61 \pm 0.92*
	Control	4.60 \pm 0.32	4.56 \pm 0.33	4.68 \pm 3.39	4.72 \pm 0.33	5.13 \pm 0.61
Hemoglobin (g/L)	HiLo	150.88 \pm 12.89	148.20 \pm 9.95	157.24 \pm 14.52*	157.04 \pm 14.68*	157.15 \pm 14.23*
	Control	145.19 \pm 8.45	143.10 \pm 9.51	146.45 \pm 10.04	147.98 \pm 9.45	145.33 \pm 9.78

*Significantly different from before hypoxic exposure in the same group ($p < 0.05$).

Table 5. Endurance performance changes before and after the experiment

	Time	Control	HiLo
$\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	Before	50.16 \pm 3.28	49.50 \pm 3.92
	After	50.54 \pm 4.63	52.20 \pm 4.06*
Yo-Yo intermittent endurance (m)	Before	1,285.71 \pm 217.17	1,240.00 \pm 281.42
	After	1308.67 \pm 350.02	1,384.62 \pm 355.73*

*Significantly different from before hypoxic exposure in the same group ($p < 0.05$). $\dot{V}O_{2max}$ = maximal oxygen uptake; HiLo = Living High Training Low group.

Table 6. Time course of red blood cells immune complex rosette test (RBC-CR1R, %) and red blood cells immune complex rosette test (RBC-ICR, %) in both groups

Group	Before	After 10 hours	After 2 weeks	After 3 weeks	After 4 weeks	
RBC-CR1R, %	HiLo	15.06 \pm 5.43	20.69 \pm 6.82*	19.63 \pm 8.02	8.19 \pm 3.35* [†]	12.19 \pm 2.30 [†]
	Control	19.38 \pm 6.51	21.69 \pm 8.29	23.06 \pm 9.65	13.19 \pm 1.44*	17.06 \pm 4.01
RBC-ICR, %	HiLo	17.75 \pm 11.52	31.00 \pm 19.65* [†]	39.75 \pm 7.62* [†]	21.75 \pm 5.42 [†]	20.44 \pm 5.99 [†]
	Control	19.17 \pm 14.80	14.44 \pm 3.01	15.31 \pm 4.37	15.75 \pm 4.89	12.13 \pm 3.84

*Significantly different from before hypoxic exposure in the same group ($p < 0.05$).

[†]Significantly different from Control group ($p < 0.05$).

[‡]Significantly different from Control group ($p < 0.01$).

HiLo = Living High Training Low group

was used for multiple comparisons within groups. The level of significance was set at 0.05.

Results and discussion

RBC count and Hb concentration

Cross-sectional studies in the Peruvian Andes (Sanchez et al. 1970; Reynafarje et al. 1959) as well as in the Colorado Rockies (Weil et al. 1968) suggested that there was an elevated red cell mass in natives living at high altitude that was proportional to the oxyhemoglobin saturation. However, some investigators failed to observe an increase in hemoglobin/myoglobin mass after brief periods of time in normobaric hypoxic environments (8–10 hours per night for 10 days to 3 weeks) and thus questioned the erythropoietic effects of moderate altitude exposure (Ashenden et al. 1999).

There was marked individual variability in the response to the training protocol. Although the genetic mechanisms responsible for determining the erythropoietic response to hypoxia in humans have not been entirely worked out, animal models suggest that this response might be transcriptionally regulated (Ou et al. 1998). Moreover, at least some individuals had genetic polymorphisms in the erythropoietin gene (Semenza et al. 1987) or the erythropoietin receptor (Prchal & Prchal 1999) that might strongly influence the erythropoietic response to hypoxia (Juvonen et al. 1991). It was possible, therefore, particularly in studies with relatively small sample sizes, that the presence of significant numbers of nonresponders could bias the study outcome.

Duration of hypoxic exposure might have an important influence. Studies using 8–10 hours of hypoxia per day have not been effective (Ashenden et al. 1999), whereas those using 16 hours of hypoxia per day have

shown an increased in hemoglobin/myoglobin mass, using the same methods (Rusko et al. 1999). In our study, the altitude-mediated RBC count and Hb increase was compelling.

$\dot{V}O_{2max}$ and Yo-Yo intermittent endurance

Some examinations of collegiate athletes (Chapman et al. 1998; Levine & Stray-Gundersen 1997) identified a 1.4% improvement in 5,000-m performance, a $2.5 \pm 2.4 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ improvement in $\dot{V}O_{2max}$, and a $1.1 \pm 0.7 \text{ g/dL}$ increase in Hb concentration after 4 weeks at 2,500 m. In elite runners, a 1.1% improvement in 3,000-m performance, a $2.3 \pm 2.6 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ improvement in $\dot{V}O_{2max}$, and an improvement of $1.0 \pm 1.1 \text{ g/dL}$ in Hb concentration after 27 days of living at moderate altitude (2,500 m) and performing high-intensity training at low altitude (1,250 m) were reported. Our results were similar to those obtained from Stray-Gundersen et al. (2001). There was a significant improvement in $\dot{V}O_{2max}$ ($\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) from 49.50 ± 3.92 to 52.20 ± 4.06 , and Yo-Yo intermittent endurance from $1,240.00 \pm 281.42 \text{ m}$ to $1,384.62 \pm 355.73 \text{ m}$ after HiLo for 4 weeks, although substantial individual variability existed within subjects. The mechanism might involve expansion of the red cell mass and an increase in circulating Hb levels, accompanied by maintenance of oxygen flux to working muscle.

Immune-adherent function of RBCs CR1

It is known that RBCs have no nucleus and are suited to the simple function of transporting respiratory gases. Notably, the immune-adherent function of RBCs was not discovered until the 1950s (Nelson 1953). It has been demonstrated that RBC immune adherence was initiated by C3b receptors on the surfaces of RBCs, also known as CR1. Furthermore, it was shown that RBCs can adhere to antigen-antibody-complement and antigen-complement complexes, the immune-adherence phenomenon led to an enhancement of phagocytosis. Siegel et al. (1981) put forward a new concept, "the red-cell immune system," in the early 1980s and suggested that RBCs were an important part of the body's immune system, and that the immune function of RBCs, just like that of white blood cells, could not be neglected.

It has been reported that RBCs contain many immune substances. Besides CR1, RBCs contain other immune

factors such as lymphocyte function-associated antigen-3 (LFA-3, CD58), decay-accelerating factor (DAF, CD55), membrane inhibitor of reactive lysis (MIRL, CD59), homologous restriction factor (HRF), phagocytosis inhibitory factor (PIF), and natural killer enhancing factor (NKEF). These factors, with CR1, have important roles in the regulation of complement activation, of immune functions of monocytes and phagocytes, of immune functions of T and B lymphocytes, as well as in the enhancement of activities of natural killer cells and lymphokine-activated killer cells. RBCs are an essential component of the immune system and they, just like white blood cells, may be considered to be an important part of the immune system (Peterson & Chao 1991; Siegel et al. 1981).

Human studies showed that untrained subjects showed greater changes in activity of RBC CR1 after exercise at 85% $\dot{V}O_{2max}$ in comparison to athletes (Hu et al. 1993). Thomsen et al. (1992) reported that 60 minutes of cycle ergometer exercise at 75% $\dot{V}O_{2max}$ did not lead to a change in concentration of RBC CR1 in highly trained athletes. Heavy-exercise training might lead to a significant decrease in RBC immune function of the body (Soong et al. 2003). The immune function of RBCs was lower at high altitude than at sea level and was attenuated with a higher altitude as a result of hypoxia and hypobaric air (Si et al. 2000; Liu et al. 1998). Altitude training could readily cause immune suppression and an increased risk of some diseases, if the body was challenged by both physical activity and exposure to hypoxia (Chang et al. 2002; Shephard 1998).

Our results indicated that a significant improvement occurred in erythropoiesis, Hb production, and $\dot{V}O_{2max}$ as well as in Yo-Yo intermittent endurance after HiLo for 4 weeks. However, after 10 hours of hypoxic exposure, the RBC-CR1R (%) and RBC-ICR (%) increased significantly, compared with values obtained before hypoxic exposure in HiLo; and the immune function of RBCs at rest showed a clinical accentuation and disturbance at this time. In HiLo after 3 weeks' hypoxic exposure, the RBC-CR1R (%) was much lower and the RBC-ICR (%) was much higher than that before hypoxic exposure, and after 3–4 weeks' hypoxic exposure, the RBC-CR1R (%) was also much lower and the RBC-ICR (%) was much higher than that in the control group. The immune function of RBCs showed a clinical secondary

descent. HiLo may lead to a significant effect on RBC immune function of the body, due to two simultaneous stimuli (physical activity and exposure to hypoxia), and, thus, it is important to pay more attention to the changes of the immune function during HiLo.

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References

- Ashenden MJ, Gore CJ, Dobson GP (1999). "Live high, train low" does not change the total haemoglobin mass of male endurance athletes sleeping at a simulated altitude of 3000 m for 23 nights. *Eur J Appl Physiol Occup Physiol* 80:479–84.
- Ashenden MJ, Gore CJ, Martin DT (1999). Effects of a 12-day "live high, train low" camp on reticulocyte production and haemoglobin mass in elite female road cyclists. *Eur J Appl Physiol Occup Physiol* 80:472–8.
- Chang Y, He ZH, Wang LR (2002). Effects of medium-level altitude training on the cell immune function of elite speed skaters. *China Sport Sci* 22:86–9.
- Peterson PK, Chao CC, Molitor T, Murtaugh M, Strgar F, Sharp BM (1991). Stress and pathogenesis of infectious disease. *Rev Infect Dis* 13:710–20.
- Chapman RF, Stray-Gundersen J, Levine BD (1998). Individual variation in response to altitude training. *J Appl Physiol* 85:1448–56.
- Guo F (1990). Methods to measure red-cell immune function. *Immunol J* 6:62–7.
- Guo F, Qian BH, Zhang LZ (2002). *Modern red blood cell immunology*. Shanghai No.2 Military Medical Academy, China, pp 61–2.
- Huang H, HaoXM, HuXY(1999). The experiment research for the effect of exercise on red-cell immune function. *Chinese J Sports Med* 18:375–7.
- Hu QC, Gou F, Weng YQ (1993). *The effects of various training intensities on red-cell immune function. New concepts of red-cell immune function*. Nanjing University Publisher, Nanjing, China, pp 98–101.
- Juvonen E, Ikkala E, Fyhrquist F (1991). Autosomal dominant erythrocytosis caused by increased sensitivity to erythropoietin. *Blood* 78:3066–9.
- Levine BD, Stray-Gundersen J (1997). "Living high-training low": effect of moderate-altitude acclimatization with low-altitude training on performance. *J Appl Physiol* 83:102–12.
- Levine BD, Stray-Gundersen J, Duhaime PG (1991). "Living high-training low": the effect of altitude acclimatization normoxic training in trained runners. *Med Sci Sports Exerc* 23:S25.
- Liu L, Chu YD, Lu TS (1998). Observation of immune function of RBC at different elevations. *J High Altitude Med* 8:53–5.
- Nelson RA (1953). The immune adherence phenomenon: an immunologically specific reaction between microorganism and erythrocyte leading to enhanced phagocytosis. *Science* 118:733–7.
- Ou LC, Salceda S, Schuster SJ (1998). Polycythemic responses to hypoxia: molecular and genetic mechanisms of chronic mountain sickness. *J Appl Physiol* 84:1242–51.
- Prchal JF, Prchal JT (1999). Molecular basis for polycythemia. *Curr Opin Hematol* 6:100–9.
- Reynafarje C, Lozano R, Valdivieso J (1959). The polycythemia of high altitudes: iron metabolism and related aspects. *Blood* 14:433–55.
- Rusko HK, Tikkanen FH, Paavolainen L, Hamalainen I, Kalliokoski K. (1999). Effect of living in hypoxia and training in normoxia on sea level $VO_{2\max}$ and red cell mass. *Med Sci Sports Exerc* 31:S86.
- Sanchez C, Merino C, Figallo M (1970). Simultaneous measurement of plasma volume and cell mass in polycythemia of high altitude. *J Appl Physiol* 28:775–8.
- Semenza GL, Ladias JA, Antonarakis SE (1987). An Xba I polymorphism 3' to the human erythropoietin (EPO) gene [Abstract]. *Nucleic Acids Res* 15:6768.
- Shephard RJ (1998). Immune changes induced by exercise in an adverse environment. *Can J Physiol Pharmacol* 76:539–46.
- Si BH, Zhang QC, Wu KZ (2000). Effects of hypoxia on immune function of red blood cells in soldiers. *J High Altitude Med* 10:37–9.
- Siegel I, Liu TG, Gleicher N (1981). The red-cell immune system. *Lancet* 2:556–9.
- Soong P, Hu QC, Huang RX (2003). The change in immune function of red blood cells in rats after an 8-week heavy exercise training. *J Exerc Physiol* 6 (3) August. Available at <http://faculty.css.edu/tboone2/asep/flldr/flldr.htm>
- Stray-Gundersen J, Chapman RF, Levine BD (2001). "Living high-training low" altitude training improves sea level performance in male and female elite runners. *J Appl Physiol* 91:1113–20.
- Thomsen BS, Rodgaard A, Tvede N (1992). Levels of complement receptor type one (CR1, CD35) on erythrocytes, circulating immune complexes and complement C3 split products C3d and C3c are not changed by short-term physical exercise or training. *Int J Sports Med* 13:172–5.
- Weil JV, Jamieson G, Brown DW (1968). The red cell mass-arterial oxygen relationship in normal man. *J Clin Invest* 47:1627–39.
- Wolski LA, McKenzie DC, Wenger HA (1996). Altitude training for improvements in sea level performance. Is the scientific evidence of benefit? *Sports Med* 22:251–63.