Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 4, 1119-1130
2024
Publisher: Learning Gate
DOI: 10.55214/25768484.v8i4.1486

The impact of hypoxic training at different altitudes on human physiology, biochemistry, and cytogenetics

Georgiy A. Demchenko[1*], Serik N. Abdreshov[2], Laura U. Koibasova[3], Makpal A. Yessenova[4], Anar N. Yeshmukhanbet[5]
[1,2,3,4,5]Laboratory of Physiology of Lymphatic System, Institute of Genetics and Physiology of the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan, Al-Farabi Ave. 93, Almaty 050060, Kazakhstan; Georgiidemchenko@Mail.Ru (G.A.D.) Snabdreshov@Mail.Ru (S.N.A.) Laura.Koibasova74@Mail.Ru (L.U.K.) Esenova.Makpal@Mail.Ru (M.A.Y.) eshmukhanbet96@mail.ru (A.N.Y.)

Abstract: This study investigated the impact of dosed hypoxic normobaric exercises on the physio-biochemical parameters of blood and lymph circulation, as well as cytogenetic changes in red blood cells (RBC), using a Sprague Dawley Rat Model. Hypoxic training was conducted in an animal hypoxic chamber. The session lasted 34-35 minutes. The O2 content was 20.5% at the beginning of the session; then, it dropped to 14.8% within 4-5 minutes, corresponding to a rise to an altitude of 2,900-3,000 m, and was maintained at that level until the end of the session. The hypoxic training cycle increased the RBC count by 4.1%, the hemoglobin count by 4.2%, the blood oxygen capacity by 8.3%, the circulating blood volume by 16.7%, and interstitial metabolism and lymph circulation by 30.6%. A cytogenetic study revealed an increase in erythropoiesis-reticulocyte count by 40.3% and a slightly higher frequency of micronucleated erythrocytes. We observed economization of the heart, respiration, activation of glucose transport by 33.5%, increased lipid utilization, and increased antioxidant protection. These parameters especially changed when hypoxic training was combined with physical activity. Hypoxic training with physical activity expands the body's compensatory and adaptive capabilities by increasing the reserves of the respiratory, cardiovascular, and lymphatic systems.

Keywords: *Blood, Exercise, Hypoxia, Lymph, Physiological adaptation, Physiological condition,*

1. Introduction

The study of the impact of hypoxic training on the human and animal body remains relevant in physiology and medicine. Hypoxia is used to treat and correct a range of diseases, improve the physiological capacities of athletes, and help master different habitats [1]. Adaptation to hypoxia is an important and multifactor process involving all body organs and systems, primarily circulatory and cardiovascular systems [2-5]. Hypoxia is interesting from the point of pathological and adaptation processes since it is characterized by a decline of molecular oxygen (O_2) and/or associated with regulating the oxygen levels during intracellular redox reactions [6]. Normobaric hypoxia results from a decline in the O_2 content in the changed gas composition of inhaled air at a normal barometric pressure of 750 mmHg (105 Pa) [7, 8]. Hypoxic training can be conducted in natural conditions of the mountains (low, middle, or high) and hypoxic chambers to increase performance and reach the peak of athletic shape before major events [9].

Systemic approaches to adaptation in mountain settings, the analysis of compensatory resources, raising the body's resistance to stressors of various levels, and the search for new criteria for assessing

* Correspondence: Georgiidemchenko@Mail.Ru

and predicting physical performance in hypoxia are topical issues in assessing and individualizing the preparation of athletes [10].

For the effectiveness of the body's adaptation reactions after work-out sessions in settings of hypoxia, the results of a detailed laboratory study of the parameters of homeostasis are necessary – biochemical, immunological, hematological, hormonal, cytogenetic, etc., which make it possible to assess the functional state of the main body systems limiting performance, which became the subject of our study – the identification of resistance factors to hypoxic exercises. The experimentally simulated hypoxia under controlled settings will help understand the internal mechanisms and test the therapy, nutrition, etc, to analyze the adaptation and pathological conditions associated with hypoxia.

The aim was to analyze the body's physiological, biochemical, and cytogenetic parameters after hypoxic exercises at 3,000 and 5,000 m altitudes above sea level (asl).

2. Materials and Methods

2.1. Hypoxic Exercises in Experimental Animals

The circulatory hypoxia was reproduced in 45 white male rats of the Sprague Dawley population with body mass 250-280 g. The studies were conducted on animals kept in a vivarium under standard settings with natural light conditions on a standard diet for laboratory animals (GOST R 50258-92). The study was approved by the Ethics Commission of the Institute of Genetics and Physiology, Almaty, Kazakhstan (Conclusion No. 5 dated July 25, 2022).

The presence of laboratory animals in the vivarium and during the experimental study met the European requirements for laboratory animals [11].

The rats were divided into three groups (15 heads per group): Group 1 – normoxia controls, Group 2 – hypoxic exercises at 2,900-3,000 m asl, and Group 3 – hypoxic exercises at 4,900-5,000 m asl. Training for Groups 2 and 3 included 2 sessions per day for 4 weeks, 48 sessions in total. The animals were placed in an Ox-100 hypoxia isolation chamber (Shanghai TOW Intelligent Technology Co., Ltd., P.R.C.). The duration of the session in Group 2 was 34-35 minutes at the concentration of O_2, which dropped from 20.5% to 14.8% in 3-4 minutes and maintained at this level for half an hour, which corresponded to a climb to an altitude of 2900-3000 m. In Group 3, after 5-6 minutes, the oxygen concentration dropped from 20.5±0.3% to 11.2±0.4%, corresponding to an altitude of 4900-5000 m asl; the session duration was 36 minutes. The reduction of O_2 and its maintenance at the appropriate level was carried out with the 10L Nitrogen concentrator, and the decline of CO_2 in the hypoxic chamber was performed in the absorber Medical Soda Lime.

The next day after the last training, the animals were examined, and their blood, lymph, and urine samples were taken. For this purpose, the animals were inhaled with ether through a mask, followed by intramuscular injections with the Ketamine anesthetic. Physiological indicators of cardiac activity, such as electrocardiogram (ECG) and heart rate (HR), were registered using a REO-Mizar rheograph (Mizar, Russia). Then, an incision was made along the white line of the abdominal muscles, the thoracic lymphatic duct at the diaphragm was dissected, and the graduated microcannula was inserted into it; subsequently, through it, the lymph outflow was determined, and the lymph was collected for examination. In the caudal part of the abdominal cavity, after the lymph collection, the abdominal aorta was dissected, into which a Teflon catheter was inserted for blood collection. Then, the animals were withdrawn from the experiment by an overdose of ether through a mask.

The blood samples were examined for the following indicators: pH, PCO_2, PO_2, BE, tCO2, HCO_3, $stHCO_3$, tHb, Hct, SO_2, Na+, K+, Cl-, AnGap using an OPTI CCA –TS2 Blood Gas and Electrolyte Analyzer (OPTI Medical Systems, USA).

Blood and lymph samples were also examined for total protein, cholesterol, triglycerides, total lipids, urea, creatinine, and bilirubin. ALT, AST, alkaline phosphatase, and amylase levels in lymph and plasma were determined using conventional methods with a Cobas Integra 400 automatic biochemical analyzer (Roche Diagnostics, Switzerland). The cellular composition of blood and lymph was

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 4: 1119-1130, 2024
DOI: 10.55214/25768484.v8i4.1486

determined using a Sysmex KX-21 hematology analyzer (Sysmex Corporation, Japan). The hormone levels in blood and lymph were identified using a Cobas e 411 Immunoassay Analyzer (Roche Diagnostics, Switzerland). Immunoglobulins IgM, IgG, and IgA were determined using the enzyme immunoglobulin method using commercial T systems (Wuhan Fine Biotech Co, China). The lipid peroxidation analysis included the determination of conjugated dienes on the Apel PD-303UV spectrophotometer (Apel Co, Japan), the analysis of malondialdehyde (MDA) was performed using the CECIL CE 1021 UV-VIS spectrophotometer (Cecil Instruments, UK), and titrimetric measurement of catalase activity [12]. The blood plasma volume was determined by hematocrit. The viscosity of blood and lymph was determined on the viscometer "VK-4" (ЗМСО, Russia).

2.2. Preparation of Agents for Micronucleus Test

Blood sampling and smear preparation to analyze micronucleated erythrocytes (MNE) frequency followed the generally accepted method. Dried peripheral blood smears were fixed in 96% ethanol for 30 minutes, then dried and stained with 4% Romanowsky-Giemsa solution for 20 minutes. The MNE frequency and cytological abnormalities in peripheral blood normochromic erythrocytes were detected using an AxioLab A.1 microscope (Zeiss, Germany) under oil immersion at 10x100 magnification. During the cytogenetic examination, all abnormalities in the structure of erythrocytes differing from the normal morphology specific to a given type were recorded. Up to 20000 erythrocytes from each sample were examined. Photo documentation was carried out on the most prominent disorders of the peripheral blood erythrocytes.

2.3. Preparation of Agents for Reticulocyte Analysis

The reticulocytes were examined to assess erythropoiesis after hypoxic training and a photon therapy course (Internal Laser Blood Irradiation). Two reticulocyte stains were used:

1. 50 μL of Brilliant cresyl blue solution + 50 μL of blood. Mix the compound thoroughly but carefully, keep for 25-30 minutes at 37ºC or 1.5 hours at room temperature (18-25)ºC, then make smears on slides using the ground glass. With this staining, erythrocytes were yellowish-greenish, and the granular-mesh substance was blue or violet-blue.
2. Add 0.4 mL of dye solution to 0.1 mL of whole blood and incubate for 30 minutes. Subsequently, thin smears are prepared from the mixture obtained. After the smear dried, they were stained with May-Grunwald fixing paint for 1 minute and washed with running water. For that purpose, the dried smear is placed for 1 minute in a container with a fixative staining agent, then removed from the container and rinsed with running water. Erythrocytes were colored bright red against the background, and the reticulofilamentous substance was colored bright blue.

2.4. Statistical Analysis

The results were analyzed using conventional measures of variation in statistics with Microsoft Excel (Microsoft Corporation, Washington, DC, USA). The arithmetic mean ± standard deviation (M±SD) was calculated and expressed in a percentage. Significance testing was performed using Fisher's t-test and Student's t-test. The threshold of statistical significance composed $p \leq 0.05$.

3. Results

Rats in experiments in a hypoxic chamber initially demonstrated pronounced anxiety tactics (running for 5-7 minutes and then concentrating together in one of the corners of the chamber throughout the training). The ECG analysis of the heart in animals with a body mass of 3 g (Figure 1) showed the heart rate fall and prolongation of the cardiac cycle after the training cycle of 3000 and 5000 m asl. In Group 2, the prolongation of the cardiac cycle was 4%; in Group 3, it was 6%, compared to the reference values. The heart rate was 444±11 beats per minute after training at 3,000 m and 436±12 after training at 5,000 m asl vs. 462±10 beats per minute in the control group.

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 4: 1119-1130, 2024
DOI: 10.55214/25768484.v8i4.1486

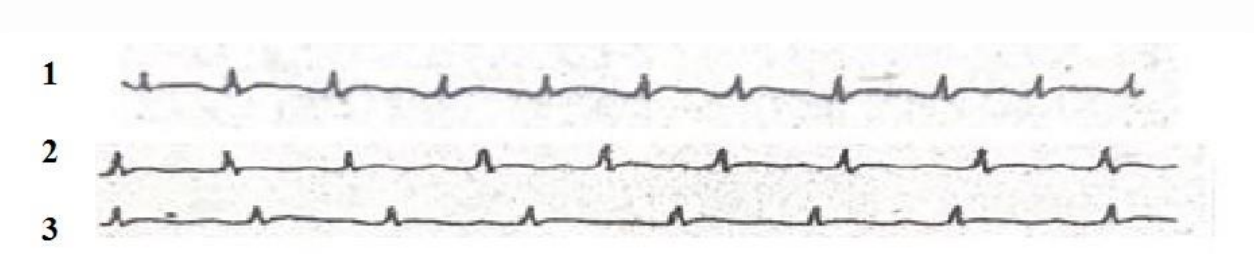

Figure 1.
ECG after normobaric hypoxic training at an altitude of 3,000 and 5,000 m asl; 1 – the control group; 2 – animals after hypoxic training at 3,000 m asl; 3 – animals after hypoxic training at 5,000 m asl. "Mitsar-REO" ECG and HR rheography sensors (St. Petersburg, Russia).

The respiratory movements (rm) decreased to 30.4±5 rm per minute after training at 2,900-3,000 m asl and 29.2±3.0 rm per minute after training at 4,900-5,000 m asl vs. 36.8±4.0 rm per minute in the control group ($p<0.05$).

The studied rats demonstrated changes in blood composition under hypoxia settings (Table 1). Thus, the RBC count increased by 4.1% in Group 2 and 5.7% in Group 3 compared to the controls. The red cell distribution width (RDW) slightly decreased above 3,000 m asl and restored after 5,000 m asl. The mean corpuscular volume (MCV) decreased by 11.7% after training at 5,000 m asl only. The hemoglobin count in blood (HGB) increased by 4.1% in Group 2 and 12.9% in Group 3. The mean corpuscular hemoglobin concentration (MCHC) added 10% in Group 3. The platelet crit count and mean platelet volume decreased against the background of hypoxia. In Group 2, these parameters decreased by 2.7% and 4.17% compared to the control. In Group 3, a decline of the platelet crit count by 10.3% and a decline of the mean thrombocyte volume by 12% were recorded compared to the control. The platelet counts in rats declined in all groups, including Group 3 (Table 1). After hypoxic training, the blood leukocytes increased by 19.3% in Group 2 and 26.3% in Group 3. The monocytes increased by 50% in Group 2 and 85% in Group 3. The granulocytes also increased by 5% in Group 2 and 10% in Group 3. The elevation of leukocytes and the entire nuclear Arneth count was associated with the periodic stress load of animals during their hypoxic training. The number of lymphocytes in Groups 2 and 3 remained within reference values (Table 1).

Table 1.
The impact of hypoxic training at an altitude of 2,900-3,000 and 4,900-5,000 m asl on complete blood count.

Parameter	Group 1	Group 2	Group 3
WBS (10^9/L)	2.85± 0.1	3.4±0.2	3.6±0.2*
LYM, 10^9/L	2.7± 0.3	2.7±0.05	2.6±0.3
LYM, %	55.5 ±3.4	55.2±6.1	52.9±6.7
MID, 10^9/L	0.07±0.001	0.14±0.004*	0.47±0.003**
MI, %	3.75±0.021	9.5±0.031**	0.55±0.09**
GRA, 10^9/L	2.0±0.010	2.1±0.04	2.2±0.03
GRA, %	2.35±0.22	11±0.07**	0.55±0.09
HGB, g/L	150.0 ±3.0	156.1±5.0	169.3±4.0*
MCH, pq	19.65±1.1	18.5±2.1	19.1±1.8
MCHC	40.4±2.1	37.9±2.4	44.45±1.8
RBC, 10^{12}/L	7.4± 0.2	7.7±0.4	7.82±0.5*
MCV, fl	48.6±5.1	48.6±4.6	42.9±4.8
HCT, %	45.0± 3.2	37.5±4.2	33.3±3.4*
RDW, cfl	36.1±0.8	34.8±1.2	35.25±1.7
RDWs, %	13.7±0.5	12.7±0.6	17.75±0.6*

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 4: 1119-1130, 2024
DOI: 10.55214/25768484.v8i4.1486

Parameter	Group 1	Group 2	Group 3
PLT, 10^9/L	525 ±14	500.3±20	469.5±29*
MPV	7.4±0.5	7.1±0.7	6.5±0.4*
PDWafl	10.35±0.7	10.2±0.9	9.25±0.4*
PDWc, %	38.65±0.7	39±3.1	36.4±3.2
PCT, %	0.50±0.04	0.45±0.05*	0.435±0.06*

Note: A statistically significant difference compared to the controls, p<0.05*, p<0.001**.

Blood pH did not change after hypoxic training. The pCO_2 in blood increased by 12.3% in Group 2 and decreased by 18.9% after training at 5,000 m asl. The pO_2 in the blood increased by 8.3% in Group 2 and 9.3% in Group 3; the buffer blood systems decreased in Group 2 and to an even greater extent in Group 3. The carbon dioxide and blood bicarbonates declined in Groups 2 and 3, but more significantly at 5,000 m asl (Table 2).

Table 2.
O_2 content, CO_2 acidity/Alkalinity, electrolytes, and hemoglobin count in blood after hypoxic training at 2900-3000 and 4900-5000 m asl.

Parameter	Group 1	Group 2	Group 3
Blood pH	7.3±0.06	7.3±0.07	7.2±0.08
PCO_2 (mmHg)	24.4±0.4	27.3±0.5	19.8±0.4*
PO_2 (mmHg)	114.6±8.5	124.1±9.6	125.3±7.8*
BE (mmol/L)	-7.5±0.3	-10.8±0.4	-11.9±0.7*
tCO_2 (mmol/L)	17.1±0.8	14.7±0.7	11.8±0.6*
HCO_3 (mmol/L)	16.4±0.9	15.8±1.1	15.5±0.5
$stHCO_3$ (mmol/L)	19.4±1.0	15.8±0.9	14.9±1.3*
tHb (g/dL)	11.9±0.8	12.4±0.9	12.6±0.4
Hct (%)	37.7±11	38.2±13	38.7±10
SO_2 (%)	96.5±12	98.0±14	98.3±1.14*
Na^+ (mmol/L)	139.4±18	132.3±15	129.1±14*
K^+ (mmol/L)	3.7±0.07	3.32±0.09	3.03±0.06
Ca^+ (mmol/L)	1.3±0.01	0.7±0.02*	0.45±0.04**
Cl^- (mmol/L)	107.6±10	104.9±12	100.5±13
AnG ap (mmol/L)	20±0.9	18.3±1.1	20.4±1.2
Bun (mmol/L)	13.5±0.8	12.9±1.2	12.3±0.6
Glu (mmol/L)	180.8±15	241.4±17*	190.5±14

Note: A statistically significant difference compared to the controls, p<0.05*, p<0.001**.

The saturation index increased in all experimental groups. The blood electrolytes (Na+, K+, Cl-) in Group 2 tended to decline, with a more significant trend at 5,000 m asl, especially for Ca^{2+} ions (Table 2). The angiotensin-converting enzyme of the blood serum slightly declined in Group 2 by 8.5%, and in Group 3 was within the control values. The state of protein metabolism (urea nitrogen) decreased, especially in Group 3, by 4.4% and 8.9%, respectively. After hypoxic training, the blood glucose after hypoxic training increased by 33.5% in Group 2 and 5.4% in Group 3; the heart mass of animals increased by 5.3% in Groups 2 and 3 (Group 1 – 0.38+0.008, Groups 2 and 3 – 0.40+0.01 per 100 g of body mass).

The lymph flow increased by 27.8% in Group 2 and 47.2% in Group 3. Diuresis intensified after hypoxic training by 7.1% in Group 2 and 42.9% in Group 3 (Figure 2).

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 4: 1119-1130, 2024
DOI: 10.55214/25768484.v8i4.1486

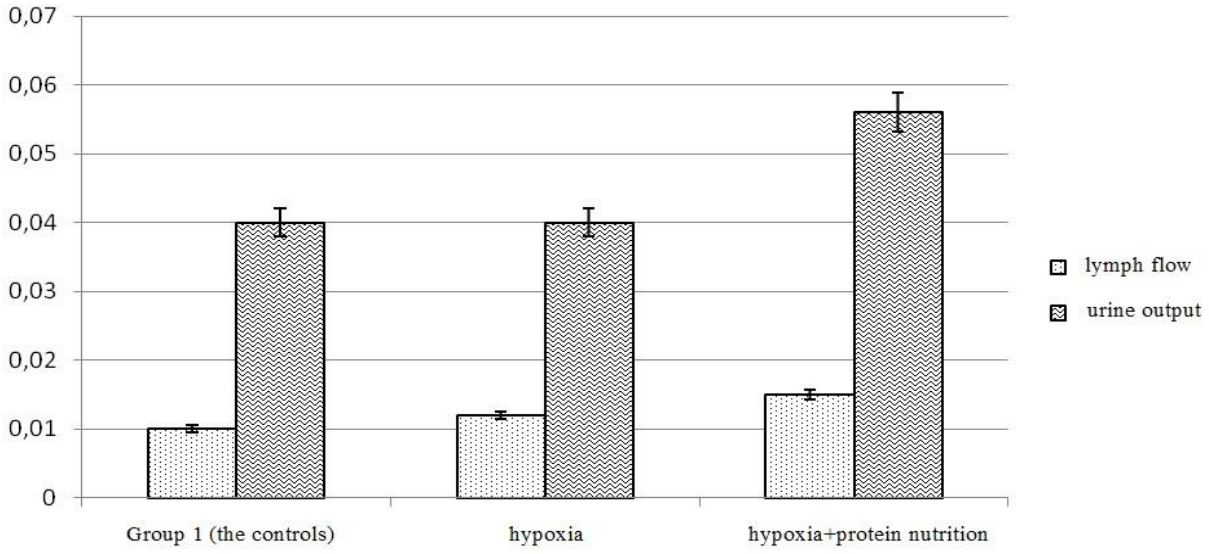

Figure 2.
Indicators of lymph and urinary flows after hypoxic training at 2900-3000 m asl and 4900-5000 m asl. Lymph flow and diuresis were measured in mL/min per 100 g of animal body mass.

ALT values increased in blood by 17.3% in Group 2 and 33.7% in Group 3, and in lymph – by 14.8% in Group 2 and 22.9% in Group 3. AST activity indicators increased in the blood – by 47.4% in Group 2 and 47.2% in Group 3, and in the lymph – by 17.6% in Group 2 and 76.5% in Group 3. Calcium levels in rats decreased after hypoxic training in the blood serum and lymph – by 34.3% and 30.96% in Group 2 and 46.7% and 48.1% in Group 3, respectively. This corresponds to the changes obtained above using a different device. Cholesterol levels in blood and lymph decreased after hypoxic training by 25.8% and 0.7% in Group 2 and by 52.6% and 53.8% in Group 3, respectively (Table 3).

Table 3.
Biochemical parameters of blood and lymph after hypoxic training at 2,900-3,000 and 4,900-5,000 m asl.

Tests	Group 1		Group 2		Group 3	
	Blood	Lymph	Blood	Lymph	Blood	Lymph
ALT (U/L)	250.9±22	80.3±6.4	294.4±11.3	92.2±10.2	335.5±21*	99.06±1*
AST (U/L)	167.6±15	76.7±9.4	247.1±17.3*	90.6±12.4	246.7±11.4	135.4±6.5
CA (mmol/L)	1.69±0.2	1.62±0.1	1.11±0.4*	1.12±0.2*	0.9±0.1*	0.84±0.2*
CHOL (mmol/L)	1.9±0.04	1.43±0.07	1.41±0.06	1.42±0.05	0.9±0.06*	0.66±0.02*
CREA (μmol/L)	49.63±4.1	7.31±8.8	48.3±5.9	5.8±4.7	51.7±4.7	4.62±3.7*
LDH (U/L)	1388±31	672±15	1478.25±41*	875±21	1708±34*	931±26**
HDLC (U/L)	0.51±0.04	0.29±0.02	0.67±0.01	0.42±0.02*	0.87±0.07	0.54±0.08
LDL (mmol/L)	1.72±0.75	0.6±0.03	2.12±0.07	-	16.5±0.04	12.2±0.06
TP (g/L)	64.3±5.1	24.7±2.3	60.7±4.9	26.8±3.3*	52.5±2.3	21.88±3.4
TBIL (μmol/L)	1.25±0.1	9.87±0.9	194.4±11.3	132.2±10.2	61.8±7.9	33.8±7.9
TRIG (mmol/L)	1.25±0.1	0.81±0.07	1.2.1±0.12*	0.48±12.4	1.34±0.46	0.54±0.14
UREA (mmol/L)	5.33+0.21	5.55+0.33	7.2±0.49*	5.82±0.31	5.83±0.36	5.83±0.32

Note: A statistically significant difference compared to the controls, p<0.05*, p<0.001**.

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 4: 1119-1130, 2024
DOI: 10.55214/25768484.v8i4.1486

The lipid profile values slightly declined. Thus, under settings of hypoxia in Groups 2 and 3, this indicator was lower by 35% in the blood and 39% in the lymph compared to the control. A significant decline in HDL indicates the effect of a stress factor (Table 3).

Table 4.
Indicators of hormonal status in rats after hypoxic training at 2900-3000 and 4900-5000 m asl.

Animal group	Cortisol (nmol/L)		Testosterone (nmol/L)		Estradiol (pmol/L)	
	Blood	Lymph	Blood	Lymph	Blood	Lymph
Group 1	46.21±2.4	23.52±1.3	9.60±0.32	8.51±0.32	18.35±0.22	18.35±2.33
Group 2	56.05±3.5*	28.42±4.1*	5.62±0.42*	5.04±3.1*	18.35±0.33	18.09±4.05
Group 3	53.9±3.7*	28.03±4.5*	4.64±0.41*	5.8±0.45*	18.35±0.15	18.35±3.11

Note: A statistically significant difference compared to the controls, p<0.05*, p<0.001**.

Cortisol in rats increased in the blood – by 21.1% in Group 2 and 16.6% in Group 3, in the lymph – by 20.8% in Group 2 and 19.2% in Group 3 (Table 4). Testosterone decreased in the blood – by 41.5% in Group 2 and 51.7% in Group 3, and in the lymph – by 40.8% in Group 2 and 31.9% in Group 3. Estradiol in blood remained unchanged in all groups.

Immunoglobulins IgM, IgG, and IgA after hypoxic training increased in the blood and lymph, both after the climb to 3000 m and after the complex of climbing at 5000 m. As shown in Table 6, the higher the altitude of the training conducted, the higher the concentration of immunoglobulins in the blood and lymph was. Significantly, the elevation of the concentration of immunoglobulins was higher in the lymph than in the blood (Table 5). The highest rise was observed for IgM (73.1% in lymph and 15.8% in blood) and IgA (73.2% in lymph and 94.1% in blood).

Table 5.
Immunoglobulins in blood and lymph after hypoxic training at 2900-3000 and 4900-5000 m asl.

Immunology	Medium	Group 1	Group 2	Group 3
Ig M (ng/mL)	Blood	125.85±9.67	138.11±9.72*	149.5±11.25*
	Lymph	44.75±0.84	245.56±17.93**	266.30±18.22**
Ig G (ng/mL)	Blood	212.35±12.44	257.37±14.05*	297.20±14.49**
	Lymph	144.24±10.65	285.44±18.76**	329.53±17.75**
Ig A (ng/mL)	Blood	16.55±1.09	194.65±12.34**	278.83±16.25**
	Lymph	76.55±14.64	71.34±16.74	285.34±17.11**

Note: A statistically significant difference: p <0.01*, p <0.001**.

This study evaluated the antioxidant properties of blood after hypoxic training. It showed a decline of catalase by 50.9% below the control indicators in Group 2 and by 61.5% in Group 3, reflecting the antioxidant protection processes at the cellular level. The free radical oxidation marker (diene conjugate) remained unchanged in Group 2 and increased by 25% in Group 3, and the oxidative stress marker (malondialdehyde) increased by 36% in Group 2 and 44% in Group 3. Blood coagulation profile (protrombine time, PT; activated partial thromboplastin time, aPTT; thrombin time, TT) indicated a decline of fibrinogen and elevation of the blood coagulation time in Groups 2 and 3 (Table 6). Against that background, the blood coagulation time increased from 3.21±0.3 minutes to 3.49 ±0.43 in Group 2 and 3.56±0.3 in Group 3, and lymph coagulation time – from 3.47±0.3 to 3.68±0.4 in Group 2 and 3.82±0.4 min in Group 3 (p<0.05). At that, the blood viscosity was 4.12±0.25 in the controls, 4.14±0.32 in Group 2, and 4.39±0.35p in Group 3 (p<0.05), and lymph viscosity amounted to 3.88±0.27 in Group 1 (the controls), 4.11±0.34 in Group 2 and 4.24±0.41 in Group 3 (p<0.05). That means a decreased blood and lymph viscosity (Figure 3).

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 4: 1119-1130, 2024
DOI: 10.55214/25768484.v8i4.1486

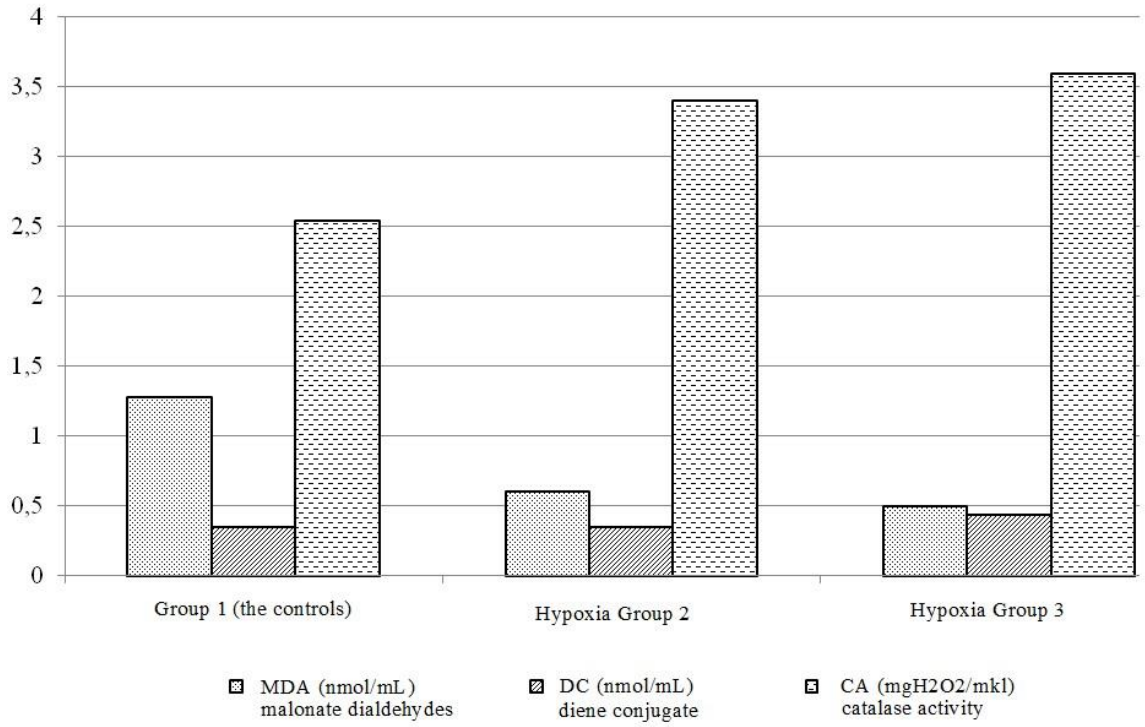

Figure 3.
The values of antioxidant activity markers after hypoxic training at 2,900-3,000 m asl and 4,900-5,000 m asl.

Table 6.
Coagulation test after normobaric hypoxic training at 2,900-3,000 and 4,900-5,000 m asl.

Indicators	Group 1	Group 2	Group 3
PT (sec)	13.04±0.8	14.7±0.7	15.05±0.8
aPTT (sec)	36.6±2.4	38.1±3.1	38.3±3.3
TT (sec)	28.5±3.1	28.8±4.3	29.5±3.4*
Fibrinogen (г/л)	1.98±0.07	1.9±0.09	1.55±0.07*

Note: A statistically significant difference compared to the controls, $p<0.05$*, $p<0.001$*.

The blood coagulation test showed a prolongation of PT, aPTT, TT, and a decline of fibrinogen in Groups 2 and 3, with more significant changes observed at 5,000 m asl (Table 6).

3.1. The Study of Changes in Cytogenetic Homeostasis of Rats after Hypoxic Training

Peripheral blood samples were taken, and cytological and cytogenetic samples were prepared after the exposure courses at different altitudes to assess the efficacy and safety of the hypoxic training cycle in model experiments of laboratory animals. MNE frequency and reticulocyte count were studied using two types of staining. Hypoxia was found to stimulate erythropoiesis since the reticulocyte count increased by 4.24±0.20% at 3,000 m asl and 3.03±0.17% at 5,000 m asl compared to the controls (2.53±0.16%). Erythropoiesis increased by 19.8% at the altitudes of 3,000 m asl and 67.6% at 5,000 m asl (Table 7).

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 4: 1119-1130, 2024
DOI: 10.55214/25768484.v8i4.1486

Table 7.
Cytogenetic parameters of rat blood after hypoxic training and proton therapy at 2,900-3,000 and 4,900-5,000 m asl.

Variant	Reticulocyte count (%)	MNE frequency (%)
Group 1	2.53±0.16	0.05±0.01
Group 2	3.03±0.17*	0.29±0.01**
Group 3	4.24±0.20**	0.35±0.02**

Note: A statistically significant difference compared to the controls, p<0.05*, p<0.001*.

Cytogenetic homeostasis has changed after hypoxic training. In particular, the MNE frequency significantly increased to 0.35±0.02% (3,000 m asl) and 0.29±0.01% (5,000 m asl), compared to 0.05±0.01% in the controls. Thus, despite increasing MNE frequency, hypoxic training stimulated erythropoiesis and strengthened adaptation processes in model experiments with laboratory animals.

4. Discussion

A decline in PaO_2 after hypoxic training triggers the mechanisms of compensation for arterial hypoxemia and tissue hypoxia. Against the background of hypoxic training, hypoxia develops in the tissues, which is accompanied by the activation of anaerobic processes.

The experimental data showed the changes in the composition of circulating blood and lymph in rats under post-hypoxic training. Hypoxic training activates the body systems responsible for oxygen transportation in the tissues, such as lung hyperventilation, a rise in the minute volume, a decline in the heart rate, and improvement of the blood supply to vital organs [13].

Hypoxic training changed the parameters of central hemodynamics, most at an altitude of 5,000 m asl, and activated the adrenergic and pituitary-adrenal systems. This non-specific adaptation component plays a role in mobilizing the circulatory apparatus and external respiration [14]. Changes in the cardiorespiratory system under recurrent hypoxia could also be attributed to the depression of function of higher brain parts. Such mobilization of systems responsible for oxygen transportation, in combination with non-specific stress syndrome and the disturbances of higher nervous activity, make a mechanism of adaptation to hypoxia conditions.

The physiological rationale for post-training hemoglobin level increase could be explained by a release of "young" erythrocytes into the blood stimulated by hypoxia. RBCs with high MCHC, all else equal, can transport more oxygen, increasing the whole blood oxygen transportation capacity. This is extremely important for the body to compensate for the raised physical activity post-training period. The occurrence of "new" erythrocytes in the blood flow at remote follow-up stages is indirectly confirmed by a significant change in their morphometric characteristics (decline in the mean cell volume) compared to the initial state. Interestingly, 5,000 m asl is likely the limit for rats since the reticulocyte count is lower at this altitude than at 3,000 m asl (Table 7).

Erythropoiesis is stimulated by the decline of oxygen delivery to tissues, which the kidneys detect. In hypoxia tissue, the kidneys secrete the hormone erythropoietin, which stimulates erythropoiesis.[2] This hormone stimulates the proliferation and differentiation of red blood progenitor cells, leading to accelerated erythropoiesis in hematopoietic tissues and elevated release of red blood cells into the blood. The decline of testosterone during hypoxic training is in some contradiction with the stimulation of erythropoiesis due to hypoxia. However, the production of erythropoietin by the kidneys and liver and red blood cells by the bone marrow is also controlled by several other hormones. In particular, the stress hormone cortisol can increase erythropoietin production by the kidneys and liver and directly stimulate the bone marrow erythroid lineage. The physiological significance lies in implementing stress reactions; raising red blood cell production and improving oxygen supply to tissues provides an advantage. An increased MNE frequency suggests a greater frequency of genetic mistakes during mitosis because, at a higher rate of erythropoiesis, the pyknotic nucleus and the surrounding chromatin fragments (Joly bodies or micronuclei) do not have time to leave the cells at the oxyphilic normocyte stage, before they enter the reticulocyte stage [15].

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 4: 1119-1130, 2024
DOI: 10.55214/25768484.v8i4.1486

Another explanation for that fact may be a slightly increased hypoxic stress since we have shown some increase in the level of cortisol – the stress hormone. It can lead to a decrease in telophases, an increase in chromosome fragility, and, as a result, an elevation of chromosomal abnormalities and MNE frequency. As for the noted trends of reduced hematocrit, such a body reaction is also not void of physiological expediency since these shifts are necessarily accompanied by optimization of the rheological properties and a decrease in blood thrombogenicity. Improvement of blood fluidity is known to significantly expand the functional capabilities of gas transport systems with increased body metabolic needs [15].

Blood components such as leukocytes, granulocytes, and monocytes significantly contribute to the state of hemostasis, which seriously impacts the transportation and utilization of oxygen by tissues and tolerance to hypoxia [16, 17]. In addition to the changes in blood already shown in the literature during the post-training period of hypoxia, we have demonstrated what happens in the lymphatic system. Increased lymphatic formation and lymphatic circulation, release of immunoglobulins into the lymph, their concentration in the lymph is higher than in the blood. There is an increase in cortisol, a stress hormone, and a decline in testosterone. These indicators depend on the altitude: at 5,000 m asl, they were more manifested than at 3,000 m asl.

Biochemical homeostasis parameters were changed but within the reference values, indicating normal energy nitrogen metabolism and activation of liver function. Hypoxia is a factor that is very natural by nature. The body begins to adapt, compensating for disorders at all levels of the body. The cycle of hypoxic training in our settings enhances the erythropoietic, metabolic, and hemodynamic functions that stimulate the synthesis of erythropoietin in serum, increase the volume of red blood cells, improve the economy of exercises, and increase the bloodstream, which leads to improved blood supply and the tissue utilization [18]. Ultimately, the cortisol content remained elevated during 1-2-day training periods, as in any case of acute stress [19], and mobilized the body for adaptation changes.

In the post-training period, the elevation of antioxidant protection at the cellular level and the decline of oxidative activity in the blood were observed. Thus, the changes in physiological, cellular, and biochemical parameters indicate an increase in the body's adaptation ability to increased loads under conditions of oxygen deficiency at an altitude of 3,000 m and 5,000 m asl [20, 21]. Adaptation reactions when staying at an altitude of 3,000 m and 5,000 m asl are reflected in the increase of functioning of the lymphatic system - the transport of water, proteins, fats, carbohydrates, electrolytes, hormones, and immunoglobulins. Stabilization of blood and lymph parameters is one of the criteria for adaptation to a certain cycle of hypoxic training. The study shows an increased mobilization readiness of the animal body to change the hematological and some biochemical parameters of blood and lymph, and the higher the altitude of hypoxic training conducted, the more significant changes observed. Hypoxic training increased blood oxygen transportation, the leukocyte count, and humoral immunity, decreased lipid metabolism, and thus improved infection resistance.

After hypoxic training, central hemodynamics parameters and external respiration were activated. The body got resistance to hypoxia, and an effective adaptation was formed. We observed activation of the hypothalamic-pituitary-adrenal system (cortisol elevation), heart hypertrophy and its hyperfunction of increasing blood oxygen capacity, capillary network enhancement, elevated hemoglobin and its chemical affinity for oxygen, and higher antioxidant system activity. The main studied parameters changed unidirectionally at 3,000 and 5,000 m asl. The changes were more pronounced after training at 5,000 m asl but remained within normal values.

A course of hypoxic training had a pronounced constructive immunomodulatory effect, which could be used for both rehabilitation and prevention. These changes rely on a hormonal-stress mechanism. A lack of oxygen triggers the stress hormones that modify the operation of organs and tissues.

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 4: 1119-1130, 2024
DOI: 10.55214/25768484.v8i4.1486

5. Conclusions

Cyclic repeated exposure to hypoxia at 3,000-5,000 m asl promoted erythropoiesis (erythrocytes, hemoglobin counts), increased the blood oxygen capacity and MNE frequency, and decreased hematocrit. The animals showed increased heart mass, lymph flow, and diuresis. Biochemical homeostasis changed within reference values. The activation of glucose transport, lipid utilization, strengthening of antioxidant protection, humoral immunity indicators, and improvement of blood and lymph flow properties were recorded. The main studied indicators changed unidirectionally at 3,000 and 5,000 m asl altitudes. The changes were more pronounced after training at 5,000 m asl but remained within normal values. According to the study, hypoxic training at 5,000 m asl is more effective in improving the physical performance of athletes. Hypoxic training at 3,000 m asl promotes rehabilitation and prevention of diseases of the lungs and cardiovascular system.

Abbreviations: aPTT, activated partial thromboplastin time; asl, above sea level; ECG, electrocardiogram; HGB, hemoglobin count in blood; HR, heart rate; M±SD, mean ± standard deviation; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MNE, micronucleated erythrocytes; O_2, molecular oxygen; PT, protrombin time; RBC, red blood cells; RDW, red cell distribution width; rm, respiratory movements; TT, thrombin time.

Funding:

This research was funded under a scientific project N 15-PTF-23-24 of the Ministry of Science and Higher Education of the Republic of Kazakhstan.

References

[1] Salyha, N., & Oliynyk, I. (2023). Hypoxia modeling techniques. Heliyon, 9(2), e.13238. https://doi.org/10.1016/j.heliyon.2023.e13238

[2] Makarenko, A.N., & Karandeeva, Y.K. (2013). [Adaptation to hypoxia as a protective mechanism in pathological conditions]. Vestnik probl biol med, 2(100), 27-33. Russian. Retrieved from https://cyberleninka.ru/article/n/adaptatsiya-k-gipoksii-kak-zaschitnyy-mehanizm-pri-patologicheskih-sostoyaniyah/viewer

[3] Storz, J.F., & Scott, G.R. (2019). Life Ascending: Mechanism and Process in Physiological Adaptation to High-Altitude Hypoxia. Annu Rev Ecol Evol Syst, 50, 503-526. https://doi.org/10.1146/annurev-ecolsys-110218-025014

[4] Cowburn, A.S., Macias, M., Summers, C., Chilvers, E.R., & Johnson, R.S. (2017)ю Cardiovascular adaptation to hypoxia and the role of peripheral resistance. eLife, 6, e28755. https://doi.org/10.7554/eLife.28755

[5] Watts, D., Gaete, D., Rodrigues, D., Hoogewijs, D., Rauner, M., Sormendi, S.M., & Wielockx, B. (2020). Hypoxia pathway proteins are master regulators of erythropoiesis. Int J Mol Sci, 21(21), 8131. https://doi.org/10.3390/ijms21218131

[6] Chen, P.S., Chiu, W.T., Hsu, P.L., Lin, S.C., Peng, I.C., Wang, C.Y., & Tsai, S.J. (2020). Pathophysiological implications of hypoxia in human diseases. J Biomed Sci, 27(63), 1-19. https://doi.org/10.1186/s12929-020-00658-7

[7] Sivam, S., Yee, B., Wong, K., Wang, D., Grunstein, R., & Piper, A. (2018). Obesity hypoventilation syndrome: early detection of nocturnal-only hypercapnia in an obese population. J Clin Sleep Med, 14(9), 1477-1484. https://doi.org/10.5664%2Fjcsm.7318

[8] Rupp, T., Mallouf Tle, R., Perrey, S., Wuyam, B., Millet, G.Y, & Verges, S. (2015). CO2 clamping, peripheral and central fatigue during hypoxic knee extensions in men. Med Sci Sports Exerc, 47(12), 2513-2524. https://doi.org/10.1249/mss.0000000000000724

[9] Charry, D., Wang, T., Mclaurin, N., Leelartapin, K., Ponlasen, S., Sukson, D., & Tanaka, H. (2023). Effectiveness of different recovery postures during high-intensity interval training. J Sports Med Phys Fitness, 63(12), 1295-1300. https://doi.org/10.23736/S0022-4707.23.15109-7

[10] Makashima, D., Fujie, S., Togo, M., Takeshita, S., Hasegawa, K., & Tabata, I. (2023). Effect of acute high-intensity intermittent exercise on serum brain-derived neurotrophic factor concentrations. J Sports Med Phys Fitness 63(6), 731-738. https://doi.org/10.23736/S0022-4707.23.14596-8

[11] Neznanov, N.G., & Babakhanyan, R.V. (2002). [Didactic and ethical aspects of conducting research on biomodels and laboratory animals]. Kachestv Klin Prakt, 1(4), 27-31. Russian. Retrieved from https://www.clinvest.ru/jour/article/view/249/256

[12] Bakhtiyarova, Sh.K., Dzhusipbekova, B.A., Zhaksymov, B.I., Makashev, E.K., Erlan, A.E., Alpysbaeva, K.K., & Khasenova, H.H. (2016). [Processes of lipid peroxidation in sturgeon fish when fed with various foods]. Mezhd Zh Prikl Fundam Issl, 5(4), 595-598. Russian. Retrieved from https://applied-research.ru/ru/article/view?id=9457

[13] Demchenko, G., Koibasova, L., Kozhaniyazova, U., Yernazarova, G., Abdualy, A., & Kolamsak, S. (2024). Physiological and biochemical indicators of the organism after normobaric hypoxic training in combination with a protein product supplementation. BIO Web Conf, 100, 01012. https:/doi.org/10.1051/bioconf/202410001012

[14] Wilber, R.L. (2007). Application of altitude/hypoxic training by elite athletes. Med Sci Sport Exerc, 39(9), 16110-1624. https://doi.org/10.1249/mss.0b013e3180de49e6

[15] Finsterbusch, M., Schrottmaier, W.C, Kral-Pointner, J.B, Salzmann, M., & Assinger, A. (2018). Measuring and interpreting platelet-leukocyte aggregates. Platelets, 29, 677-685. https://doi.org/10.1080/09537104.2018.1430358

[16] Baikovsky, S.E. (2010). [Factors that determine an athlete's training in high and mid-altitude conditions]. Moscow: TVT Divizion. Russian.

[17] Lodge, K.M., Cowburn, A.S., Li, W., & Condliffe, A.M. (2020). The impact of hypoxia on neutrophil degranulation and consequences for the host. Int J Mol Sci, 21, 1183. https://doi.org/10.3390/ijms21041183

[18] Chapman, R.F., Karlsen, T., Resaland, G.K., Ge, R.L., Harber, M.P., Witkowski, S., Stray-Gundersen, J., & Levine B.D. (2014). Defining the dose of altitude training: How high to live for optimal sea level performance enhancement. J Appl Physiol, 116(6), 595-603. http://dx.doi.org/10.1152/japplphysiol.00634.2013

[19] Obernikhin, S.S., Yaglova, N.V., Nazimova, S.V., Yaglov, V.V., & Timokhina, E.P. [Transcriptional regulation of morphogenesis of rat adrenal zona glomerulosa during postnatal development]. Clin Exp Morphol 2019;8:48-54. Russian. https://doi.org/10.31088/2226-5988-2019-30-2-48-54

[20] Drzazga, Z., Schisler, I., Poprzecki, S., Michnik, A., & Czuba, M. (2019). Serum autofluorescence and biochemical markers in athlete's response to strength effort in normobaric hypoxia: A preliminary study. BioMed Res Int, 2019(3), 1-11. http://dx.doi.org/10.1155/2019/5201351

[21] Yan, C., Tian, D., Zhang C. Zhang, Q., & Sun, Y. (2023). Evaluation of blood cellular and biochemical parameters in rats under a chronic hypoxic environment at high altitude. Ann Med, 55(1), 898-907. https://doi.org/10.1080/07853890.2023.2184859

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 4: 1119-1130, 2024
DOI: 10.55214/25768484.v8i4.1486