Physiological and biochemical indicators of the organism after normobaric hypoxic training in combination with a protein product supplementation

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Abstract. The article shows studies conducted to study physiological and biochemical blood parameters after normobaric hypoxic training in combination with protein nutrition. The studies were conducted on 45 white male Sprague Dawley rats weighing 250-280 g. Hypoxic training in combination with protein nutrition led to an increase in red blood cells, hemoglobin, oxygen capacity of the blood, and a decrease in hematocrit. There was also an increase in plasma volume, lymph flow and urine output, while heart weight values remained within the normal range. Indicators of biochemical homeostasis of plasma and lymph do not go beyond the reference values, which indicate the normal maintenance of protein and mineral metabolism and the absence of pathological processes in the body. Studies have shown that the combination of normobaric hypoxic training at an altitude of 2800-3000 m above sea level with protein nutrition in animals improved the physiological state of the body, which will allow timely adjustment of nutrition to optimize the training process.

1 Introduction

Training under hypoxic conditions is actively used to improve performance and reach peak athletic fitness before major competitions. For this purpose, the natural conditions of the mountains (low mountains, middle mountains, high mountains), and special equipment (pressure chambers, climatic chambers, hypoxicators) are used [1]. It is known that hypoxia is a condition characterized by a decrease in molecular oxygen (O_2) or associated with the problem of regulating oxygen levels during intracellular redox reactions [2]. To assess the individual adaptive reactions of the body under conditions of hypoxia, the results of a detailed laboratory study of the parameters of homeostasis – biochemical, immunological, hematological, hormonal, etc., are necessary, which make it possible to assess the functional state of the main performance-limiting systems of the body. The balance of stress-induced and anabolic factors is of great importance to support the performance of

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athletes' bodies [3]. Adequate nutrition of athletes plays an important role in ensuring the training process and the first phase of pharmacological support for athletes. At the same time, the role of nutrition during normobaric hypoxic training is poorly understood. It was of interest to study the effect of normobaric hypoxic training on the body at an altitude of 2800-3000 m above sea level in combination with a new type of protein nutrition.

The purpose of the study is to study the physiological and biochemical parameters of the body during normobaric hypoxic training in combination with a new protein product.

2 Materials and Methods

The work was carried out within the framework of scientific project №15-TFP-23-24 MHES RK.

Circulatory hypoxia was reproduced on 45 white male Sprague Dawley rats weighing 250-280 g. The studies were carried out on animals under standard vivarium conditions on a standard diet (GOST R 50258-92). The study was conducted with the approval of the Ethical Commission Institute of Genetics and Physiology (Conclusion №5 of July 25, 2022). The presence of laboratory animals in the vivarium met the European requirements for working with laboratory animals [4].

The rats were divided into 3 groups (15 animals per group): 1 - control group, 2 - hypoxic training group at an altitude of 2800-3000 meters above sea level, 3 - hypoxic training group + protein nutrition at the same altitude (4 weeks, number of trainings - 48). Hypoxic training was carried out in a hypoxic chamber for animals (Ox-100), session duration was 34-35 minutes at an O_2 concentration that dropped from 20.5% to 14.5%, which corresponded to an increase to an altitude of 2800-3000 m above sea level. O_2 reduction and maintenance was carried out using a 10L Nitrogen concentrator device, and CO_2 reduction in the hypoxic chamber was carried out using a Medical Soda Lime absorber.

Animals in the 3rd group were supplemented with standard vivarium food with energy protein food in the form of a bottle containing the following components: crushed activated porous buckwheat, powdered cream, dried camel milk, maltodextrin, dried mare's milk, crushed almonds, etc. (registration number KZ7500361.24.01.03089). 8 grams per animal product after each workout in a hypoxic chamber. The rat ate 400 grams of the product during the training period.

To obtain blood, lymph and urine samples, animals were inhalation anesthetized with ether through a mask and the anesthetic Ketamine was administered intramuscularly. The thoracic lymphatic duct near the diaphragm was dissected and a graduated microcannula was inserted into it, the outflow of lymph was determined and the lymph was collected for research. After lymph collection, the abdominal aorta was prepared, into which a Teflon catheter was inserted to collect blood. The following was determined in blood samples: pH, PCO_2 , PO_2, BE, tCO_2, HCO_3, $stHCO_3$, tHb,Hct, SO_2 , Na^+, K^+,Cl^-,AnGap using the OPTI CCA –TS2 Blood Gas and Electrolite Analyzer. The content of total protein, cholesterol, triglycerides, total lipids, urea, creatinine and bilirubin was determined in blood and lymph samples. The levels of ALT, AST, alkaline phosphatase and amylase in lymph and blood plasma were determined by standard methods using an automatic biochemical analyzer Cobas Integra 400 (Roche Diagnostics). The cellular composition of blood and lymph was determined using a hematological analyzer Sysmex KX-21. Determination of biochemical parameters and urine cells was carried out using an analyzer (CL-50Urine Analyzer. High Technology. USA). The content of hormones in the blood and lymph was determined on a RocheCobase 411 Immunochemical Analyzer. The muscle response, ECG and heart rate in animals were recorded through the sensors of a REO-Mizar rheograph. In blood and lymph

samples, the viscosity was determined using a "VK-4" viscometer (Moscow) and the coagulability of these biological fluids.

The results obtained were statistically processed using traditional methods of variation statistics in Microsoft Excel (Microsoft Corparation, Redmond, Washinfton, DC, USA). The arithmetic means and its deviation (M±SE) in % were calculated. The significance of differences in mean values was assessed using the Fisher and Student t-test. The threshold for statistical significance was $p \leq 0.05$.

3 Results and Discussion

After hypoxic training, there was a slight increase in red blood cells in the blood. The hemoglobin content in erythrocytes remained at the figures as in the control group. But there was an increase in the oxygen capacity of the blood (Table 1, 2).

Table 1. Indicators of O_2, CO_2 acidity/alkalinity, electrolytes and hemoglobin status in the blood after normobaric hypoxic training

Indicators	Control group №1	Group with hypoxia №2	Group C of hypoxia + protein nutrition №3
pH	7.3±0,06	7.3±0,07	7,2±0,05
PCO_2 (mmHg)	24.4±0,4	27.3±0,5*	28,2±0,6*
PO_2 (mmHg)	114.6±8,5	124,1±9,6	129,7±7,6*
BE (mmol/l)	-7.5±0,3	-10.8±0,4	-9,9±0,6
tCO_2 (mmol/l)	17.1±0,8	14.7±0,7	16,0±0,9
HCO_3 (mmol/l)	16.4±0,9	13.8±1,1	15,1±0,8
$stHCO_3$ (mmol/l)	19.4±1,0	15.8±0,9	15,6±0,8
tHb (g/dl)	11.9±0,8	12.4±0,9	14,8±0,6*
Hct (%)	37.7±11	38,2±13	44,6±10
SO_2 (%)	96.5±12	98,0±14	98,3±12
Na^+ (mmol/l)	139.4±18	132,3±15	132,5±16
K^+ (mmol/l)	3.7±0,07	3,32±0,09	3,5±0,06
Ca^+ (mmol/l)	1.3±0,01	0,7±0,02*	0,60±0,03*
Cl^- (mmol/l)	107.6±10	104,9±12	100,4±11
AnG ap. (mmol/l)	20±0,9	18,3±1,1	19,3±0,8
Bun (mmol/l)	13.5±0,8	12,9±1,2	13,0±0,7
Glu (mmol/l)	180,8±15	241,4±17*	237,8±16*

Note: significantly compared with the control, *p<0.05, **p<0.001.

A slight increase in leukocytes in the blood was observed (Table 3). With hypoxia, there are regular phase changes in pulmonary ventilation in group 1 of 38 ± 4d.d. per minute, and after training at 2nd group 30.4 ± 5d.d per minute, in 3rd group 32.3±4 d.d. per minute. Breathing is adjusted to a lower level of pO_2, which maintains a constant level of hyperventilation and thereby ensures normal blood oxygenation (Table 1).

The heart rate after hypoxic training in animals was reduced. The first group has 460 ± 12 min., the second 455± 11 and the third group has 448± 10 min. An ECG after hypoxic training showed an increase in the cardiac cycle and an expansion of the QS complex.

An analysis of the rheogram in rats after hypoxic training showed a change in perfusion parameters in the microcirculatory bed of the hind limb - a decrease in the 2nd, as well as in the 3rd groups. Repeated hypoxic effects improve myocardial circulation without causing its

hypertrophy. The weight of the heart in 1st group 0.383 ±0.06, 2nd group 0.374±0.07, 3rd group 0.392± 0.07 per 100 g of animal weight (Table 1).

Table 2. The effect of normobaric hypoxic training on the total blood count at an altitude of 2800-3000 supercharged meters

Indicators	Control group №1	Group with hypoxia №2	Group C of hypoxia + protein nutrition №3
WBS (10^9/L)	2,85± 0,1	3,4±0,2*	3,3±0,3*
LYM (10^9/L)	2,7± 0,3	2,7±0,05	3,0±0,4
LYM %	55,5 ±3,4	55,2±6,1	61,8±5,4
MID (10^9/L)	0,07±0,001	0,15±0,05*	0,2±0,06*
MI %	3,75±0,021	9,5±0,031**	6,0±0,04*
GRA (10^9/L)	0,02±0,001	0,02±0.04	0,06±0,001
GRA %	2,35±0,22	2,1±0.07	2,1±0,12
HGB g/L	150,0 ±3,0	141,1±3,1	149,9±3,2
MCH pq	19,65±1,1	18,5±2,1	19,3±1,9
MCHC	40,4±2,1	37,9±2,4	38±3,2
RBC(10^{12}/L)	7,4± 0,2	7,7±0,4*	7,7±0,6
MCV fl	48,6±5,1	48,6±4,6	50,7±5,7
HCT %	45,0± 3,2	37,5±4,2	39,3±6,1
RDWcfl	36,1±0,8	34,8±4,2	38,3±0,9
RDWs %	13,7±0,5	12,7±0,6	13,7±0,4
PLT (10^9/L)	525 ±14	500,3±20	440,6±28*
MPV	7,4±0,5	7,1±0,7	7,2±0,6
PDWafl	10,35±0,7	10,2±0.9	10,4±0,8
PDWc %	38,65±0,7	39±3,1	39,9±3,5
PCT %	0,50±0,04	0,35±0,05	0,24±0,07

Note: significantly compared with the control, *p<0.05, **p<0.001.

Hypoxic training led to a slight increase in lymph circulation from 0.0036±0.0004 to 0.0047±0.0005 ml and in group 3 to 0.0052±0.0005ml (p<0.05) per 100 grams of body weight in 1 min (Fig. 1). An increase in urination was observed from 0.014±0.003 to 0.021±0.004 ml and in group 3, 0.020±0.004 (p<0.05) ml per 100 grams of body weight (Fig. 1). After hypoxic training, we observed a slight decrease in the clotting properties of blood, as well as lymph. We observed a decrease in blood and lymph viscosity after training, in the 2nd and 3rd groups and a decrease in the number of platelets (Table 2). The electrolyte composition of the blood was stable, with the exception of Ca, which decreased in groups 2 and 3 (Table 1). Hypoxic effects caused moderate stress activation of the body – an increase in cortisol, both in blood and lymph, testosterone decreased. Biochemical parameters of blood and lymph are shown in Tables 1,2,3. They did not reveal pathological abnormalities. Only the lipidogram indicators decreased slightly. Activation of protein metabolism was also revealed. Cortisol group 1 46.2±2.4 blood; 23.5±1.3 lymph; group 2 56.1±3.5; 28.4±4.1; group 3 54.5±4.1; 31.9±3.4 p<0.05. Testosterone group 1 9.60±0.32 blood; 8.51±0.32 lymph; group 2 5,62±0,42*; 5,04±3,1*; 3 the group 4,67±0,33*; 4,02±0,34*.

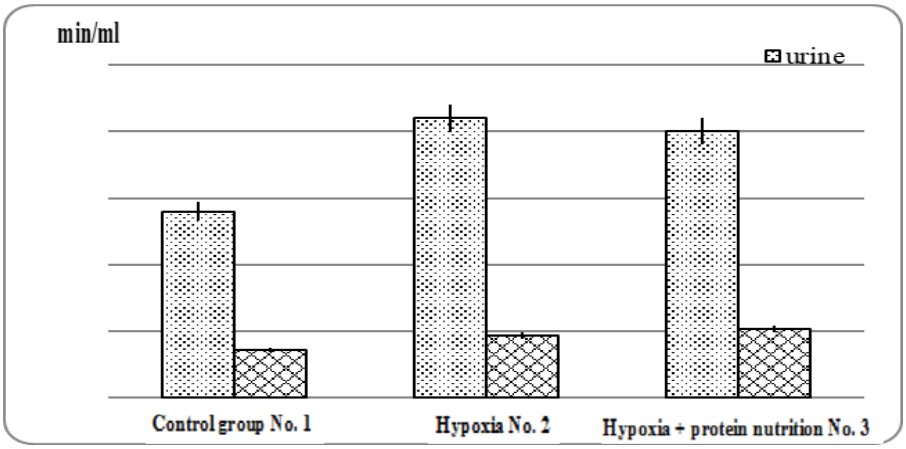

Fig.1. Lymph flow and urination during hypoxic training.

Urine analysis (leukocytes, ketone body, protein, erythrocytes, pH, urobilin, specific gravity) did not reveal significant changes (Table 3).

Table 3. Biochemical parameters of blood and lymph after normobaric hypoxic training at an altitude of 2800-3000 above sea level.

Tests	Groups of animals					
	Control group №1		Group with hypoxia №2		Group C of hypoxia + protein nutrition №3	
	Blood	Lymph	Blood	Lymph	Blood	Lymph
ALT (ME/L)	250,9±22	80,3±6,4	194,4±11,3	92,2±10,2	235,1±21	125,5±9,1
AST (ME/L)	167,6±15	76,7±9,4	197,1±17,3*	90,6±12,4	240,4±14*	96,3±8,5
CA (mmol/L)	1,7±0,2	1,62±0,1	1,41±0,4*	1,4±0,2	1,5±0,3	1,4±0,5
CHOL (mmol/L)	1,9±0,0	1,43±0,1	1,41±0,1*	1,3±0,2*	1,1±0,05*	1,3±0,0
CREA (mkmol/L)	49,6±4,1	13,16±8,8	48,3±5,9	5,8±4,7	72,7±7,8*	6,0±5,3
LDH (ME/L)	138,8±3,0	67,2±1,5	127,2±4,3*	45,1±1,7*	137,3±5,1	65,5±2,1
LDL (mmol/L)	0,5±0,0	0,29±0,0	0,67±0,0	0,2+0,02	0,6±0,04	0,2±0,0
TBIL (mkmol/L)	1,7±0,85	0,6±0,0	1,92±0,1	0,4±0,0*	1,9±0,03	0,5±0,0
TP(g/L)	64,3±5,1	24,7±2,3	60,7±4,9	22,8±3,3	69,3±5,9	31,8±2,8
TRIG (mmol/L)	1,3±0,1	0,87±0,7	1,2±0,0	0,5±0,1*	1,2±0,04	0,6±0,1
UREA (mmol/L)	5,3±0,2	5,55±3,3	7,2±0,2*	5,8±0,1	8,3±0,82	6,3±0,9

Note: significantly compared with the control, *p<0.05, **p<0.001

4 Conclusion

Hypoxic training led to a restructuring of breathing, slowing it down and deepening it during the post-training period. This helped oxygenate a larger volume of blood in one breathing movement and increase the maximum oxygen consumption over a given period of time. A decrease in the coagulation activity of blood and lymph and its anticoagulation properties is a positive phenomenon, especially since the pH does not change. The most important and rapid adaptive effect of hypoxia is the expansion of capillaries in the brain, heart and lungs, which runs in parallel with increased blood circulation, and therefore the minute volume of blood circulation increases [5]. Hypoxia is a condition that occurs due to a lack of oxygen in the body's tissues. The reasons for impaired oxygen starvation can be completely different, but in any case, with oxygen deficiency, the synthesis of adenosine triphosphate (ATA), which is necessary for the normal functioning of the whole organism, is disrupted, but the body prevents these processes by adaptive mechanisms [6]. Under conditions of exposure to hypoxia, a number of important compensatory changes occur in the body, including the initialization of the respiratory and cardiac systems, as well as the restructuring of the vascular system, shunting of blood in the lungs, expansion of the alveolar surface, an increase in the number of regional vessels, which stimulates increased blood supply to the heart and liver, for due to a decrease in blood supply to skeletal muscles [7]. We detected a decrease in capillary circulation on the rheogram of the femoral muscle. The training effect of hypoxic training changes the parameters of central hemodynamics along with changes in the functions of the peripheral circulation, primarily the resistive, capacitive and microcirculatory sections. Redistribution of extracellular fluid and increased functioning of the lymphatic system help maintain systemic hemodynamics in these new conditions. The lymphatic system, like the cardiovascular system, is involved in the body's adaptive reactions [8]. Biochemical changes in the composition of lymph are associated with an increase in liver enzymes, lipase, and a decrease in lipid and protein metabolism. A significant increase in glucose was shown. What is positive that it is not the immediate changes in the body, but the duration of preservation of the identified effects [9]. The body has an extensive arsenal of behavioral, physiological, and biochemical tools for restoring adequate oxygen conservation, and this arsenal can be successfully activated and increased with the help of hypoxic training. The oxygen status of the body includes a hypoxic state as an integral regulatory reaction [10]. The new protein product did not reveal any deviations from the norm in biochemical and physiological parameters; it only had a beneficial effect on animals. In our study, we did not find large differences between the group of animals with training in a hypoxic chamber and the group of animals that had enhanced protein and vitamin nutrition. Perhaps we will get differences in the future with physical training in a hypoxic chamber. Experimentally simulated hypoxia under controlled conditions made it possible to understand the internal mechanisms and test a new protein product to accelerate adaptation and prevent conditions associated with hypoxia.

Thus, the applied dosed hypoxic training with additional protein nutrition made it possible to significantly improve the parameters of hemolymphodynamics and cardiorespiratory systems. Non-medicinal methods of influencing the body in the form of hypoxic training are aimed at increasing the functional level of adaptive processes, which increase physical capabilities and provide a good therapeutic and prophylactic effect.

Authors contribution

All authors participated in planning the experiment, interpreting the results, and reviewing the article; D.G.A, K.L.U and K.U.N drafted the manuscript, conducted the study, and interpreted the study; Y.G.I, A.A. and S.K.U participated in the development of the concept

and design of the study. All authors have read and agreed to the published version of the manuscript.

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