E.D. Oкhlopkova, L.I. Konstantinova, L.D. Olesova, G.E. Mironova, E.I. Semenova, A.I. Yakovleva, A.V. Efremova, Z.N. Krivoshapkina, V.A.Alekseev, V.M. Nikolaev, S.D. Efremova

THE CORRECTION OF PEROXIDATION DURINGPHYSICAL ACTIVITYBY EXOGENOUS FACTORS IN THE CONDITIONS OF COLD CLIMATE OF YAKUTIA

ABCTRACT

The effect of the vitamin-mineral complex and one-day koumiss on the imbalance of the LP-AOS organism of athletes of Yakutia was studied. It is established that in winter during the period of intense physical activity the intake of the vitamin and mineral complex helps to reduce the intensity of lipid peroxidation and has a supporting effect of the body's antioxidant defense during the 20-day period. The antioxidant effectiveness of koumiss in summer is manifested by an increase in the activity of the enzymatic link already on the 10th day of administration and is an effective means for accelerating the body's recovery processes

Keywords: lipid peroxidation, antioxidant system, physical activity, cold, Yakutia.

Introduction

In the Far North, where winter lasts for more than 6 months, cold is one of the main factors affecting metabolic changes in a human body. It is known, that one of the adaptive reactions of the organism to the cold climate is an acceleration of metabolic processes, including theoxidation of free radicals[1,5,11]. At the same time, intense physical loads contribute to the intensification of peroxidation processes and formation of free radicals [9].

In modern sports, with an increased level of physical activity at all stages of training process, the prevention of fatigue, the acceleration of recovery processes and the increase in general and physical performance through various exogenous additives is becoming increasingly popular [9]. In the conditions of Yakutia, the recovery processes can be accelerated with the help of bio-additives from local raw materials of plant and animal origin [2]. Products with antioxidant effect can stimulate the body's defenses, increase overall stability and vitality, physical and mental performance. Therefore, study of properties of biological additives that reduce negative impact of negative environmental factors, has special role in prevention of development of pathological conditions associated with intense physical exertion not only among athletes, but also in population engaged in physical labor in extreme cold conditions of Yakutia.

The aim of the study was to evaluate the effectiveness of the vitamin and mineral complex and koumiss in maintaininglipid peroxidation (LP) and antioxidative defense (AOD) systems ofathletes under intensive physical exertion in Yakutia.

Materials and methods

The study involved 79 volunteers (freestyle wrestlers) of Yakut nationality, which were divided into four separate groups. The first and the second group consisted of 39 highly qualified athletes from the School of Higher Sportsmanship in Yakutsk and students of the Institute of Physical Training and Sports of Northeastern Federal University, at the age of 21 to 24. The first group consisted of 21 sportsmen who took «Valtek-SP Active» drink prepared from a dry mixture supplemented with vitamins and minerals once a day after evening workout during 20 days. The second group consisted of 18 sportsmen who took similar drink that did not contain vitamin and mineral additives made by ValetekProdimpexas a placebo. Biochemical studies in these groups were performed three times (before the drink administration, after 10 days and after 20 days of administration). The study was carried out in the beginning of the competition period, during the winter period (November-December).

The third and the fourth group consisted of 40 athletes from the School of Olympic Reserve aged 13-16 years. The study was conducted during the beginning of the recovery period in the summer sport camp «Rodnik».

The third group included 20 athletes who took 250 ml of kumis drink everyday 4 times a day (3 times 20-30 minutes before meals and once before the bedtime) during 10 days; the fourth group consisted of 20 sportsmen who did not take kumis during the recovery period. The study was performed twice, before the start of kumis administration and on the 11th day after the end of kumis administration.

The vitamin-mineral supplemented drink was prepared by dissolving 10 g of the dry mixture in 250 ml of bottled water at room temperature. The drink contained following vitamins and minerals: A (0.39 mg), E (7.5 mg), D3 (5.35 μg), C (64.0 mg), B1 (0.95 mg), B2 (1.1 mg) , B6 (1.2 mg), B12 (1.6 μg), PP (9.65 mg), K1 (48.5 μg), pantothenic acid (2.9 mg), folic acid (0.38 mg) , biotin (16.0 μg), calcium (140 mg), magnesium (100 mg), succinic acid (50 mg), carbohydrates (6.7 g). Certificate of Conformity of the Russian Federation No. C-RU.AE84.V.06712 TR 0497019 and «Dry mixture with vitamins and minerals «Valetek-SP Active»(cherry and orange) in the form of a drink (certificate of state registration RU.77.99. 11.003.E.045369.11.11 issued 03.11.2011) produced by ZAO ValetekProdimpeks. Kumis was manufactured byOAO Sakhaplemobedineniyefrom mare's milkwith the use of standard technology TU 9222-001-55673105-2009.

The research material was heparinized blood and serum. Blood sampling was performed in the morning on an empty stomach from the ulnar vein. The study protocol was approved by the local committee on biomedical ethics at the Yakutsk Scientific Center of Complex Medical Problems (Minutes

No. 24 of June 29, 2010). The samples were collected after voluntary informed agreement of the participants.Studies of the lipid peroxidation rate and the state of antioxidative system were carried out on erythrocyte suspension using spectrophotometric method on Specord 40 spectrophotometer. Lipid peroxidation was determined by the accumulation of thiobarbiturate-active substances(TBAS) [12].

Antioxidative protection was measured by superoxide dismutase (SOD)[10] and catalase [4] activities, as well as by the total content of low molecular weight antioxidants (LMWA) [7]. The content of ascorbic acid in blood serum was measured using titrimetric method. The statistical analysis was done using statistics software Statistica 6.0 and SPSS 19.0 for Windows. The normality of independent variables was confirmed by nonparametric Kolmogorov-Smirnov test.

The reliability of the mean differences between the groups with an abnormal distribution was determined by the Mann–Whitney t-test. Probability of the validity of the null hypothesis was assumed for $p < 0.05$.

Results and discussion

The results of our study demonstrated that the intensity of lipid peroxidation and the state of antioxidative system in the first, third, second and fourth groups before taking the vitamin-mineral drink and kumis on the first day of the study were significantly different. The concentration of TBAS in the first and second groups was reduced by 45 and 41%, compared to the third and fourth groups, respectively (Fig. 1-2). The initial values of the total LMWA content of the athletes of the first and the second group were higher by 75 and 69%, SOD by 71%, and catalase activity in both groups was reduced by 33 and 43%, compared to the third and fourth groups, respectively. The level of vitamin C in all groups of athletes was normal, but was lower in the third group (Table 1-2).

Changes in indicators were associated with different levels of load during training periods and with the time of the year during the study and were consistent with previously published data [6,8]. On the 10th day, the following changes were recorded in the study groups: the concentration of TBAS in the first, second, and the fourth group did not change, and in the third group it was significantly decreased by 35% ($p<0.05$) compared to the first day of the study 1-2). The total content of LMWA during this period increased in all four groups, but the nature of the increase had some differences. In the first and the third group LMWA levels were significantly increased - by 44 and 41%, respectively, which was due to the positive effect of the synthetic vitamin-mineral complex and kumis drink. In the second and the fourth group, the increase was insignificant - by 6 and 19% respectively, compared to the first day. The activity of antioxidative defense enzymes varied in different ways. A decrease in catalase activity in all groups was observed. The greatest decrease was by 11 and 19% in the first and the third group respectively, which is probably due to a parallel increase in SOD activity, and in the second and the fourth group the decrease was by 8 and 11% respectively. The activity of SOD in the first group did not change, and in the third group it was increased by 80%, in the second group the enzyme activity remained at the initial level, and in the fourth group it increased by 24% compared to the first day of the study. Nutritional factors that reduce catalase activity include lack of vitamin B, folic acid, biotin, vitamin A. The reduction of catalase levels in the third group was more pronounced, similar to the effect of excess of methionine, tyrosine, cystine, copper and zinc, which are contained in kumis. Catalase prevents the accumulation of hydrogen peroxide in the cell, which is formed during aerobic oxidation of reconstituted flavoproteins and also from oxygen. The decomposition of peroxide requires very small amount of enzyme, the reaction

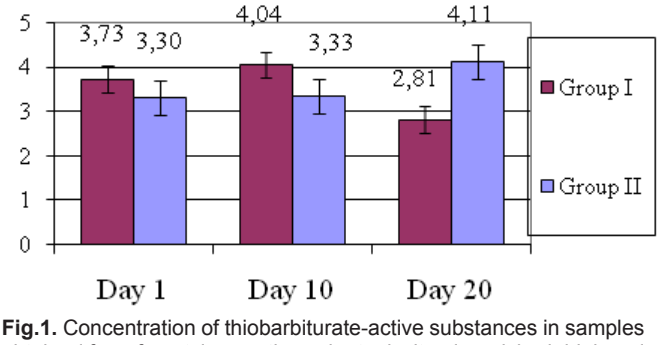

Fig.1. Concentration of thiobarbiturate-active substances in samples obtained from freestyle wrestlers who took vitaminenriched drink and a placebo for 20 days (nmol/L).

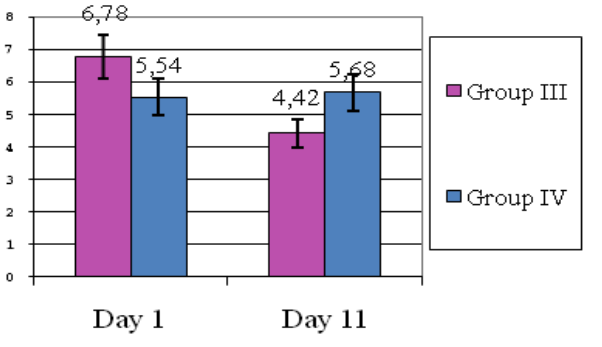

Fig.2. Concentration of thiobarbiturate-active substances in samples obtained from freestyle wrestlers who drank kumis for 10 days and did not drink kumis (nmol/L).

Table 1

The indicators of the antioxidative system of freestyle wrestlers before and after administration of the vitamin-mineral drink

	GroupI (People who were administered the drink) (n=21)			GroupII (People who were administered placebo) (n=18)		
	Day 1	Day 10	Day 20	Day 1	Day 10	Day 20
LMWA,mg×equvalent /ml erythrocyte	0,065±0,00	0,098±0,00**	0,130±0,01***	0,068±0,00	0,072±0,00	0,087±0,00
SOD, μM/min×ml	0,061±0,00	0,060±0,05	0,061±0,00	0,061±0,01	0,062±0,00	0,061±0,00
CAT cat/L	0,700±0,03++	0,625±0,05*	0,592±0,05**++	0,509±0,05	0,467±0,04	0,251±0,03
Vitamin C, 0.7-1.5 mg/dL	1,01±0,09	1,51±0,07	1,76±0,06*+	1,01±0,12	1,24±0,17	1,04±0,20

*) $p<0,5$; **)$p<0,001$; ***)$p<0,0001$ in the first group compared to the first day of study; +) $p<0,5$; ++) $p<0,001$; +++)$p<0,0001$ compared to the second group that was administered a placebo.

Table 2

The indicators of the antioxidative system of freestyle wrestlers who were and were not administered kumis

	Group III (people who drank kumis) (n=20)		Group IV (people who did not drank kumis) (n=20)	
	Day 1	Day 10	Day 1	Day 10
LMWA, mg×equvalent /ml erythrocyte	0,016±0,00	0,027±0,00*	0,021±0,00	0,026±0,01
SOD, μM/min×ml	0,018±0,00	0,090±0,05**	0,019±0,01	0,022±0,00
CAT cat/L	0,915±0,04++	0,750±0,05*	0,899±0,01	0,804±0,05
Vitamin C, 0.7-1.5 mg/dL	0,87±0,08	1,38±0,09*	1,03±0,10	0,95±0,08

*) p <0.5; **) p <0.001; in the first group, in comparison with the first day of the study.

speed is determined by diffusion and does not require energy for its activation [3]. It should be noted that catalase is the second link of antioxidative defense system, therefore, the reduced activity of the enzyme in athletes of the 3rd and 4th groups can be related to more efficient work of the first link of the antioxidative defense system, in particular, SOD and non-enzymatic antioxidants. On the 20th day of the study in the 1st group of athletes who were taking the vitamin drink, the concentration of TBAS decreased by 24%, which indicates a decrease in the intensity of lipid peroxidation in this group. In contrast, in the second group of athletes who were administered a placebo, the concentration of TBAS, increased by 20%, compared to the first day, which is an unfavorable sign and shows the tension of adaptation processes during the period of competition (Fig. 1). The total content of LMWA increased in both groups of athletes: in the first group by 50 (p <0.0001) and in the second group by 22%. Catalase activity in both groups decreased in comparison with the first day of the study, but in the first group of athletes the decrease in activity was insignificant - 15% (p <0.001), while in the second group, 51% (p <0.001). The SOD level remained unchanged throughout the study period. The content of vitamin C in the first group increased by 57%, while in the second group it remained at the same initial level (Table 1). Consequently, «Valtek-SP Active» vitamin-mineral complex had a significant antioxidative effect on the 20th day of the study. To determine the efficacy of vitamin-mineral drink and kumis, a comparative analysis of pro- and antioxidant equilibrium was performed in all groups, which was calculated with the formula:

CAOD / LP = LMWA + SOD + CAT / TBAS.

At the beginning of the study, the coefficient of pro- and antioxidant balance in the athletes of the first group was 30% higher than in the third group (Fig. 3), which can be explained by the relatively low level of TBAS (Fig. 1). Thus, the lower intensity of lipid peroxidation and the best state of antioxidative system (increased level of SOD activity, content of LMWA and ascorbic acid (Table 1)), was common for the beginning of the competition period. In the third group, a decrease in CAOD/LP value was attributed to a high level of TBAS and a decreased antioxidativedefence, as the athletes were fatigued and overtrained at the initial stage of the recovery period. On the 10th day of the study, the three groups showed a decrease in CAOD/LP index: in the first group, by 10, in the second group by 6 and the fourth group by 12%. The third group, demonstrated a significant 30% increase in CAOD/LP (Fig. 3), which is a favorable sign in the recovery period and is associated with a decrease in TBAS levels (Figure 2). The increase in LMWA, SOD and vitamin C levels (Table 2) indicate ongoing regenerative processes in the body and adaptation of the organism. On the 20th day in the first group there was 20% increase in CAOD/LP value compared to the first day and 36% on the 10th day (Fig. 3), which was caused by statistically significant decrease in TBAS by 24%, an increase in the content of LMWA by 15, vitamin C by 57% and with the supportive action on the enzymatic link. Catalase activity associated with increasing competition loads decreased by only 15%, and SOD activity did not change (Table 2). In the second group CAOD/LP value decreased by 50 and 53%, respectively, compared to the first and the tenth day, indicating a decrease in antioxidativedefense levels (nonenzymatic and enzymatic part) and the prevalence of prooxidative processes.

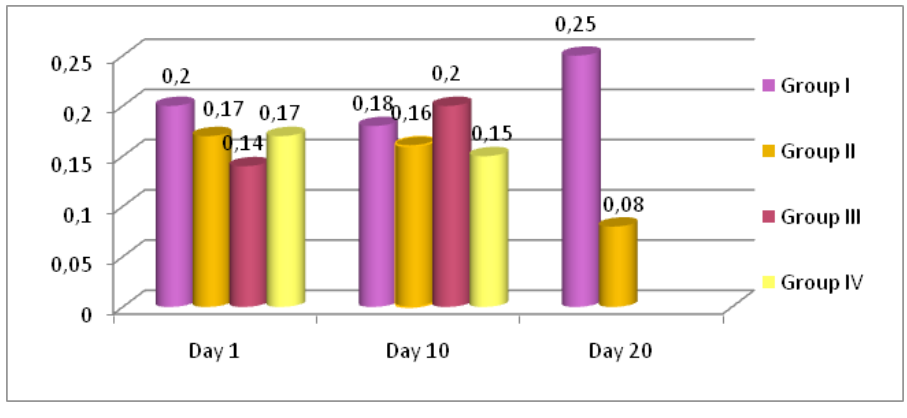

Fig. 3. The level of antioxidant balance before and after administration of the vitamin-mineral complex and kumis

References

1. BoikoE.R. Fiziologo-biokhimicheskie osnovy zhiznedeiatelnosti cheloveka na Severe [Physiological and biochemical basis of human activity in the North]. Ekaterinburg: UrO RAN, 2005. p. 190.

2. Kershengolts B.M. Zhuravskaia A.N. Shein A.A. i dr. Biopreparaty iz prirodnogo severnogo syria kak sredstva korrektsii ekologicheskogo neblagopoluchiia [Biological preparations from natural northern raw materials as agents of ecological trouble correction] Izvestiia Samarskogo nauchnogo tsentra Rossiiskoi akademii nauk [Bulletin of the Samara Scientific Center RAS], Yakutsk, 2010, Issue12, No. 1 (8), p. 2041-2046.

3. Kazimirko V.K. Maltsev V.I. Antioksidantnaia sistema i ee funktsionirovanie v organizme cheloveka [Antioxidant system and its functioning in the human body [Med. Gazeta Zdorove Ukrainy [Med. Newspaper], 2004, Issue. No. 98. Novosti meditsiny [News of Medicine].

4. Koroljuk M.A. Ivanov L.I., Mayorova I.G. Method for determination of catalase activity [Metod opredeleniia aktivnosti katalazy] Lab. delo [Lab. Business], 1988. No. 1, p.16– 19.

5. Mironova G.E. Vasilyev E.P., Velichkovskii B.T. Khronicheskii obstruktivnyi bronkhit v usloviiakh Krainego

Severa znachenie oksidantnogo statusa bolnogo i antioksidantnoi terapii [Chronic obstructive bronchitis in the Far North (the oxidative status of the patient and antioxidant therapy]. Krasnoyarsk, 2003, p.169.

6. Okhlopkova E. D. G.E. Mironova Sostoianie pro i antioksidantnogo ravnovesiia u bortsov volnogo stilia i bokserov na raznykh etapakh trenirovochnogo tsikla [State of the pro - and antioxidant balance in freestyle wrestlers and boxers in various stages of training cycle] Iakutskii meditsinskii zhurnal [Yakut medical journal], 2009, No. 4(28), p.60-64.

7. Rogozhin V. V. Metody biokhimicheskikh issledovanii [Biochemical research methods]. Iakutsk, 1999, p. 91–93.

8. Konstantinova L.I. Mironova G.E. Okhlopkova E.D. i dr. Sostoianie pro- i antioksidantnogo ravnovesiia u bortsov volnogo stilia Iakutii v raznye sezony goda [The state of pro and antioxidant equilibrium in wrestlers of the free style of Yakutia in different seasons of the year] Iakutskii meditsinskii zhurnal [Yakut Medical Journal], 2016, No. 2, p.39-42.

9.Morozov V.I. i dr. Snizhenie povrezhdeniia myshts pri fizicheskoi nagruzke i uvelichenie rabotosposobnosti s pomoshchiu biologicheski aktivnykh dobavok BAD antioksidantnoi napravlennosti [Reduction of muscle damage during physical activity and increase in working capacity with the help of biologically active additives (BAA) of antioxidant orientation] Sbornik nauchnykh trudov Sankt-Peterburgskogo nauchno-issledovatelskogo instituta fizicheskoi kultury Itogovaia nauchnaia konferentsiia [Collected scientific works of the Spb. Scientific Research Institute of Physical Culture. Final scientific conference] Spb., 2005, p. 128-133.

10. Danilova I. A. Spravochnik po laboratornym metodam issledovaniya [Handbook of laboratory methods] Pod red. Danilovoy L. A. St. Petersburg: Piter, 2003. 541 p.

11. Krivoshapkina Z.N. Mironova G.E. Semenova E.I. i dr. Urovni nizkomolekuliarnykh anti- oksidantov u korennykh i prishlykh zhitelei iakutii [Levels of low-molecular antioxidants in native and alien inhabitants of Yakutia] Iakutskii meditsinskii zhurnal [Yakut Medical Journal], 2014, No. 2, p.83-87.

12. Uchiyama M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test / M. Uchiyama, M. Michara //Anal. Biochem. - 1978. - Vol.86. - №1. - P. 271-278.

The authors

1. Yakutsk Scientific Center for Complex Medical Problems, 677010, Yakutsk, Sergelyakhskoye Highway, 4 km.

2. North-East Federal University. M.K. Ammosova, 677000, the city of Yakutsk, ul. Belinsky, 58.

3. Okhlopkova Elena Dmitrievna - Candidate of Biological Sciences, Leading Researcher - Head of Immunology Laboratory, FGBICU «YANC KMP», elena_ohlopkova@mail.ru

4. Konstantinova Lena Ivanovna - Researcher at the Laboratory of Biochemistry, FGBICU «YANC KMP», konstanta.l@mail.ru

5. Olesova Lyubov Dygynovna - Candidate of Biological Sciences, leading researcher - head of the laboratory. Biochemistry, FGBNU «YaNC KMP», oles@mail.ru

6. Mironova Galina Egorovna - Doctor of Biological Sciences, Professor of Biological Branch of the IAN FGAOU VOFU. M.K. Ammosov, mirogalin@mail.ru

7. Semenova Evgenia Ivanovna - Ph.D., Senior Researcher of the laboratory. biochemistry, FGBNU «YAC CMP», kunsuntar@mail.ru

8. Yakovleva Alexandra Ivanovna - a research fellow at the laboratory. biochemistry, FGBNU «YAC CMP», sashyak@mail.ru

9. Efremova Agrafena Vladimirovna - Ph.D., Senior Researcher of the laboratory. biochemistry, FGBNU «YaNC KMP», a.efremova01@mail.ru

10. Krivoshapkina Zoya Nikolaevna - Ph.D., senior research fellow of the laboratory. Biochemistry, FGBNU «YaNC KMP», zoyakriv@mail.ru

11. Alekseev Vladislav Amirovich - junior research fellow of the laboratory. Immunology, FGBNU «YaNC KMP», vldsvalekseev@gmail.com

12. Nikolaev Vyacheslav Mikhailovich - Ph.D., Chief Researcher - Head of the Department for the Study of Adaptation Mechanisms, FGBICU «YANC KMP», Nikolaev1126@mail.ru

13. Efremova Svetlana Dmitrievna - junior research fellow of the laboratory. immunology, FGBNU «YaNC KMP», esd64@mail.ru