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Original Article

Comprehensive assessment of structural and metabolic peculiarities of blood plasma in highly qualified athletes

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Abstract:
Highly qualified athletes' successful performance depends on the level and quality of the training process and athletes' training timely medical support. ***Research aim*** is to study the state of oxidative metabolism, physical and chemical blood properties in highly qualified athletes. ***Materials and methods***. 262 athletes aged 19-29 engaged in cyclic sports took part in the scientific project. Screening data of healthy, non-exercising volunteers of the same age (n=35) were used for control. The blood serum of all participants was examined to determine the level of 8-isoprostane, oxidized low-density lipoproteins, the activity of superoxide dismutase, glutathione peroxidase and reductase. The levels of α- and β-carotenes, α- and γ-tocopherols, lycopene, lutein and zeaxanthin were studied. ***Results***. It was found that the absolute majority of the parameters of metabolism components in qualified athletes differ significantly from the non-training people's indicators characteristic; it demonstrated that athletes have shifts in oxidative metabolism and physical-chemical properties of blood serum caused by professional sports activities. This fact was manifested in changes in the state of the pro- and antioxidant systems of the blood and in shifts in the crystallogenic activity of biofluids. ***Conclusions***. The data obtained confirmed the development of oxidative stress in highly qualified athletes as a result of long-term sports practice. The method of the structural and metabolic peculiarities complex assessment of blood plasma in qualified athletes is an effective and efficient way of medical screening for defininig physiological changes in athletes' body, especially during the pre-competition period of the annual training macrocycle program in any sport.
Key Words*:* **athletes, physical loads, metabolism and crystallogenic blood peculiarities, oxidative stress**

Introduction

Elite sport is one of the national priorities, the successful implementation of which requires the creation of a comprehensive system of athletes' training and medical control (Amap et al., 2018; Tummala et al., 2018; Goodman et al., 2018; Vynohradov et al., 2021). The creation of structural and metabolic peculiarities of blood plasma protocols in athletes can be used to monitor their health and physical performance (Reneker, 2018; Lucas de Albuquerque Freire et al., 2020), for planning rehabilitation measures (Chiriac Paul Bogdan et al., 2021) and reducing sports injuries (Mardiana et al., 2021). Such blood plasma studies can be used in monitoring the state of the body of various groups in the population, among which there are both athletes and untrained persons (Bocharin et al., 2021c). The results of monitoring an athlete's blood and metabolism indicators enable sports specialists to track changes in the state of the body's functional reserves (Bocharin et al., 2021a; Bocharin et al., 2021b; Siti Baitul Mukarromah et al., 2021) and adjust them.

The medical screening system is mainly focused on identifying the athlete's reserve capabilities and managing them. At the same time, much less attention is paid to the study of the athlete's body's ability to adapt to extreme physical exertion (Maman et. al., 2018; Tummala et. al., 2018). The adaptation process includes all types of adaptability of the human body, which is realized at the morphological, physiological and biochemical levels (Wagner et. al., 2010; Gryaznykh et. al., 2021).

Oxidative metabolism of the entire human body and its functional components are supported by a combined complex of pro-oxidant and antioxidant systems, including enzymatic and non-enzymatic components. This complex is supported by a variety of regulatory mechanisms, which are represented by both internal and

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external effectors and affect the enzymatic and non-enzymatic components of this complex of systems (Halliwell et al., 1999; Soria et al., 2015).

Oxidative metabolism determines its participation in the pathogenesis of the absolute majority of diseases. On the other hand, this participation provides ample opportunities for physiological processes correction in the human body. Dreißigacker et al. (2010), Arbnore Ibrahimaj Gashi et al. (2020) consider the maladaptation of oxidative metabolism (oxidative stress) to be an individual syndrome. The scientific literature does not fully reflect the issues of a comprehensive assessment of the structural and metabolic peculiarities of blood plasma in highly qualified athletes. In our opinion, the relevance of studying the appearance of the oxidative stress state will allow us to quickly make adjustments to the training process of highly qualified athletes to improve the effectiveness of their performances at competitions of any complexity. **Research aim** is to study the state of oxidative metabolism, physical and chemical blood properties in highly qualified athletes.

Material & methods

The blood of 262 highly qualified athletes (Russia) aged 19 to 29, engaged in cyclic sports (a group of athletes) was examined. In parallel, the blood of healthy people of the same age (n=35) who do not do sports (control group) was examined. In each group, blood samples were examined for the content of 8-isoprostane using an enzyme immunoassay kit (Usbiological, USA). The analysis of oxidized low-density lipoproteins (OLDL) was carried out by competitive enzyme immunoassay on a microplate using an automated immunoassay analyzer Evolis (Bio-Rad, Germany-USA) with Biomedica Gruppe reagents. The activity of superoxide dismutase by inhibiting adrenaline autoxidation in the carbonate buffer (pH=10.0) was studied with the addition of blood hemolysate samples (1:50). α- and β-carotene, α- and γ-tocopherol determination was carried out by a standard method using analyzers. The analysis of non-tissue-specific antioxidants (lycopene, lutein and zeaxanthin) was carried out by chromatography-mass spectrometry. To obtain serum, all blood samples were centrifuged for 15 minutes (2500 g; 1S-R multiphase centrifuge). Then 100 mql of serum was applied to the microscope slide. The crystallogenic activity of the biofluid was evaluated according to the author's method of sample preparation and description proposed by Martusevich, Kamakin (2007), Martusevich, Peretyagin (2013).

The character of the crystalline elements density in three simultaneously obtained facies, the structure index, the degree of destruction of the facies and the clarity of the boundary zone of the facies were considered, on the basis of which the parameter values were calculated. Image samples were obtained using the Levenhuk kit. The data obtained during the research were processed using the licensed Statistica 6.1 software package for Windows. Arithmetic mean (X), mean error (m) and mean absolute deviation (σ) were calculated for each sample. The normal distribution of features was determined using the Shapiro-Wilk test. To compare experimental and control sampling units, the method of one-sided analysis of variance was used. The differences were considered significant at $p<0.05$. The study was approved by the Ethics Committee of the Federal Medical Biophysical Center. Burnazyan, Russia, No. 18 of October 12, 2015) and complies with the international ethical standards of the Human Rights Committee (WMA Declaration of Helsinki, 2008).

Results

We have found that the absolute majority of the studied parameters of the metabolism components differ significantly in qualified athletes compared to the indicators characteristic of non-training volunteers. Athletes showed significantly higher levels of 8-isoprostane in blood plasma (Fig. 1); and this value is 1.25 times higher compared to the control group ($p<0.05$). Currently, the concentration of 8-isoprostane in blood plasma is considered as an integral laboratory marker of oxidative stress in the human body. Therefore, the results obtained by us from a blood test in a group of athletes indicate excessive stimulation of the process of free radical oxidation caused by intense physical exercises and training loads (Figure 1).

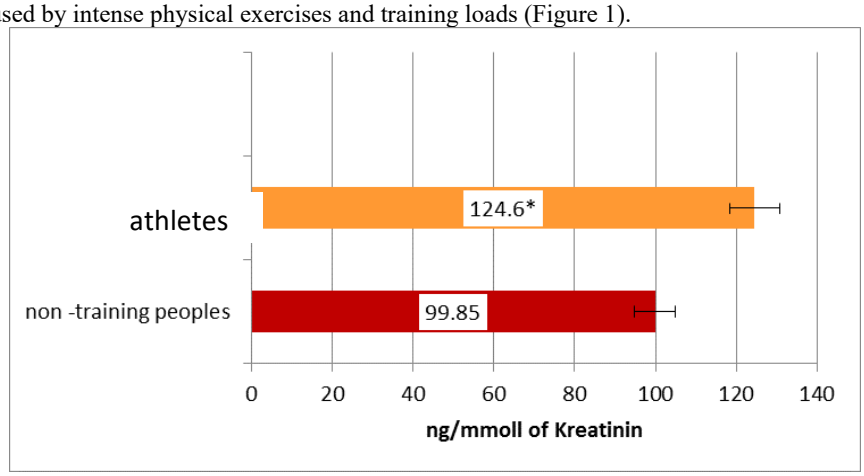

Fig. 1. Level of 8-isoprostane in blood serum of athletes and non-training people

At the same time, the level of oxidized low-density lipoproteins in qualified athletes was 16% lower than in non-training volunteers, $p<0.05$ (Figure 2). This is probably due to the predominant effect of the studied factor not on lipid peroxidation, but on oxidative damage to other macromolecules, in particular, on oxidative modification of protein.

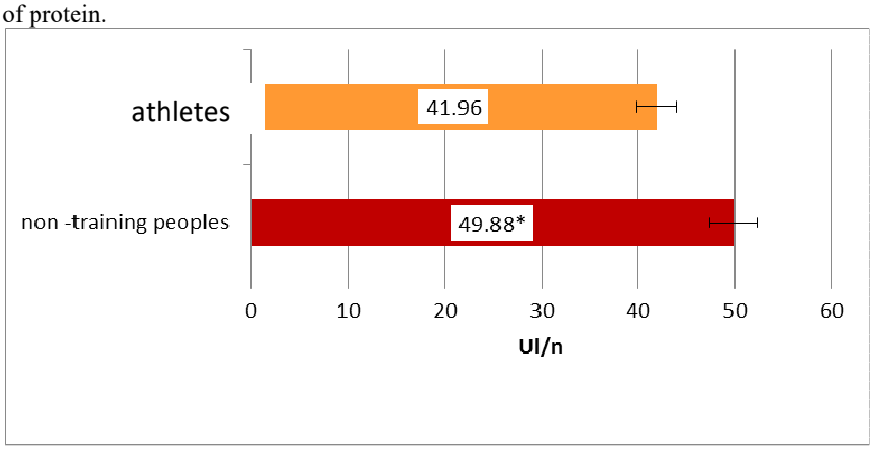

Fig. 2. Level of oxidized lipoproteins of low density in blood serum of athletes and non- training people

This is indirectly confirmed by the nature of changes in the activity of superoxide dismutase in qualified athletes, as one of the main antioxidant enzymes. In this group of project participants, a moderate inhibition of the level of catalytic abilities of this enzyme was recorded by 8% ($p=0.072$), compared with the level found in the control group.

The above-mentioned shifts in the level of parameters indicate the active participation of the superoxide dismutase enzyme in free radicals (superoxide anion radicals) removal from the qualified athletes' biological fluid and may reflect a partial modification of the superoxide dismutase enzyme in the form of a large protein molecule.

In addition, a change in the concentration of non-tissue-specific non-enzymatic antioxidants in the blood plasma indicates a pronounced activation of free radical processes in qualified athletes. According to our data, the levels of α- and γ-tocopherol in people who regularly engage in intense physical exercise and training loads were significantly lower than in the group of people who do not engage in physical exercise and sports (Figure 3).

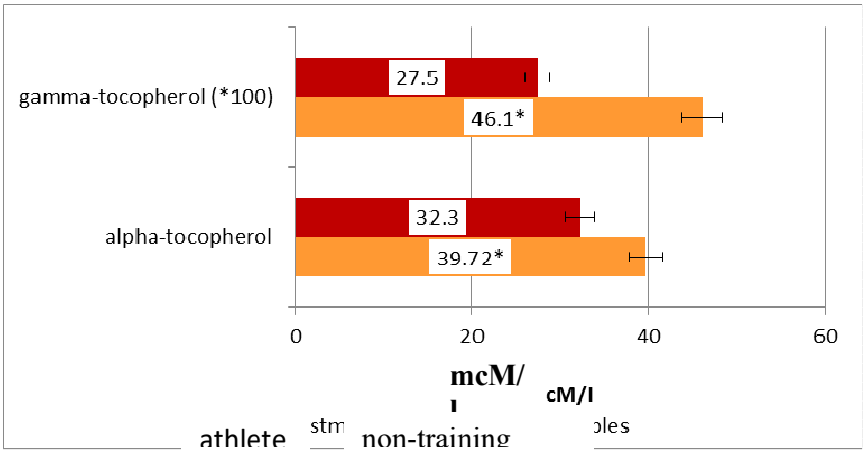

Fig. 3. Level of alpha- and gamma-tocopherols in blood serum of athletes and non- training people

The results of our research project showed that the concentration of γ-tocopherol in the athletes' blood serum was 1.68 times lower, α-tocopherol level was 1.23 times lower than in the control group ($p<0.05$ for both parameters). We observed not only an absolute decrease in the levels of both tocopherols, but also a decrease in the ratio of vitamin E/cholesterol concentration in plasma, which was 1.25 times lower in trained individuals compared with the results in the control group ($p<0.05$).

Similar, but less pronounced changes were registered for another group of non-selective non-enzymatic antioxidants (carotenes). After studying the qualified athletes' blood plasma, it was found that the concentrations

of α-carotene decrease more significantly than the concentrations of β-carotene (by 1.3 and 1.1 times, respectively), $p<0.05$ for both parameters. This fact once again confirms that with continuous intensive physical exercises and regular sports, a deficiency of antioxidant potential develops and a state of oxidative stress occurs.

This trend fully applies to tissue-specific antioxidants. In particular, plasma levels of zeaxanthin, lycopene and lutein were significantly (1.9 times) reduced in qualified athletes compared to non-training volunteers, $p<0.05$.

Simultaneously with the assessment of parameters reflecting the oxidative metabolism state, we examined athletes' and non-training individuals' individual blood serum samples for the crystallogenic activity of blood plasma, which reflects the general physical and chemical properties of the biological substrate and its composition. Evaluation of the biological fluids crystallization parameters allows speaking about the conjugation of the biological medium and oxidative metabolism crystallogenic properties (Figures 4 and 5).

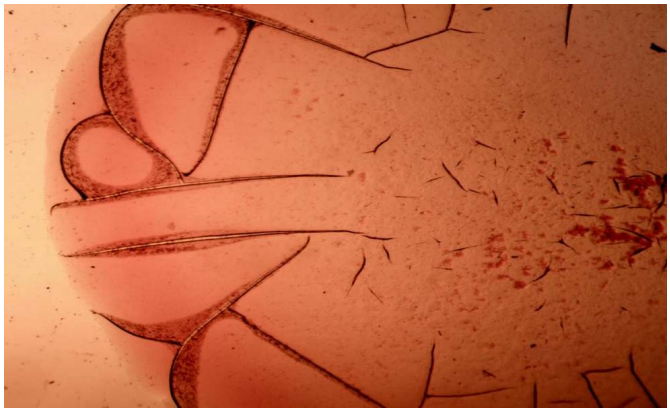

Fig. 4. An example of crystalloscopic picture of the blood plasma in athletes (magn. x60)

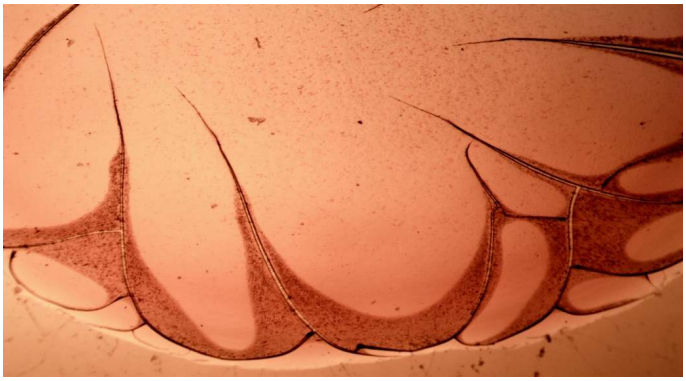

Fig. 5. An example of crystalloscopic picture of the blood plasma in non-trained persons (magn. x60)

We found changes in all the main visual parameters of the athletes' blood plasma facies compared with people who do not engage in regular physical exercise and sports.

In the group of athletes, in comparison with the control one, we observed a tendency to increase crystallizability by 1.22 times ($p<0.05$), which is a quantitative characteristic of the biological fluid structuring and the demonstration of the crystallogenesis process activity. We believe this is due to significant concentrations of under-oxidized products of free radical processes in the athletes' blood plasma, the stimulation of which is confirmed by the above data. These compounds in the biological fluid can play the role of crystallization centers. The presence of these compounds contributes to the organic-mineral clusters formation, which are visually described as crystalline elements.

The proposed hypothesis is also confirmed by visual changes in parameters reflecting the qualitative aspect of crystallogenesis, in particular, the structural index. This index allows taking into account the complexity of the structure of elements formation in a dried blood plasma sample.

Qualified athletes demonstrated higher (1.3 times) values of this parameter compared to non-training people, $p<0.05$. These deviations indicate that metabolic adaptation during prolonged and intensive physical exertion causes not only quantitative stimulation of the crystallogenic activity of the biological substrate, but also

complication of the formed structures. This indicates significant changes in the physical and chemical properties of the biosubstrate.

Especially interesting is the assessment of the degree of facies destruction, which is the main criterion for the crystallogenesis correctness and is currently considered an indicator of maladaptivity degree of the biological substrate. We found a phenomenon when dried blood serum samples from non-training individuals in the control group had moderate signs of destruction of facies elements. Subtotal destruction of the blood plasma microslides structures was recorded in the qualified athletes' samples. At the same time, the level of the facies destruction degree in untrained and trained individuals was 0.64 ±0.14 and 2.12± 0.36 points, respectively ($p<0.05$). This fact indicates pathological changes in the crystals formation in the athletes' blood caused by regular intense physical exercise (metabolic maladaptation). We observed the opposite trend with respect to the clarity of the marginal zone of blood plasma crystallograms. The level of this parameter was lower in qualified athletes, since in this group the marginal zone of the microslide was narrowed.

The study of blood serum crystallogenic properties, together with the oxidative metabolism modification determination in the biological substrate, confirmed the presence of negative shifts in the athletes examined by us caused by regular intensive physical exercises and training loads with an adequate vector of the blood biochemical response to such an impact. This fact is also confirmed by the analysis of our results, when the presence of multiple relationships between changes in metabolic parameters reflecting the state of pro- and antioxidant systems of the blood and crystalloscopic parameters indicate changes in the physical and chemical properties of biomass in qualified athletes.

Dicussion

The optimal level of fitness choice and the training process organization is associated with many factors affecting the functional state of the human body with any level of physical fitness. These indicators are predictors of various states of overtraining, overwork and overexertion (Lucas de Albuquerque Freire et al., 2020; Chiriac Paul Bogdan et al., 2021; Vynohradov et al., 2021). One of the key factors is the process of maintaining optimal functioning of regulatory systems, which depends on the intensity of free radical processes and the degree of physiological adaptation (Arbnore Ibrahimaj Gashi et al., 2020). The analysis of oxidized lipoproteins, the activity of superoxide dismutase, tissue-specific antioxidants, as well as the morphological assessment of dried samples of biological fluid by crystalloscopy are among the most integral parameters allowing to characterize the degree of oxidative stress or its absence, taking into account the level of fitness of the human body (Goodman et al., 2018; Saraykin et al., 2019). Consequently, the study of oxidative metabolism and physico-chemical properties of blood serum state makes it possible to assess the degree of a person's regulatory capabilities at a certain stage of his physical and athletic improvement (Martusevich, & Peretyagin, 2013). Significant differences were noted between the parameters studied by us in the groups of qualified athletes and untrained individuals. A significantly higher level of 8-isoprostane in the blood plasma of trained people indicates the stimulation of free radical processes caused by enhanced training. At the same time, the level of oxidized lipoproteins in athletes was slightly lower, which indicates the active participation of superoxide dismutase in the elimination of free radicals. At the same time, when analyzing the crystalloscopic facies of biological fluids in this contingent of individuals, an increase in the level of crystallizability and the structural index was found. At the same time, according to the degree of facies destruction, athletes showed a moderate tendency to change this parameter, relative to a stronger destruction in untrained individuals.

Based on the known molecular mechanisms of structure formation in dried droplets of biological fluids (Martusevich, & Peretyagin, 2013), protein macromolecules preserving structure, physicochemical properties and conformation (i.e. native) are concentrated in the corresponding region of the sample. This fact suggests a decrease in the amount of native protein during intense physical exertion, which corresponds to the literature data (Shenoy et al., 2017). It is due to the fact that shifts in oxidative metabolism lead not only to activation of fat peroxidation in the blood and membranes of shaped elements, but also to oxidative modification of proteins (Halliwell et. al., 1999; Mardiana et al., 2021), which in turn leads to loss of functionality, physical and chemical properties of proteins. The data obtained by us as a result of research work indicate that an increase in the severity of oxidative stress in the examined athletes' body manifests itself in the form of an increase in the level of 8-isoprostane and concentrations of oxidized low-density lipoproteins and a decrease in the total antioxidant activity of plasma, the activity of superoxide dismutase and the level of non-enzymatic antioxidants. These changes occur simultaneously with negative shifts in the crystallogenic properties of blood serum, such as increased structure formation activity, increased degree of elements destruction in the facies and reduction of the native part of the biological fluid proteome.

Conclusions

After finishing the project work, it was found that in the group of qualified athletes, the level of 8-isoprostane in blood plasma was 1.25 times higher, the level of oxidized low-density lipoproteins was 16% lower, the level of γ-tocopherol in athletes' blood serum was 1.68 times lower, α-tocopherol was 1.23 times lower, the ratio of vitamin E/cholesterol concentration in plasma was 1.25 times lower, the levels of zeaxanthin,

lycopene and lutein in blood plasma were 1.9 times lower, it was found moderate (8%) inhibition of the level of catalytic abilities of the enzyme superoxide dismutase, the structural index was 1.3 times higher. There is a tendency to increase crystallizability by 1.22 times, subtotal destruction of the blood plasma micro preparations structures was recorded, compared with the results obtained in the control group in untrained individuals.

The results of our comprehensive study of the structural and metabolic peculiarities of blood plasma have demonstrated the presence of negative shifts in oxidative metabolism and physico-chemical properties of blood serum in highly qualified athletes, caused by regular high-intensity cyclic sports.

We believe that the method of complex assessment of the structural and metabolic peculiarities of blood plasma in qualified athletes is an effective and efficient way of medical screening to detect hysiological changes in the athletes' body, especially during the pre-competition period of the annual training macrocycle program in any sport.

Conflicts of interest. The authors declare no conflict of interest.

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