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Red blood cells state and calcium content of in the blood plasma during physical activity in marathon runners of various qualifications

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Abstract

The aim of the work is to investigate the morpho-functional state of peripheral blood erythrocytes and the content of Ca^{2+} in the blood plasma during physical exercise in marathoners of different qualifications.

Material and methods. The studies were conducted in three groups of marathoners aged 20–25 years old with conditionally high, medium and low performance. Exercise was performed for 90 minutes on a bicycle ergometer with a given power. The study of peripheral blood erythrocytes was performed before and after exercise. The osmotic resistance of erythrocytes, the content of Ca^{2+} and free fatty acids in the blood were determined. Morphological study of erythrocytes was performed using a scanning electron microscope and stained by standard techniques. The red blood cell form index was determined by a special formula. Changing the content of Ca^{2+} enhances lipid melting, increases membrane fluidity and increases surface tension, with the result that the discocytes are stretched and thinned, the central pits deepen and the surface roughness increases, the volume and number of conical pores, and peripheral blood erythrocytes density.

Results. Marathon runners of different groups after physical exercise differed in terms of the frequency of cardiac sweeps, the content of free fatty acids and the osmotic resistance of red blood cells. This is closely correlated with changes in the electrolyte and metric composition of erythrocytes and the presence of their reversible and irreversible forms in the general circulation after exercise.

Conclutions. Physical activity causes structural and functional reorganization of erythrocytes, which is based on a change in their microelement composition, a decrease in osmotic resistance, mainly in poorly trained marathoners, and the appearance of various forms of erythrocytes against the background of the destruction of degenerative forms and an increase in the active release of young forms, which is a sign of high adaptive capacity in highly skilled athletes.

Key words: physical loading, red blood cells, osmotic resistance, calcium, marathon runners.

Анотація

Олиференко І.А., Попель С.Л. Стан еритроцитів і вміст кальцію в плазмі крові при фізичному навантаженні у марафонців різної кваліфікації

Мета роботи – дослідити морфо-функціональний стан еритроцитів периферичної крові і вміст Ca^{2+} в плазмі крові при фізичному навантаженні у марафонців різної кваліфікації. **Матеріал і методи.** Дослідження проводилися в трьох групах марафонців у віці 20-25 років з умовно високою, середньою і низькою працездатністю. Фізичне навантаження виконувалася протягом 90 хв на велоергометрі із заданою потужністю. Дослідження еритроцитів периферичної крові проводили до і після фізичного навантаження. Визначали осмотичну резистентність еритроцитів, вміст Ca^{2+} і вільних жирних кислот в крові.

Результати. Марафонці різних груп після фізичного навантаження розрізнялися за показниками частоти серцевих скорочень, вмістом вільних жирних кислот та осмотичної резистентності еритроцитів. Це тісно корелює зі зміною електролітного і метричного складу еритроцитів та наявності їх оборотних і необоротних форм в загальному кровотоці після фізичного навантаження. Зміна вмісту Ca^{2+} підсилює плавлення ліпідів, збільшує плинність мембран і підвищує поверхневий натяг, в результаті чого дискоцити витягуються і витоншуються, центральні ямки поглиблюються і збільшується шорсткість поверхні, обсяг і кількість конусоподібних пор, а також щільність еритроцитів периферичної крові.

Висновки. Фізичне навантаження викликає структурно-функціональну перебудову еритроцитів, в основі якої лежить зміна їх мікроелементного складу, зниження осмотичної резистентності, в основному у мало тренованих марафонців і появі різноманітних форм еритроцитів на тлі деструкції дегенеративних форм і посиленням активного викиду молодих форм, що є ознакою високих адаптаційних можливостей у висококваліфікованих спортсменів.

Ключові слова: фізичне навантаження, еритроцити, осмотична резистентність, кальцій, марафонці.

Анотация

Олиференко И.А., Попель С.Л. Состояние эритроцитов и содержание кальция в плазме крови при физической нагрузке у марафонцев разной квалификации

Цель работы – исследование морфо-функциональное состояние эритроцитов периферической крови и содержание Ca^{2+} в плазме крови при физической нагрузке у марафонцев разной квалификации.

Материал и **методы**. Исследования проводились в трех группах марафонцев в возрасте 20-25 лет с условно высокой, средней и низкой работоспособностью. Физическая нагрузка выполнялась в течение 90 мин на велоэргометре с заданной мощностью. Исследование эритроцитов периферической крови проводили до и после физической нагрузки. Определяли осмотическую резистентность эритроцитов, содержание Ca^{2+} и свободных жирных кислот в крови.

Результаты. Марафонцы различных групп после физической загрузки различались по показателям частоты сердечных покращений, содержанию свободных жирних кислот и осмотической резистентности эритроцитов. Это тесно корелирует с изменением электролитного и метрического состава эритроцитов при наличии их обратимых и необратимых форм в общем кровотоке после физической нагрузки. Изменение содержания Ca^{2+} усиливает плавление липидов, увеличивает текучесть мембран и повышает поверхностное натяжение, в результате чего дискоциты вытягиваются и истончаются, центральные ямки углубляются и увеличивается шероховатость поверхности, объём и количество конусовидных пор, а также плотность эритроцитов периферической крови.

Выводы. Физическая нагрузка вызывает структурно-функциональную перестройку эритроцитов, в основе которой лежит изменение их микроэлементного состава, понижение осмотической резистентности, в основном у мало тренированных марафонцев, и появлении разнообразных форм эритроцитов на фоне деструкции дегенеративных форм и усилением активного выброса молодых форм, что является признаком высоких адаптационных возможностей у высококвалифицированных спортсменов.

Ключевые слова: физичекая загрузка, эритроциты, осмотическая резистентность, кальций, марафонцы.

Introduction

Currently, one of the main tasks in the practice of sports is the timely correction of the educational process in order to optimize it [1]. In this regard, the question arises of choosing the most informative indicators that reflect different levels of adaptation of the athlete's body to physical activity, especially for the development of general endurance in sports that present special requirements for this physical ability [2].

According to modern scientific data, such studies must be carried out at different levels of the organization of the human body. In the vast majority of cases, the leading role in endurance studies belongs to the identification of factors contributing to the activation of energy metabolism, especially oxygen in the transport system [3]. At the same time, studies of cellular reactions in the process of training associated with an increase in the overall endurance of the athlete's body are ignored. The peripheral blood red blood cells in this case are a convenient model for this kind of research, since they take part in the processes associated with maintaining homeostasis at the level of the whole organism [4, 5, 6]. In addition to their inherent specific gas transport function, these cells take part in the regulation of the acid-base state, water-electrolyte balance, micro-rheological status of the blood, in immune responses, the binding and transfer of amino acids and lipids, which is of direct interest in the development of the general endurance of the body [7].

One of such indicators can be the presence of circulating and non-circulating forms of peripheral blood red blood cells, the erythrocyte deformability index, their macro-microelement composition, osmotic resistance of red blood cells, which, according to modern concepts, are integrative indicators of the state of cell membranes in the whole body and are very sensitive to action physical activity [6, 8, 9]. An important role in the stabilization of the membrane can be played by calcium ions [10], which also have a wide spectrum of action on various physiological processes: regulation of cell sensitivity to various stimuli, control of the functional activity of the cell and the cellular metabolism system.

The purpose of the work is to study the morphological and functional state of peripheral blood red blood cells and the concentration of Ca^{2+} in blood plasma during physical activity in marathon runners of various qualifications.

Material and methods

Studies were conducted in three groups of marathon runners aged 20-25 years. The first group, with a conditionally high working capacity, is highly qualified athletes involved in marathon running (masters of sports of international class, masters of sports). The second group, with average working capacity, is represented by marathon runners with a qualification level at the level of the CCM and grade I. The third group with conditionally low working capacity is students of the Faculty of Physical Education and Sports of the Carpathian National University named after V. Stefanik who are involved in a marathon and do not have a sports category.

Physical exercise was performed for 90 minutes on a bicycle ergometer with a given power of 17 kgm / min per 1 kg of weight, which corresponds to 50-60% of the MPC. At rest, during exercise and at the 2nd minute of recovery, ECG was used to determine the heart rate (HR). The studies were conducted on heparinized blood taken from the pulp of the fourth finger of the left hand before and after exercise. The osmotic resistance of red blood cells was determined by the method of ND Vasilevskaya [7]. The Ca^{2+} content in the blood was measured by a direct potentiometric method using membrane ion-selective electrodes in combination with an EV-74 ionomer and a B7-22 digital millivoltmeter [10].

The content of free fatty acids in the blood was studied by the enzymatic method on a Humalyser 2000 biochemical analyzer (Germany) using a set of reagents from Human [14, 19].

A morphological study of peripheral blood erythrocytes was performed using a JEOL-25M-T220A scanning electron microscope (Japan) according to generally accepted methods [6, 13, 18]. Electron microscopic images were analyzed in the ImagerJ editor.

The shape index was determined as the ratio of the maximum and minimum diameters of peripheral blood red blood cells according to the formula:

$$IF = D_{max}/D_{min},\ [16]$$

where:
IF - form index;
Dmax - maximum diameter
Dmin - the minimum diameter.

Samples of the values obtained for all types of measurements had a normal distribution. This allowed the use of Student's t-test when comparing intergroup differences. The differences were considered significant at $p < 0.05$.

Results

The differences between the three groups in the duration of the work and the reaction of the cardiovascular system in response to the FN give reason to say that the studied groups differ in performance. The change in heart rate under the influence of physical activity in athletes of group I was 92.0% (67.1±2.93 beats·min^{-1} at rest, 142.0 ± 3.35 beats·min^{-1} after physical load), in athletes of group II - 102, 0% (respectively 72.5 ± 3.67 beats·min^{-1} and 154.0 ± 7.28 beats·min^{-1}), in group III the highest heart rate rise was 137.0% (respectively 76.3 ± 4.45 beats·min^{-1} and 171.1 ± 5.33 beats·min^{-1}). Not all the subjects coped with physical activity, designed for 90 min: in group I of 8 people all managed (average work time 90 min), in group II of 8 persons 3 (average work time 84 min), in group III out of 8 persons, only 4 coped (average work time - 74 min).

Before exercise, the content of free fatty acids was 0.28 mmol·l^{-1} in group I, in group II - 0.44 mmol·l^{-1}, and in group III - 0.89 mmol·l^{-1}. The study of the content of free fatty acids in the blood of athletes of groups I and II in response to physical activity increased on average by 112.8 ± 1.42% and 76.3 ± 1.11%, respectively ($p<0.05$), whereas In group III, it amounted to only 3.1 ± 0.33% ($p> 0.05$) (Tab. 1).

Calculation of the average values of osmotic hemolysis showed that peripheral blood erythrocytes in group II are most stable. The breakdown of peripheral blood erythrocytes in two other groups was significantly higher, while its value was almost the same in both the I and III groups (Table 1, Fig. 1).

Table 1

Indicators of osmotic hemolysis of peripheral blood erythrocytes in marathon runners of various qualifications before and after physical activity (%)

Saline concentration, %	I group, n=6		II group, n=6		III group, n=6	
	Before physical load	After physical load	Before physical load	After physical load	Before physical load	After physical load
0,4	76,9 ±2,92	78,9 ±2,53	80,1 ±2,25	69,1 ±2,49	78,5 ±3,01	82,8 ±3,52
0,42	52,6 ±2,34	54,9 ±3,11	31,6 ±1,02	35,7 ±1,81	53,2 ±2,44	56,2 ±2,64
0,44	36,1 ±2,45	38,1 ±2,27	22,4 ±1,66	15,2 ±1,14	38,5 ±2,37	31,2 ±2,68
Hypotension 50% red blood cell destructuring	0,421± 0,001	0,423 ±0,003	0,412 ±0,003	0,409 ±0,004	0,423 ±0,005	0,422 ±0,005

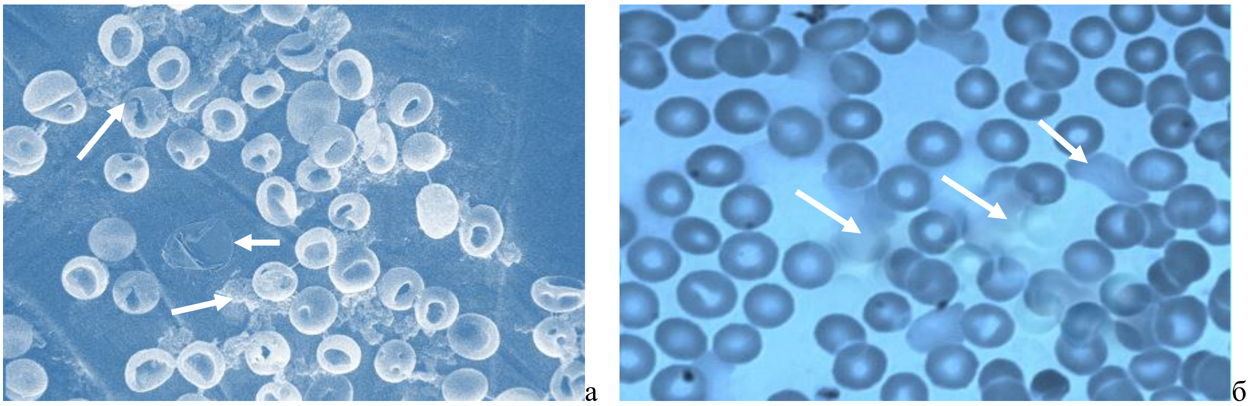

Fig. 1. Specific lytic forms of red blood cells (shown by arrows) in a blood smear of athletes of group I (a) and III (b). Method: a - scanning electron microscopy, b - Romanovsky staining. Uv .: a - x 1500, b - x 1350

The results of the study showed that in different groups there are significant differences in the content of Ca2 + in the blood, and its level was highest among athletes of group I, while among athletes of group II it was lower and in group III it was the smallest (Fig. 2). Changes in the concentration of Ca2 + in the blood under the influence of physical activity are multidirectional, nevertheless, according to average data, one can speak of a tendency to its decrease in the first group of athletes and an increase in the third group, while in the second group the level of Ca2 + practically did not change.

At the same time, a significant (p <0.05) increase in the ability of peripheral blood erythrocytes to deformity was revealed in comparison with the initial values to FN in athletes of groups I and II.

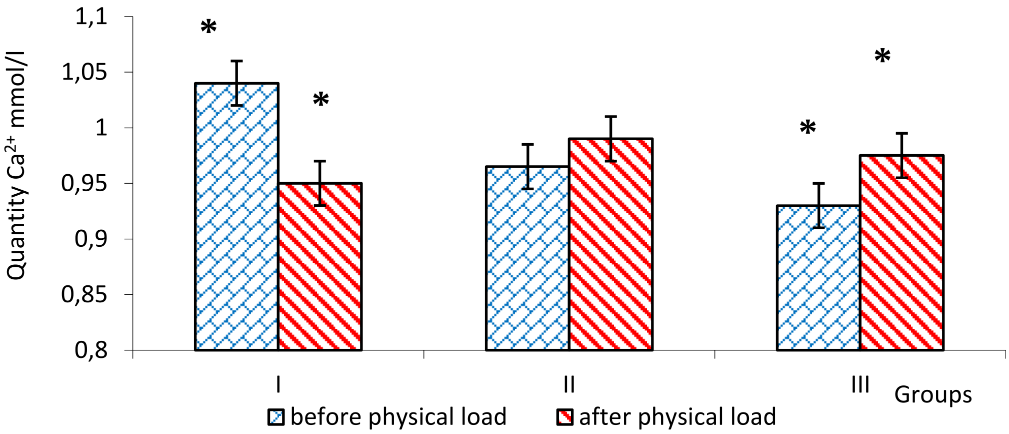

Fig. 2. The content of Ca^{2+} in blood plasma before and after prolonged physical activity:
* - p <0.05 between groups I and III

The linear dimensions of peripheral blood red blood cells during physical exertion are characterized by small, but systemic shifts. In particular, the maximum sizes of red blood cells of peripheral blood differ only in the form of tendencies to increase diameters. Ho, shorter axes show more significant and statistically significant differences (Tab. 2).

Table 2

Morphometric indicators of red blood cells of peripheral blood of marathon runners after exercise, $\bar{x} \pm S$

Parameter	Group		
	I	II	III
Diameter of disks, mcm	7,0±0,02	7,0±0,03	6,6±0,02
Disc height, mcm	0,79±0,004	0,61±0,003	0,37±0,001*
Form index, rel. units	1,01±0,001	1,06±0,002	1,12±0,008*
Pore Diameter, nm	100,9±3,03	120,7±3,51	181,5±11,23*

Note: * – p<0,05

These tables indicate rather small deviations of each of the parameters (within 5-7%), but they accumulate and take on a systemic character. This makes these shifts functionally significant in post-exercise.

In contrast to group I, where the erythrocytogram has the appearance of a normal distribution (Fig. 3), in group III, the histogram of the distribution of peripheral blood red blood cells by size is asymmetric and multipolar due to an increase in the number of varying classes of the small-sized (left) wing of cellular elements (Fig. 4) . A comparative analysis shows that for marathon runners of groups I and II, the size class size in the left wing is only 7.0%, while in group III it is about 70.0%. An increase in the amount of EPA of the left wing of the histogram is accompanied by a significant (p <0.05) decrease in the median and mode values.

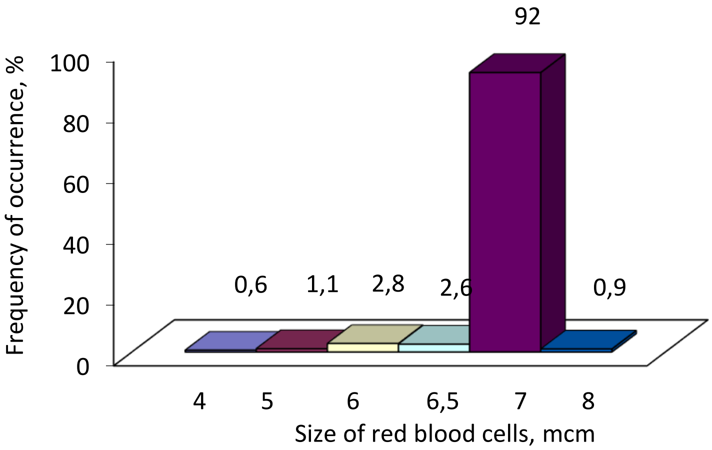

Fig. 3. Distribution of erythrocytes of peripheral blood of marathon runners I and II groups by size classes. Parameters: median 7.0 ± 0.3 mcm, mode 6.8 ± 0.3 mcm, dispersion 0.5, kurtosis 2.2, interval 1.0 mcm

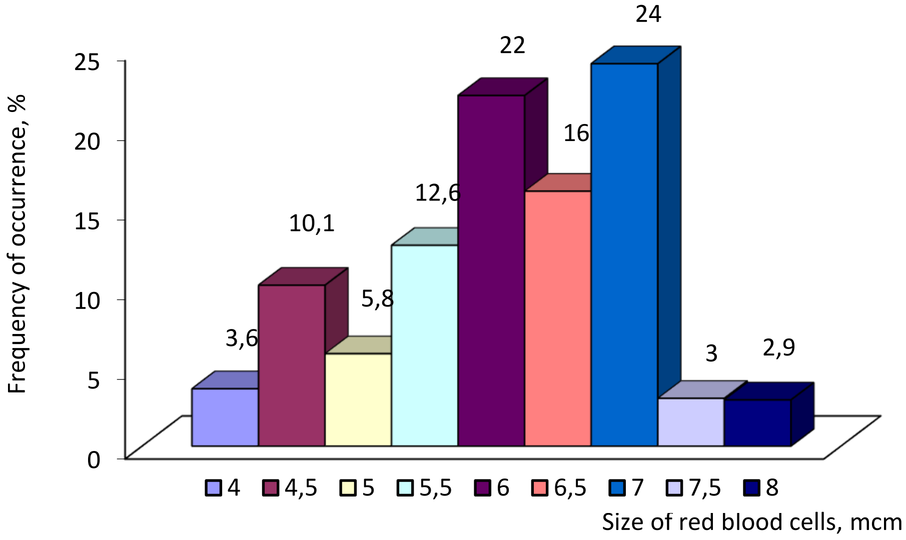

Fig. 4. Distribution of red blood cells of peripheral blood of marathon runners of group III according to size classes. Parameters: median 6.2± 0.2 mcm, mode 5.8 ± 0.2 mcm, dispersion 0.5, excess k 2.2, interval 0.5 mcm

Discussion

Our studies have shown that the body of athletes of different qualifications reacts differently to physical activity. It is known that an untrained organism responds to physical activity with a pronounced stress reaction, accompanied by a large release of catecholamines into the blood [19].

In the process of formation of long-term adaptation to physical activity, the apparatus of hormonal regulation is being restructured [17, 18].

An increase in the level of catecholamines in the blood in response to physical activity is much less. One of the mechanisms that allow the body to function sparingly in conditions of prolonged physical activity may be a change in the sensitivity of tissues to hormones [12]. It has been established that Ca^{2+} can regulate the sensitivity of adrenergic receptors to catecholamines [19]. With the participation of Ca^{2+} in this process, differences in the content of these ions in people with different levels of physical performance may be associated. In addition, an increase in the concentration of Ca^{2+} promotes the activation of α-adrenergic receptors of cell membranes and deactivates β-adrenergic receptors. Conversely, a decrease in the extracellular concentration of Ca^{2+} activates β-adrenergic receptors and decreases the sensitivity of α-adrenergic receptors [8]. The change in the sensitivity of various structures to hormones is probably due to the concept of Labori [5], with the predominance of one of the metabolic pathways in metabolic processes with the predominant use of fats or glucose as an energy substrate.

An increase in the concentration of Ca^{2+} that occurs in untrained people under the influence of physical activity can cause deactivation of β-receptors of fat cells, which can lead to inhibition of lipolysis, insufficient mobilization of fats, which are the main source of energy during prolonged work [14]. The decrease in Ca^{2+} concentration observed in most cases in highly qualified athletes can contribute to the intensive course of lipolysis, effective mobilization of energy resources [15]. This is confirmed by our analysis of the lipid composition of the blood: the content of free fatty acids in the blood of athletes in response to physical activity increased mainly in athletes of groups I and II, while in group III it was statistically unreliable. As part of lipoproteins, which are thus the transport form of fatty acids, they are delivered to target organs, in which fatty acids serve either as energy sources (cardiac and striated muscles), or as precursors for the synthesis of tissue triglycerides, followed by deposition in organ cells (in the form of a lipid depot) [19]. At rest, free fatty acids are oxidized mainly in the liver and heart muscle. Under load, oxidation increases in skeletal muscle. It is believed that free fatty acids are not only high-energy “fuel”, but they are signaling molecules. A change in their concentration is a factor affecting the intensity of glucose utilization in muscles, especially during prolonged physical activity [9].

Physical activity can cause both an increase in the osmotic resistance of red blood cells, and its decrease [8]. Calculation of average values revealed a tendency to increase this indicator in group II, while in the other two groups the changes were insignificant.

Similar results were obtained by Kremmyda et al., [7] in the study of osmotic resistance of red blood cells in groups with different levels of physical performance. It has been established that the content of total cholesterol in blood plasma in individuals with high and low physical performance is approximately the same and that in people with moderate physical performance it is higher [5, 9].

Other authors [11], when studying hemorheological criteria, such as viscosity and blood adhesion in laboratory animals, showed that their change in response to extreme physical activity was characterized in animals in the phase of “achieving full adaptation” in the absence of significant differences from control (untrained animals) and differed sharply in animals at the training stage [8]. In another laboratory, the same relationship was found between the rate of erythropoiesis and physical performance. The rate of erythropoiesis was almost equal in the control group and among highly qualified athletes, while in individuals with average working capacity it was significantly higher. However, the indicated regularity of the dependence of the values of the studied indicators on the degree of fitness has not found a satisfactory explanation for today within the framework of existing theories of the adaptation process.

People with varying degrees of fitness are at different levels of adaptation. The long-term adaptation that develops during training is characterized by shifts in functional indicators towards more economical work and is a consequence of the restructuring that occurs at the cellular and subcellular levels of the body. This process, designated as the formation of a structural trace [21, 22], includes activation of the synthesis of nucleic acids and proteins and subsequent selective growth of cell structures, due to which the capacity of the system responsible for adaptation to physical activity is increased. The response to physical work in this case will be expressed in the mobilization of physiological functions to the maximum attainable level occurring under stress. In the process of training, the body enters the stage of restructuring the structural basis of the functional system. It is at this stage that the mechanisms that affect the deep level of functioning of the body are connected, associated with changes in permeability, modification of membranes and cellular metabolism [2, 4]. Therefore, we note an increased erythrocyte resistance compared to the first group in individuals with an average degree of fitness who are at the stage of transition to long-term adaptation. But the structural trace is still imperfect to a certain extent; it is generalized, redundant. Many processes are involved in perestroika, one way or another connected with ensuring functional activity in extreme conditions [3]. Such a mechanism cannot underlie the economical and efficient functioning of the body, which is characteristic of highly trained athletes. An alternative model is to selectively strengthen or inhibit the work of strictly defined elements of a functional system. Nonspecific stress response is minimized. Selective strengthening of the dominant links in the system allows many indicators to return to normal. Due to this, the osmotic resistance of red blood cells in highly qualified athletes and untrained people are similar. Apparently, the osmotic resistance of erythrocytes is an indicator by which we can judge the dynamics of the formation of a structural trace - the basis of long-term adaptation [8].

The indicated features of the histogram of the distribution of peripheral blood erythrocytes by size classes mean that small erythrocytes primarily respond to physical activity in response to exposure, which is typical for short-term, rapidly developing reactions that obviously occur as a result of the release of the “first echelon of stress response” cells from the reserve pool of total blood flow [1].

At the same time, the central fossa of the discs deepen, but at the same time, the thickness of the discs decreases by 50–75 nm, which is due to a decrease in the thickness of the protein-globular layer of the membrane of peripheral blood erythrocytes [2]. This is adaptive, as it expands the plastic capabilities of red blood cells when passing through the capillary segment of the hemomicrocirculatory bed.

The distribution of red blood cells among athletes of the second and especially third groups becomes more extreme: the number of intervals increases, the volumes of individual classes vary due to jumps, and the achievement of the values of the central parameters occurs in waves (in the control according to a monotonously upward trend). The wavy shape of the histogram, apparently, reflects the unstable-vibrational state of peripheral blood erythrocytes in the period of active training of the body [3, 5].

In conclusion, it must be emphasized that the determination of Ca^{2+} and morphological changes in peripheral blood erythrocytes can serve as an informative test to assess not only the functional state of the body, but also its fitness and development of the general endurance of athletes of different specializations and qualifications.

Conclusions

1. In the acute phase of physical activity, peripheral blood erythrocytes undergo structural and functional transformations, the pathogenetic basis of which is a flicker-resonant increase in the amplitude and frequency of oscillating vibrations of the fluid, membranes and cells as a whole.

2. A change in the content of Ca^{2+} enhances the melting of lipids, increases the fluidity of the membranes and increases the surface tension, as a result of which the discocytes stretch and thin, the central fossa deepen and the surface roughness, volume and number of cone-shaped pores, as well as the density of red blood cells increase.

3. Due to the multidirectional action of the deforming forces, a violation of the regular disk-shaped form of red blood cells is observed, and changes in the structure and physico-mechanical properties initiate apoptosis and enhance their aggregation interactions.

After the training camp is over, systemic transformations of the peripheral blood erythrocyte pool occur due to the degradation of degenerative forms and increased regenerative capabilities due to the active release of young forms of red blood cells with increased fluidity of membranes, which is a positive adaptive adaptation for the body as a whole.

Conflict of interest. Authors declare that there is no conflict of interest.

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