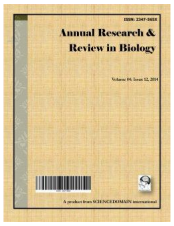

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Physiological Peculiarities of Erythrocytes' Rheological Characteristics in Persons of the Second Mature Age at the Start of Regular Exercises after Lasting Hypodynamia

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Authors' contributions

This work was carried out in collaboration between all authors. Authors NVV and EVS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author AVS managed the analyses of the study. Author TVS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Rheological characteristics of erythrocytes mostly determine the processes of microcirculation and metabolism in tissues. Investigation of their peculiarities in untrained people who began regular adequate exercises, can help to understand rehabilitation mechanisms after hypodynamia.
Aim: To determine the changes of erythrocytes' microrheological properties in those persons of the second mature age who had avoided exercises earlier and then began regular athletic training.
Materials and Methods of Research: The group of observation was composed of 45 healthy people of both sexes of the second mature age who had avoided physical loads earlier and began regular exercises in the athletic section thrice a week. The 1st control group was composed of 42

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people of both sexes of the same age who had regularly trained in the athletics section thrice a week for not less than 10 years. The 2nd control group was composed of 46 people who had daily walked not less than 6 km in the course of the last 10 years. There was applied biochemical, hematological and statistical methods of investigation.
Results: The start of regular exercises in persons with hypodynamia was accompanied by quantity lowering of acylhydroperoxides in their plasma in 3 months of observation by 14.8%, and in 6 months – by 23.4% reaching the level of both control groups. It took place against the background of strengthening of their antioxidant plasma activity in 6 months by 10.2%. During 6 months of physical training the group of observation was noted to have imbalance decrease of arachidonic acid metabolites: the level of thromboxane B_2 in their plasma lowered by 10.7% and the level of 6-keto-prostaglandin $F_{1\alpha}$ increased by 8.7%. It was accompanied by increase of nitric oxide metabolites by 8.9% in their plasma. Erythrocytes of persons from the group of observation in the course of 6 months were noted to have lowering of cholesterol level by 9.1% and acylhydroperoxides by 19.9% at the rise of common phospholipids in them by 6.2%. In 6 months the group of observation was noted to have the increase of erythrocytes-discocytes by 8.2% at quantity lowering of erythrocytes' reversibly and irreversibly modified forms by 18.5% and in 2.1 times, respectively. At the same time, to the end of observation they were found to have the lowering of erythrocytes' involvement into aggregates by 17.3% and the quantity of these aggregates by 39.3% at the increase of free erythrocytes by 13.5%.
Conclusion: Persons of the second mature age who began regular exercises after lasting hypodynamia are characterized by gradual improvement of erythrocytes' microrheological properties reaching the level of control groups.

Keywords: The second mature age; hypodynamia; erythrocytes; aggregation; membrane's surface properties; athletic loads.

1. INTRODUCTION

Low physical activity is very common in modern society [1]. It causes realization of many variants of hereditary predisposition to pathology [2]. It was traced in different categories of population in industrially developed countries [3,4] and confirmed in experiment [5,6,7]. Evident consequences of hypodynamia in a man can manifest themselves already in his young age by frequency increase of episodes of temporary disability because of weakening of functional reserves of the whole body [8,9]. While aging low physical activity leads to aggravation of many diseases and their chronic course [10,11]. Lasting hypodynamia alters many blood indices [12]. It is connected with the fact that low muscle activity causes the development of some functionally unfavorable changes in a body [13]. As previous researches showed, low physical activity was accompanied by microrheological dysfunctions and promotes hypoxia in tissues [14]. Developing of these conditions in chronic oxygen deficiency worsened the course of anabolic processes in the whole body and weakened its common vitality [15,16]. All this formed the basis for pathology development in the internals [17,18] and promoted the appearance of steady vessels' spasm [19]. These changes become especially noticeable in the second mature age when hereditary predisposition to various diseases starts to get realized. It was observed that conditions for the increase of blood pressure could often be created in the second mature age against the background of hypodynamia. It led to gradual development of arterial hypertension [20]. Moreover, hypodynamia in the second mature age aggravated the course of already existing cardiovascular pathology and contributed to the formation of resistance to ongoing drug therapy in these patients [21,22]. Taking into account the severity of hypodynamia negative effects on able-bodied persons of the second mature age, it becomes urgent for the society to find ways out of this state which can improve microrheological properties of red blood cells. Athletics is currently considered to be one of the safest options for physical activity. It has a mild tonic effect on the body, which provides general recovery [10]. In this respect it seems to be important to estimate the impact of exercises (as the most available and safe physical loads) on erythrocytes' microrheological properties in persons of the second mature age who had avoided regular physical loads within lifetime. The following aim was put in this research: to determine the changes of erythrocytes' microrheological properties in those persons of the second mature age who had avoided exercises earlier and then began regular athletic training.

2. MATERIALS AND METHODS

The research was conducted at the expense of the authors. The conduction of the research was approved by the local Ethics Committee of the South-West State University in May, 25th, 2016 (Record №5). All the examined persons gave written informed consent on participation in the conducted research. The work was performed on people of the second mature age (men - 35-60 years old, women - 35-55 years old), living in Kursk and Kursk region. The group of observation was composed of 45 people of both sexes (23 men and 22 women) of the first mature age (mean age 44.9±2.2 years) who had purposefully had no regular exercises within lifetime. All the persons who formed the observation group, had missed the lessons of physical training while studying at school and some higher educational institution. Men had not served in the army. No one in the observation group had attended fitness classes. All of them tried to move by transport but not to walk. They started regular trainings in the athletic section thrice a week with the duration for not less than an hour. The 1st group of control was composed of 42 healthy persons of both sexes (22 men and 20 women) of the second mature age (mean age – 42.8±2.6 years) who had regularly trained for not less than 10 years in the athletic section thrice a week with the duration of each training for not less than an hour [23]. The first control group was composed of 42 healthy persons of both sexes (22 men and 20 women) of the second mature age (mean age 42.8±2.6 years) who had regularly trained in athletics section thrice a week for not less than 10 years with the duration of each training for not less than an hour. The second control group was composed of 46 people of the second mature age (mean age 43.7±2.3 years) who had tried to walk daily the distance of 6 km and had had no additional exercises. Existing in some persons from the group of observation and the control groups chronic diseases (chronic bronchitis, chronic tonsillitis, chronic cholecyctitis) had been in the state of stable remission for not less than 2 years. All the enrolled into the research persons were once observed and examined.

The activity of the processes of lipids' peroxidation (LPO) in blood plasma was determined according to the content of thiobarbituric acid (TBA)-active products in it by a kit of the firm "Agat-Med" (Russia) and according to the level of acylhydroperoxides (AHP). Antioxidant plasma activity was registered [24]. The content of thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$ in plasma of the examined persons was determined with the help of immune-enzymatic analysis by a kit of the firm "Enzo Life science" (USA). Summary content of nitric oxide metabolites was also determined in plasma [25].

After erythrocytes' washing and resuspending we quantitatively estimated the levels of cholesterol (CS) by enzymatic colorimetric method with the help of a kit produced by the firm "Vital Diagnostikum" (Russia) and common phospholipids (CPL) according to the quantity of phosphorus in them [26]. The evidence of the processes of intra-erythrocyte LPO was found out in washed and resuspended erythrocytes according to the concentration of malon dialdehyde (MDA) and AHP quantity [24].

The state of erythrocytes' microrheological properties was judged by their cytoarchitecture and aggregation. The quantity of normal and modified erythrocytes' forms in blood was determined with the help of light phase-contrast microscopy [27]. Erythrocytes' capacity to spontaneous aggregation was established with the help of light microscopy by calculating the quantity of erythrocytes' aggregates, the number of aggregated and non-aggregated erythrocytes [27] in Gorjaev's box.

In the group of observation the persons were examined at the beginning and in 3 and 6 months of trainings. All the persons from both control groups were observed and examined once.

The results were processed by Student's criterion (t). Statistical processing of received information was made with the help of a program package "Statistics for Windows v. 6.0", "Microsoft Excel". Differences in data were considered reliable in case of $p<0.05$.

3. RESULTS

Persons from the group of observation had strengthening of LPO processes (Table). The quantity of AHP and TBA-products in their plasma surpassed the values in the 1st control group by 40.6% and 33.0%, in the 2nd control group – by 38.1% and 30.9%, respectively. It took place against the background of weakening of plasma antioxidant activity in them - in comparison with the 1st control group by 18.5%, in comparison with the 2nd control group – by 16.9%.

Table. Hematologic parameters in people who started regular physical activity after prolonged low physical activity

Indicators	Persons who started physical training after prolonged hypodynamia, n=45, M±m			Control 1 [23], n=42, M±m	Control 2 [23], n=46, M±m
	Initial state	**3 months**	**6 months**		
Acylhydroperoxides of plasma, D_{233}/l ml	2.32±0.57 $p<0.01$ $p_1<0.01$	2.02±0.64 $p<0.01$ $p_1<0.01$	1.88±0.49 $p<0.05$ $p_1<0.05$	1.65±0.20	1.68±0.17
Thiobarbituric acid-products of plasma, mkmol/l	4.19±0.59 $p<0.01$ $p_1<0.01$	3.75±0.32 $p<0.01$ $p_1<0.01$	3.41±0.20 $p<0.05$ $p_1<0.05$	3.15±0.23	3.20±0.26
Antioxidant activity of plasma, %	26.5±0.48 $p<0.01$ $p_1<0.01$	27.8±0.43 $p<0.01$ $p_1<0.01$	29.2±0.47 $p<0.05$ $p_1<0.05$	31.4±0.46	31.0±0.37
thromboxan B_2, pg / ml	208.5±0.72 $p<0.01$ $p_1<0.01$	197.4±0.69 $p<0.01$ $p_1<0.01$	188.4±0.61 $p<0.05$ $p_1<0.05$	162.2±0.72	165.1±0.68
6-keto-prostaglandin $F_{1α}$, pg / ml	83.0±0.35 $p<0.01$ $p_1<0.01$	86.4±0.39 $p<0.05$ $p_1<0.05$	90.2±0.42 $p<0.05$ $p_1<0.05$	95.0±0.45	94.3±0.54
nitric oxide's metabolites, umol/l	31.2±0.28 $p<0.01$ $p_1<0.01$	32.7±0.22 $p<0.01$ $p_1<0.01$	34.0±0.25 $p<0.05$ $p_1<0.05$	37.2±0.32	35.8±0.32
cholesterol of erythrocytes, mkmol/10^{12} erythrocytes	1.07±0.012 $p<0.01$ $p_1<0.01$	1.03±0.010 $p<0.05$ $p_1<0.05$	0.98±0.009	0.94±0.010	0.95±0.014
common phospholipids of erythrocytes, mkmol/10^{12} erythrocytes	0.64±0.010 $p<0.01$ $p_1<0.01$	0.66±0.012 $p<0.05$ $p_1<0.05$	0.68±0.008	0.73±0.015	0.71±0.008
acylhydroperoxides of erythrocytes, D_{233}/10^{12} erythrocytes	4.03±0.017 $p<0.01$ $p_1<0.01$	3.71±0.014 $p<0.01$ $p_1<0.01$	3.36±0.020 $p<0.05$ $p_1<0.05$	3.11±0.016	3.14±0.011
malonic dialdehyde of erythrocytes, nmol/10^{12} erythrocytes	1.89±0.009 $p<0.01$ $p_1<0.01$	1.69±0.011 $p<0.01$ $p_1<0.01$	1.52±0.008 $p<0.05$ $p_1<0.05$	1.36±0.014	1.41±0.009
erythrocytes-discocytes, %	77.8±0.27 $p<0.01$ $p_1<0.01$	80.6±0.22 $p<0.05$ $p_1<0.05$	84.2±0.25	87.3±0.18	86.5±0.12
reversibly modified erythrocytes,%	14.1±0.19 $p<0.01$ $p_1<0.01$	12.6±0.15 $p<0.01$ $p_1<0.01$	11.9±0.21 $p<0.05$ $p_1<0.05$	9.5±0.10	10.1±0.07
irreversibly modified erythrocytes,%	8.1±0.10 $p<0.01$ $p_1<0.01$	6.8±0.12 $p<0.01$ $p_1<0.01$	3.9±0.11	3.2±0.15	3.4±0.10
sum of all the erythrocytes in an aggregate	40.7±0.14 $p<0.01$ $p_1<0.01$	37.2±0.16 $p<0.01$ $p_1<0.01$	34.7±0.18	31.5±0.10	32.1±0.07
quantity of aggregates	8.2±0.09 $p<0.01$ $p_1<0.01$	7.5±0.07 $p<0.01$ $p_1<0.01$	7.0±0.06	6.0±0.07	6.2±0.09
quantity of free erythrocytes	245.0±0.45 $p<0.01$ $p_1<0.01$	262.8±0.52 $p<0.05$ $p_1<0.05$	278.1±0.49	296.1±0.35	292.3±0.29

Conventions: p – the significance of differences in the parameters of those surveyed who have inactivity and control 1 groups; p_1 – the significance of differences in the parameters of those surveyed who have inactivity and control 2 groups; the significance of differences between indicators 1 and 2 are not detected

Blood of the examined persons with hypodynamia in the initial state was noted to have imbalance of arachidonic acid metabolites: the level of thromboxane B_2 in their plasma turned out to be higher in comparison with the 1st control group by 28.5%, in comparison with the 2nd control group – by 26.3%. The level of 6-keto-prostaglandin $F_{1α}$ in the group of observation at that was lower in comparison with the 1st control group by 14.4%, in comparison with the 2nd control group – by 13.6% (Table). It was accompanied in them by content lowering of the quantity of nitric oxide summary metabolites in plasma (by 19.2% in comparison with the 1st control group and by 14.7% in comparison with the 2nd control group).

At the beginning erythrocytes' membranes of persons with hypodynamia were noted to have the rise of CS level by 13.8% in comparison with the 1st control group and by 12.6% in comparison with the 2nd control group. It was accompanied by CPL lowering in them by 14.1% and 10.9%, respectively. Erythrocytes of physically untrained persons were found to have the rise of AHP (by 29.6% in comparison with the 1st control group and by 28.3% in comparison with the 2nd control group) and MDA in comparison with the 1st control group by 38.9%, in comparison with the 2nd control group – by 34.0%.

Persons having low physical activity within lifetime, were noted to have lowering of erythrocytes-discocytes' percentage in blood (in comparison with the 1st control group by 12.2%, in comparison with the 2nd control group – by 11.2%) (Table). The quantity of erythrocytes' reversibly and irreversibly modified forms in their blood was increased in comparison with both control groups by more than 39.0% and in 2.3 times, respectively.

At the same time, in the initial state the examined persons with hypodynamia were found to have strengthening of erythrocytes' aggregative properties. It was pointed at by the index increase of summary erythrocytes' involvement into aggregate by 29.2% and 26.8% (in comparison with the level of the 1st and the 2nd control groups) and quantity increase of these aggregates by 36.7% and 32.2% at decrease of freely lying erythrocytes by 20.8% and 19.3%, respectively.

The start of regular athletic loads was accompanied by positive dynamics of accountable indices. The group of observation was noted to have weakening of LPO processes against the background of physical loads (Table). The quantity of AHP and TBA-products in plasma of persons from the group of observation lowered in 3 months of observation by 14.8% and 11.7%, and in 6 months – by 23.4% and 22.9% in comparison with the beginning. However, the level of both control groups wasn't reached. It took place against the background of strengthening of plasma antioxidant activity in them in 3 months by 4.9%, in 6 months – by 10.2% thus approaching the control levels.

The blood of persons with hypodynamia who began physical trainings was noted to have the lowering of imbalance evidence of arachidonic acid metabolites: the level of thromboxane B_2 in their plasma lowered in 3 months of observation by 5.6%, in 6 months – by 10.7%. At that, the level of the derivative of its functional antagonist – 6-keto-prostaglandin $F_{1\alpha}$ in the group of observation rose in 3 months by 4.1% in comparison with the beginning, in 6 months – by 8.7% (Table). It was accompanied in them by content rise of the quantity of nitric oxide summary metabolites in plasma (by 4.8% - by the 3rd month of trainings and by 8.9% - by the 6th month of trainings).

Erythrocytes' membranes of persons from the group of observation in the course of physical loads were noted to have lowering of CS level by 3.9% in 3 months and by 9.1% in 6 months of observation. It was accompanied by CPL rise in them by 3.1% and 6.2%, in these terms respectively. At the same time, the erythrocytes of persons who began physical trainings, were found to have lowering of AHP (by 8.6% - in 3 months and by 19.9% - in 6 months) and MDA in 3 months of physical trainings by 11.8%, in 6 months – by 24.3%.

The persons who started to have regular physical loads were noted to have the increase of erythrocytes-discocytes' percentage in blood in comparison with the beginning (in 3 months – by 13.6%, in 6 months – by 8.2%) (Table). The quantity of erythrocytes' reversibly and irreversibly modified forms in blood of persons from the group of observation gradually lowered. Summary lowering of these indices by the end of observation was equal to 18.5% and 2.1 times, respectively. At the same time, those persons who had hypodynamia earlier, were found to have weakening of erythrocytes' aggregative properties against the background of athletic loads. It was pointed at by the lowering of erythrocytes' summary involvement into aggregates in 3 and 6 months by 9.4% and 17.3%, and the quantity of these aggregates by 39.3% and 17.1% at the increase of freely lying erythrocytes by 7.3% and 13.5%, respectively.

4. DISCUSSION

Lasting maintenance of physiological optimum in a human body and its effective rehabilitation are possible only at high muscle activity [28]. It was noted long ago that hypodynamia could cause prepathological state and strengthen existing pathology [29]. Last decades notwithstanding the successes of medicine and propaganda of medical knowledge hypodynamia remains rather widespread among population of developed countries. Its consequences at that continue to damage economically because of high frequency of working capacity lowering [30] against its background. It's noted that formation of hypodynamia is accompanied by not only worsening of musculoskeletal system state but also lowering of metabolism intensity. Given situation negatively influences the functioning of the whole body [31].

There is some basis to suspect that great significance in the formation of hypodynamia consequences belongs to disturbances of regular blood elements' microrheological properties and, first of all, to their most numerous population – erythrocytes [32]. At the same time, weakening of a body's antioxidant protection leading to the increase of LPO intensity in its plasma and cells, has special significance for their development. The products' surplus of lipids' peroxidation in plasma and erythrocytes causes changes in membranes of these cells from the inside and outside what worsens their functions [33]. It is aggravated at hypodynamia by the development of lipid imbalance in erythrocytes' membranes which additionally promotes worsening of these regular blood elements' functioning [34]. Forming changes of phospholipids' and cholesterol's quantity and ratio in their membranes are, evidently, functionally unfavorable. They damage selective permeability in erythrocytes and membrane's viscosity and negatively influence membrane-bound proteins in the result of their secondary structure's modification. Given situation rather negatively influences the state of most erythrocytes' membranes.

Found strengthening of erythrocytes' aggregation at hypodynamia can be estimated as the result of the combined impact of low physical activity's consequences on microrheological processes. Reliable number increase of reversibly modified erythrocytes and the increase of their irreversibly modified forms inevitably lead to quantity rise of erythrocyte aggregates in blood of these people and to the increase of the involvement degree of new erythrocytes into them. Found evidence increase of erythrocytes' aggregation in conditions of hypodynamia can be mostly explained by strengthening impact of catecholamines on them. Their level reliably rises in blood at any dysfunction [35]. Quantity increase of catecholamines in blood of persons from the group of observation should be mostly estimated as the mechanism of metabolism intensification in tissues [36].

The synthesis of biologically active substances which can limit erythrocytes' aggregation, lowers in vascular wall of persons with hypodynamia. At the same time, the level of pro-aggregants rises in their blood. So, noted in group of observation intensification of thromboxane formation and production weakening of its functional antagonist – prostacyclin – forms imbalance of arachidonic acid metabolites. Given disturbances are aggravated in them by developing weakening of NO production in vascular wall. Probably, it takes place in the result of endeothelial NO-synthase suppression by strengthened LPO in plasma [37]. Forming in these conditions erythrocytes' microrheological dysfunctions can worsen microcirculation processes and weaken trophism themselves, including vascular walls and production of disaggregants in them.

In present research the persons with hypodynamia were appointed regular adequate athletic physical loads for rehabilitation of a body. Against their background the examined persons had quantity decrease of lipids' peroxidation products in plasma and erythrocytes what promoted state optimization of these cells' membranes on the outside and inside. The effect was strengthened in the course of hypodynamia overcoming by developing improvement of lipid composition of erythrocytes' membranes which mostly stimulated the functioning of these regular blood elements [38]. Positive changes of phospholipids' and cholesterol's quantity and ratio in their membranes turned out to be functionally favorable and approximated the selective permeability and membranes' viscosity to the optimum in erythrocytes. They also improved the state of secondary structure of membrane-bound proteins of most erythrocytes. As the conducted research showed, the start of physical exercises in persons who had lasting hypodynamis, is characterized by content lowering of erythrocytes' reversibly and irreversibly modified forms in their blood against the background of an upward quantity trend of their discoid forms.

Noted weakening of erythrocytes' aggregation in the course of athletic trainings of persons with previous hypodynamia can be estimated as the result of the impact of physical activity rise on microrheological processes. Number lowering of reversibly modified erythrocytes at that and the decrease of their irreversibly modified forms led to quantity decrease of erythrocyte aggregates in blood of these people and to lowering of involvement degree of new erythrocytes into them. Detected weakening of erythrocytes' aggregation in the course of hypodynamia overcoming can be mostly explained by weakening of catecholamines' impact on them, the level of which always lowers in their blood against the background of exercises [39].

The synthesis of biologically active substances able to limit erythrocytes' aggregation strengthened in vascular wall of persons who had hypodynamia earlier, against the background of started regular exercises. It was accompanied by level lowering of pro-aggregants in their blood. So, noted in the group of observation weakening of thromboxane formation and production strengthening of its functional antagonist – prostacyclin – was the start of imbalance restoration of arachidonic acid metabolites. Given process was supported by developing against this background strengthening of NO production in vascular wall [40]. Probably, it took place in the result of activation of endothelial NO-synthase on behalf of progressive LPO weakening in plasma [41].

5. CONCLUSION

Persons of the second mature age who began athletic trainings after lasting hypodynamia are characterized by weakening of lipids' peroxidation processes in plasma and erythrocytes. It is accompanied by number lowering of erythrocytes' modified forms in their blood and weakening of their aggregative capacity. Found changes can improve the processes of blood rheology in capillaries and activate trophism in all the internals promoting the common rehabilitation. To restore microrheological disorders in people of the second mature age who had previously avoided physical loads, exercises should be performed at least thrice a week for at least an hour a day. These trainings should become part of their lives.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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