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ОРИГИНАЛЬНОЕ ИССЛЕДОВАНИЕ
ORIGINAL RESEARCH

Differences in the ratio of the peripheral blood phospholipid fractions among residents of different climatic and geographic territories

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Abstract. Relevance. A key element of human adaptation to the complex of extreme ecological factors is intensification of the energy metabolism. Mobilization of energy resources may lead to modification of the biological membranes and, as a consequence, to change their functional activity. Evaluation of the persistent imbalance degree on the level of phospholipid fractions may contribute to early diagnosis of the maladaptive states. **Aim:** an analysis of the levels of phosphatidylserine (PS), phosphatidylcholine (PC), phosphatidylethanolamine (PE) and sphingomyelin (SM) in the peripheral blood of the population in the Arctic and subarctic region of Russia, as well as the Southern Caucasus inhabitants. **Materials and Methods.** From 2010 to 2018, 687 people of both sexes aged 22 to 60 years were examined. The participants were divided into the following groups: 1) natives of the Russian Arctic region (AR); 2) residents of the subarctic region of Russia (SR); 3) residents of the South Caucasus (JUR). The level of the phospholipids was assessed using the thin-layer chromatography method. The results are presented in percentage terms. **Results and Discussion.** The residents of the AR revealed lower PS and higher PE levels compared to those of SR and JUR natives. At the same time, the AR individuals in the phospholipid spectrum reveal a relative decrease in the share of PC. Unlike the AR natives, the low PS content in the SR residents' peripheral blood did not affect the overall pool of the phospholipids, which is within similar variation limits regarding the natives of the JUR. The SM medians in the compared groups had no statistically significant differences. **Conclusion.** The complex of adaptive alterations in the human body under Arctic conditions is associated with mobilization of mechanisms aimed at increasing physical fluidity of the biological membranes, which may indicate a significant increase in their permeability and intensification of receptor and enzymatic activity in cells.

Keywords: phosphatidylethanolamine, phosphatidylcholine, sphingomyelin, phosphatidylethanolamine, Arctic region, subarctic region, South Caucasus

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a significant contribution to the development of the concept and manuscript writing, read and approved the final version before publication.

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Ethics approval. The study was approved by the Ethics Committee of the IPNA UrB RAS and the Ethics Committee of the FECIAR UrB RAS (dated February 2, 2009, February 4, 2023, and November 9, 2016).

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Consent for publication. Voluntary written consent was obtained from the patients for the investigation and publication of relevant medical information according to WMA Declaration of Helsinki — Ethical Principles for Medical Research Involving Human Subjects, 2013.

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Introduction

Assessing the impact of latitudinal factor on human health is an important eco-physiological task. The latitude-zone variations have a significant impact on the formation of physiological strategy of the adaptation. At the same time, prolonged exposure to sub-extreme and extremal natural and climatic conditions may lead to depletion of compensatory mechanisms, which contributes to the occurrence and development of the chronic diseases, which begin to manifest long before the onset of clinical symptoms [1].

As the living area expands towards higher latitudes, the negative effects (such as significant fluctuations in atmospheric pressure, cold weather, changes in daylight hours, geomagnetic activity) increase. It is established that such climatogeographic features, being to some extent extremal for the human life activity, initiate a characteristic set of adaptive changes at the level of the body's metabolic, morphological and regulatory systems [2]. However, the key element in the process of acquired adaptation is a shift in the energy homeostasis system, primarily driven by lipid metabolism components, which are inherently linked to cellular structural elements. This indicates

that intensification of the lipid oxidation process may lead to the modification of biological membranes and, consequently, to changes in their functional activity. A system of such transformations can lead to increasing the degree of persistent metabolic imbalance which may involve other environmentally-related criteria for its assessment [3].

The phospholipids are a key element in ensuring the structural and functional integrity of the biological membranes. A number of critical physiologic processes depend on their dynamic equilibrium, including maintenance of cellular homeostasis, regulation of the cell cycle as well as adaptation of cells to changes in the environment [4].

Available empirical data, obtained during observational studies, provide convincing evidence of a specific phospholipid profile in Arctic residents [5–8]. However, there is a lack of information in the available scientific literature concerning the comparative assessment of the fractional composition of peripheral blood phospholipids in population at different climatic and geographical zones. Research in this area is fragmentary. In the available data, metabolic parameters of the population of the North are often analyzed in isolation from similar indicators of natives of southern

regions. We believe this approach distorts understanding of adaptive restructuring occurring within serum phospholipid fractions under extreme natural-climatic influences.

Mentioned aspects highlight the need to develop criteria for assessing the phospholipid profile in humans under contrasting extreme climatic and geographical conditions. This will not only enhance diagnostic capabilities for pre-pathological states but also help design rational preventive measures and correctional strategies addressing metabolic disorders associated with extreme environmental impacts. Therefore, it seems important to conduct a comparative analysis of ratios between phosphatidylserine, phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin in peripheral blood serum samples from Russian Arctic and subarctic Okrug populations compared to Southern Caucasus residents.

Materials and methods

The present study was conducted as part of a comprehensive expedition program that took place from 2010 to 2018. During the winter-spring season, 687 individuals were examined — men and women aged between 22 and 60 years. Separate groups were formed based on the location of birth and residence of the volunteers. The first group consisted of 264 persons — natives of the Arctic region of Russia (AR; 71–65°N) — villages: Gyda, Soyakha, Antipayuta, Krasnoselkup; Tazovskiy settlement and Nadym town in the Yamalo-Nenets Autonomous Okrug. The second group included 244 representatives of the local population of the subarctic region of Russia (SR; 64°N) — Pinega settlement of the Arkhangelsk region and Arkhangelsk city. The third group comprised 179 natives of South Caucasus (JUR; 42°N) — Tskhinvali city, Republic of South Ossetia. The average age of participants was as follows (mean ± SD): AR — 42.41 ± 11.06 years; SR — 42.56 ± 10.85 years; JUR — 39.40 ± 11.09 years.

The Arctic is a territory with harsh natural and climatic conditions that are unsuitable for permanent human habitation. The climate of the Arctic zone

in the Yamalo-Nenets Autonomous region is highly uncomfortable. The unique climatic conditions are characterized by low temperatures and a combination of factors, including permafrost, Arctic air circulation, and the immediate proximity of the Kara Sea. The annual mean temperature: about –10 °C [9].

Climatic and geographical features of the subarctic regions, despite considerable mitigation of the extreme conditions inherent in the Arctic, still create a significant level of discomfort for the local population. (The city of Arkhangelsk and the Pinega settlement are situated in the Atlantic-Arctic sector of the temperate zone. The meteorological regime of these territories is determined by the complex interaction of advective processes, in particular — outgoing from the northern seas. The average annual temperature varies from 0,1 to 2,0 °C [10].

South Ossetia is characterized by a variety of ecological factors due to the complex topography of the region. The ecological and climatic situation of the city of Tskhinvali, where the study was conducted, can be described as physiologically favorable for human life. The region under consideration is located in the foothills of the Greater Caucasus, at an altitude of about 600 meters above sea level. The climatic conditions in this area are characterized as subtropical, with an average annual temperature fluctuating around 11,2 °C [11].

Within the framework of the study, a targeted sampling of the respondents was performed among the local residents, compliant with the criteria of health groups I and II. To reduce the likelihood of influencing of the pathological processes on the results of the analysis, all volunteers were in a state of remission from their chronic diseases. The examinations were conducted in strict accordance with the ethical standards regulated by the WMA Declaration of Helsinki — Ethical Principles for Medical Research Involving Human Subjects, 2013. The studies were conducted based on the written consent of each participant, obtained after careful explanation of all aspects of the procedure, potential risks and expected benefits. The study protocols were approved by the Biomedical Ethics Commission of the IPNA UrB

RAS and the Ethics Committee of the FECIAR UrB RAS (dated February 2, 2009, February 4, 2023, and November 9, 2016).

The examination process included a questionnaire, designed to determine age, anthropometric data, the presence of chronic diseases, work experience, ethnicity, bad habits, level of physical activity, and diet. In the morning, between 08:00 and 10:00 local time, venous blood was collected from the cubital vein. The procedure was performed exclusively under fasting conditions, which implied observing a strict time interval of 10–12 hours from the last meal.

Lipids from blood serum were extracted and purified using the liquid extraction method [12]. To achieve optimal phospholipid separation, an eluent was used in the following proportions: 6.5 parts chloroform, 2.5 parts methanol, and 0.5 parts aqueous ammonia solution with a mass fraction of NH_3 of at least 25%. The total volume of the system was 3 cm^3 . The identification of phosphatidylcholine (PC), sphingomyelin (SM), phosphatidylethanolamine (PE), and phosphatidylserine (PS) was performed by comparing with R_f values of standard samples provided by Sigma (USA). The densitometer «DenSkan» (Russia) was used as a tool for measuring the optical density of chromatographic spots. The analysis of chromatographic data was carried out using the peak area normalization method implemented using the «Dens» software version 14–12–03 beta (Russia).

The statistical analysis of the obtained material was carried out using the IBM SPSS Statistics 22.0 program (USA) [13]. The hypothesis of a normal distribution of a random variable was tested using the Shapiro-Wilk test. Due to the revealed anomalies in the distribution of the studied features, the median (Me) indicators were used as the central trend, and the values of the first (Q_1) and third (Q_3) quartiles were used to estimate the range of variability. The Kruskal-Wallis test (H-test) was used to assess the degree of differences between the compared groups. The Mann-Whitney test (U-test) was applied to a posteriori comparisons. In order to minimize the risk of false positives, the Bonferroni correction was applied when testing hypotheses. The found changes were considered

statistically significant if the probability of error in accepting the null hypothesis was less than 5%.

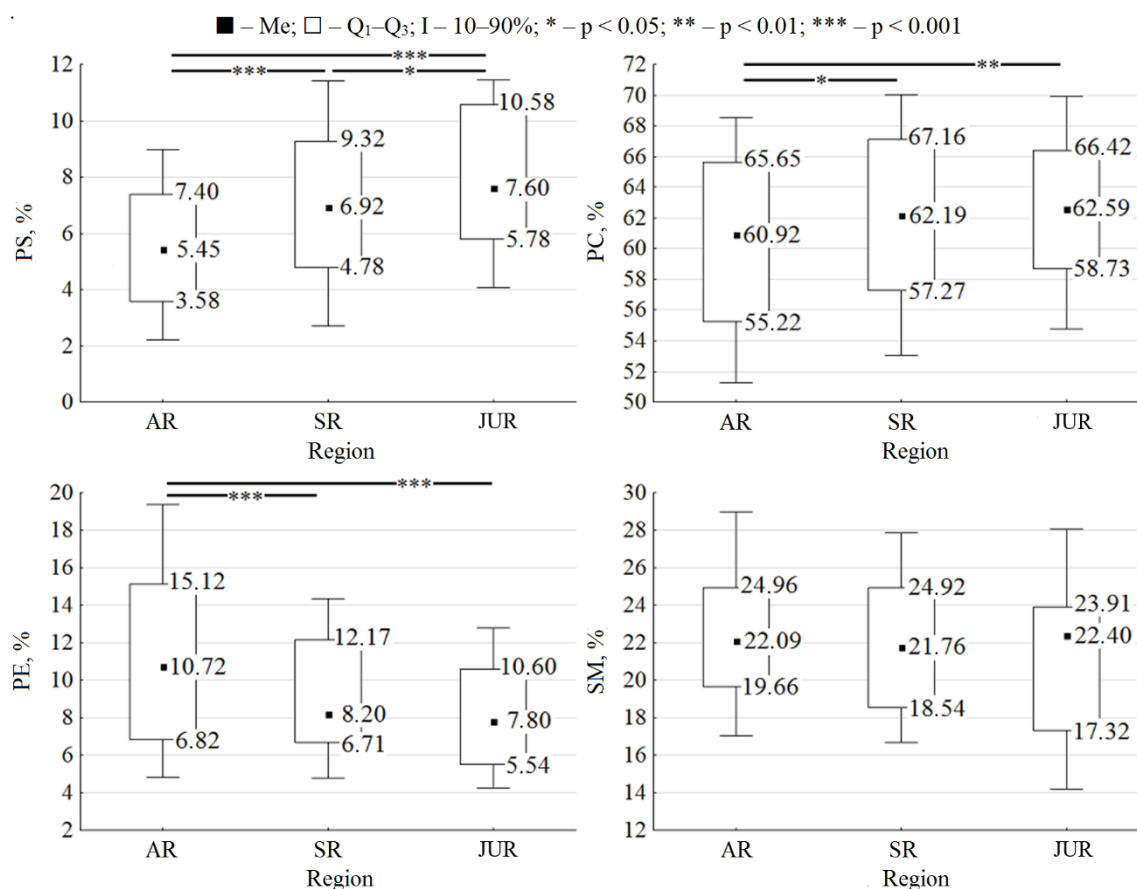
Results and discussion

The phospholipid composition of peripheral blood is largely determined by the intensity of synthesis as well as breakdown of these compounds in the tissues of the liver, kidneys, and lungs as well as in blood cells and in the vascular walls. Therefore, the ratio of the phospholipid fractions in blood serum not only reflects a generalized picture of their metabolism, but it can also serve as an indirect indicator of the structural and functional organization of the cell membranes of many organs and tissues [14].

A preliminary analysis of the data obtained during the comparison of three independent groups revealed statistically significant differences in the PS indicators ($H = 57.36$; $p = 3.50 \times 10^{-13}$), PE ($H = 36.48$; $p = 1.20 \times 10^{-8}$), PC ($H = 13.78$; $p = 1.02 \times 10^{-3}$), SM ($H = 6.45$; $p = 3.98 \times 10^{-2}$).

The subsequent calculation was performed using the method of a posteriori comparisons (Figure).

The analysis of the obtained results showed that the profile of phospholipid fractions in the peripheral circulation system of residents of the JUR region differs in a statistically significant high level of PS relative to individuals living in the SR and AR of Russia ($p = 4.58 \times 10^{-2}$; $p = 5.69 \times 10^{-13}$ accordingly). In the same time, in 90% of the subjects examined in the Arctic, the proportion of this fraction in the total pool of phospholipids did not exceed 9.0%, which is a statistically significant low content compared to SR natives ($p = 1.36 \times 10^{-6}$) and JUR natives. According to the data obtained on the PC, it was found that the level of this indicator is statistically significantly lower among the residents of AR than relative to the natives of SR and JUR ($p = 1.85 \times 10^{-2}$; $p = 1.50 \times 10^{-2}$, respectively). It is also important to note that the range of PC fluctuations in the total phospholipid spectrum of 80% of the individuals examined in the Arctic was between 51.3% and 68.6%. At the same time, the SR and JUR groups had statistically significantly lower concentrations of PE than the AR group ($p = 1.00 \times 10^{-4}$; $p = 4.75 \times 10^{-8}$, respectively), where 80% of the



Analysis of the fractional composition of phospholipids in peripheral blood serum of the population of natives of the Russian Arctic region (AR) and residents of the subarctic region of Russia (SR) regions of Russia, as well as residents of the South Caucasus (JUR)

participants had a relative value range of 4.82–19.46%. At the same time, the median value of SM did not differ statistically significantly between the compared groups. In particular, the residents of the AR and JUR the probability of differences in the obtained SM values in the statistical trend zone ($p = 5.55 \times 10^{-2}$).

It is believed that the metabolic pathways of the PS, PE and PC are closely related [15]. It has been established that the main metabolic pathway from PS to PE is the carbon dioxide elimination reaction catalyzed by phosphatidylserine decarboxylase. This enzyme is a transmembrane protein whose active site is located on the outer side of the inner mitochondrial membrane. In this regard, it plays a key role in the production of mitochondrial PE. It has been noted that PE synthesized in mitochondria is actively

exported to other cellular organelles, including the plasma membrane. Alternatively, PE can be obtained by synthesis on the surface of the endoplasmic reticulum, which involves the phosphorylation and activation of exogenous ethanolamine, followed by the condensation of diacylglycerol [16]. Given that the human adaptation process is associated with the intensification of metabolic processes, it can be assumed that the identified changes in the residents of the AR represent an element of specific adaptive-compensatory reaction caused by increased enzymatic activity. This reaction may be typical for Arctic natives in response to prolonged and intense exposure to stressful environmental factors. On the other hand, this may be a consequence of the relationship between the substrate and the reaction

product, which is related to the exogenous supply of the substrate.

The next significant stage of the PE metabolic cycle is its methylation process involving S-adenosylmethionine. In this reaction, PE acts as a precursor to PC and can serve as a source for its synthesis [17]. In this regard, it is likely that the high level of PE in the population of the Arctic territories serves as a reserve that supports the synthetic capacity for producing PC. According to the data presented in the work by E.R. Boyko and co-authors [6], the decrease in the PC indicators in individuals, living in the climatic and geographical conditions of the North, may have several causes, among which the process of activating cholesterol esterification reactions is the main factor. According to the authors, the active use of PC in this process is an adaptive reaction of the body that helps to eliminate this atherogenic lipid from the vascular bed.

Another important factor that, in our opinion, may limit the synthesis of PC in high-latitude residents is the high degree of unsaturation of fatty acids in lipids. The current data indicates that the PS and PE fractions, unlike the PC esters, are directly associated with the polar transport form of fatty acids that have four or more double bonds in the hydrocarbon chain [18]. At the same time, the length and degree of unsaturation of the acyl components usually depend on the fatty acid composition of the food [19]. In addition, the content of fatty acids in the diet can exogenously affect the rate, balance, and direction of phospholipid metabolism [20, 21]. So, in a study aimed at studying the fatty acid composition of blood plasma lipids and erythrocytes among the population of the Chukotka Autonomous Okrug and the city of Moscow, it was revealed that the content of polyunsaturated fatty acids of the n-3 family in these media in representatives of Chukotka, especially in coastal areas, significantly exceeds similar indicators in Muscovites. At the same time, in the subfractions of high-density lipoproteins, residents of Chukotka have a lower proportion of PC and a higher percentage of SM and PE compared to Muscovites [22]. Concurrently, a decrease in the proportion of PS in the total pool of serum phospholipids in individuals in

the AR compared to those in the SR and JUR leads to a corresponding relative increase the PE percentage. Their data demonstrate that the level of n-3 fatty acids in the PE fraction of Arctic residents significantly exceeds similar indicators in the control group formed from representatives living in Vancouver. Moreover, it was found that the occurrence of these fatty acids in PE is higher than in PC, which may also indicate their specific distribution and significance in the context of adaptation to a cold climate [23].

Based on the presented data, it is possible to make a reasonable assumption that the direction of metabolic processes occurring in the human body is not only associated with the qualitative characteristics of his diet, but is also largely determined by the climatic and geographical factors of the territory of his permanent residence. These parameters, in turn, have a complex effect on the dynamics and structural distribution of phospholipid fractions, both in the peripheral circulation system and in the organization of cellular membranes of organs and tissues. In particular, the results of the study indicate enrichment of the cell membranes of Arctic residents with phospholipid fractions characterized by a high degree of unsaturation of their acyl chains. On the one hand, this leads to a decrease in the antioxidant properties of membrane phospholipids, and on the other hand, it can contribute to an increase in the liquid properties of biological membranes, expanding the kinetic and thermodynamic characteristics of enzymes and receptors. As a result, such changes in the total pool of phospholipids in the peripheral blood of local residents can reflect the adaptive orientation of metabolic processes to the extreme environmental conditions of the Arctic, which occurs at the level of cell membranes.

Conclusion

The results of the study show that residents of the AR have the lowest level of PS in the peripheral blood phospholipid spectrum, while residents of the JUR have the highest concentration. At once, a decrease in the proportion of PS in the total pool of serum phospholipids in individuals in the AR compared to those in the SR

and JUR leads to a corresponding relative increase of the PE percentage. However, in volunteers examined in AR, the observed changes in the ratio of phospholipid fractions are accompanied by a relative decrease in the concentration of PC. At the same time, the concentration of SM in the groups under consideration did not differ statistically significantly.

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Оценка различий в соотношении фосфолипидных фракций периферической крови у жителей различных климатогеографических территорий

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Аннотация. *Актуальность.* Ключевой элемент адаптации человека к комплексу экстремальных экологических факторов — интенсификация энергетического обмена. Мобилизация энергетических ресурсов может приводить к модификации биологических мембран и, как следствие, изменять их функциональную активность. Оценка степени устойчивого метаболического дисбаланса на уровне фракций фосфолипидов может способствовать ранней диагностике дезадаптивных состояний. Цель работы заключалась в анализе фосфатидилсерина (ФС), фосфатидилхолина (ФХ), фосфатидилэтаноламина (ФЭА) и сфингомиелина (СФМ) в сыворотке периферической крови населения арктического и приарктического регионов России, а также жителей Южного Кавказа. Материалы и методы. С 2010 по 2018 год обследовано 687 лиц обоего пола в возрасте от 22 до 60 лет. Среди участников были сформированы следующие группы: 1) уроженцы арктического региона России (АР); 2) лица, проживающие в приарктическом регионе России (ПР); 3) жители Южного Кавказа (ЮР). Уровень фосфолипидов оценивался с помощью метода тонкослойной хроматографии. Результаты представлены в процентном соотношении. *Результаты и обсуждение.* У жителей АР по сравнению с уроженцами ПР и ЮР выявлено низкое содержание ФС и высокое — ФЭА. При этом у лиц из АР в фосфолипидном спектре отмечается относительное снижение доли ФХ. В отличие от уроженцев АР у жителей ПР низкое содержание ФС в периферической крови не оказывало влияние на общий пул сывороточных фосфолипидов, который находится в схожих лимитах варьирования относительно уроженцев ЮР. Медианы СФМ в сравниваемых группах не имели статистически значимых различий. *Выводы.* Сложный комплекс адаптационных изменений в организме человека в условиях Арктики ассоциирован с мобилизацией механизмов, направленных на повышение физической текучести биологических мембран, что может указывать на значительное увеличение их проницаемости, а также интенсификацию рецепторной и ферментативной активности в клетках.

Ключевые слова: фосфатидилсерин, фосфатидилхолин, сфингомиелин, фосфатидилэтаноламин, арктический регион, приарктический регион, Южный Кавказ

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