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Original article

Seasonal changes in the profile of blood plasma fatty acids as a mechanism of human adaptation to the extreme conditions of the North

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Abstract
Extreme environmental factors lead to changes in the metabolism of the body and, in particular, to the predominant use of proteins and fats. The aim of our study was to reveal seasonal differences in the blood plasma fatty acid profile of people with evolutionarily developed mechanisms of adaptation to the specific conditions of the North. The subjects of the study were young male aborigines of the North (Yakuts), virtually healthy volunteers whose mean age was 19.1 ± 2.2 (n = 26). Venous blood samples were collected in the morning from 8:00 am to 9:00 am in different seasons (summer, fall, and winter). Temperature variations during these seasons were more than 100 °C. Identification and determination of fatty acid (FA) concentrations in the blood plasma samples were performed using gas chromatography-mass spectrometry (GC-MS). Statistical analysis of blood plasma lipid profile data was performed using MetaboAnalyst 5.0. The results showed that the ratio of unsaturated fatty acids (USFA) to saturated fatty acids (SFA) increases by 1.8 times in winter compared to other periods (summer, and autumn). The leading role of polyunsaturated fatty acids (PUSFA) in the adaptation of the human body to interseasonal changes has been revealed. The most important role is played by the winter increase of 11,14,17-eicosatrienoic acid (omega-3) and the winter decrease of arachidonic acid, cis-11,14-eicosadienoic acid and 8,11,14-eicosatrienoic acid.
Keywords: seasonal changes, chronobiology, fatty acids, adaptation, cold, metabolomics, lipidomics, student, indigenous people, blood plasma
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Оригинальная статья

Сезонные изменения профиля жирных кислот плазмы крови как механизм адаптации человека к экстремальным условиям Севера

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Аннотация
Экстремальные факторы окружающей среды приводят к изменениям в метаболизме организма и, в частности, к преимущественному использованию белков и жиров в обмене веществ. Целью нашей работы было выявить сезонные различия в содержании жирных кислот в плазме крови людей с эволюционно развитыми механизмами адаптации к специфическим условиям Севера. Объектами исследования были молодые мужчины-абориге-

ны Севера (якуты), практически здоровые добровольцы, средний возраст которых составил 19,1 ± 2,2 года (n = 26). Образцы венозной крови брали в утренние часы с 8:00 до 9:00 в разное время года (лето, осень, зима). Колебания температуры в течение этих сезонов составляли более 100 °C. Идентификацию и определение концентрации жирных кислот (ЖК) в образцах плазмы крови проводили методом газовой хроматографии-масс-спектрометрии (GC-MS). Статистический анализ данных липидного профиля плазмы крови проводился с использованием платформы MetaboAnalyst 5.0. Результаты показывают, что отношение ненасыщенных жирных кислот (UFA) к насыщенным жирным кислотам (SFA) зимой увеличивается в 1,8 раза по сравнению с другими периодами (летом, осенью). Выявлена ведущая роль полиненасыщенных жирных кислот в адаптации человеческого организма к межсезонным изменениям. Наиболее важную роль играют зимнее увеличение содержания 11,14,17-эйкозатриеновой кислоты (омега-3) и зимнее снижение арахидоновой кислоты, цис-11,14-эйкозадиеновой кислоты и 8,11,14-эйкозатриеновой кислоты.

Ключевые слова: сезонные изменения, годовой ритм, жирные кислоты, адаптация, экстремальные условия, метаболомика, липидомика, север, коренные народы, плазма крови

Финансирование. Работа выполнена в рамках госзадания Минобрнауки РФ по проекту «Физиологические и биохимические механизмы адаптации растений, животных, человека к условиям Арктики/Субарктики» (код научной темы: FWRS-2021-0025; гос. регистр. номер: АААААА-А21-121012190035-9), а также с использованием оборудования ЦКП ФИЦ «ЯНЦ СО РАН».

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Introduction

Seasonally recurring patterns in physiology and behavior are common in nature [1–4]. They often reflect adaptive, proactive responses to annual energy constraints [5–8]. The North-East of Russia is one of the harshest regions of the globe, where a complex of environmental factors of extreme nature affects a living organism: specific photoperiodism (polar day and polar night), cold, sharply continental climate, and geomagnetic disturbances. In this regard, there is a need to study the ecological, physiological and biochemical mechanisms of adaptation of the body to the complex effects of extreme factors both during short-term and long-term human residence in the North. The question of how the constancy of the internal environment of the body is maintained in various seasons of the year in extreme conditions is especially relevant. Extreme environmental factors lead to changes in all types of metabolic processes and, above all, to the predominant use of proteins and fats in metabolism, as well as to a significant decrease in the proportion of carbohydrates [9]. Fatty acids (LC), being structural elements of lipids and their derivatives, are involved in almost all the most important physiological processes, thereby ensuring the vital activity of the human body and its optimal interaction with the environment. LC are involved in the processes of synthesis and energy formation, maintaining the integrity of cell membranes, regulation of inflammatory processes, regulation of transcription and intracellular signaling. In recent years, more and more attention has been paid to the study of the role of LC as biomarkers of the functional state of the body. At the same time, there is currently insufficient information about the presence of seasonal changes in the fatty acid profile of human blood plasma adapted to the extreme conditions of the North.

The aim of this study was to identify seasonal differences in the profile of fatty acids in the blood plasma of people with evolutionarily developed mechanisms of adaptation to specific conditions of the North.

Materials and methods

A longitudinal study was conducted during three seasons (summer, autumn, and winter) in 2019. The object of the study was young aboriginal men of the North (Yakuts), practically healthy volunteers, whose average age was 19.1 ± 2.2 (n = 26). All the subjects at the time of the study had no signs of the disease and were found to be practically healthy. Venous blood sampling was carried out from 8:00 am to 9:00 am after night fasting in summer from June 21 to June 22, in autumn – September 23, in winter – December 21–22. Temperature differences in these seasons were more than 100 °C. The study was conducted in full compliance with the ethical recommendations of the Helsinki Declaration of the World Medical Association and the "Fundamentals of the Legislation of the Russian Federation on Public Health Protection" (1993).

Identification and determination of the concentration of fatty acids (FAs) in blood plasma samples was carried out by gas chromatography with mass

spectrometry (GC-MS) [10]. To obtain methyl esters of FAs, the method of acid hydrolysis was used, 100 ml of serum was placed in sealed containers, 1 ml of 2.5 % methanol H_2SO_4 solution was added and placed for one hour in a thermoshaker at 80 °C. and 1000 rpm. After cooling to room temperature (20 °C), 1 ml of 0.9 % NaCl solution was added to the resulting solution. Next, methyl esters of fatty acids were extracted with 0.5 ml of hexane. The resulting mixture was placed in a shaker for 1 min, then centrifuged for 1 min at 6.5 g. methyl esters of FAs were taken by decantation from the infusion liquid. 200 µl were taken for analysis.

Hexane extract of FAs esters was placed in the autosampler of the MAESTRO 7820/5975 chromatograph based on the Agilent 7820 gas chromatograph and the 5975 mass spectrometric detector of the same manufacturer. An HP-INNOWax capillary column (30 m, 0.25 mm, 0.25 microns) was used for separation; the velocity of the carrier gas (helium) was 2 ml/min. A non-separating insert was used to inject samples with a volume of 10 µl; the injector temperature was 2700°C. Temperature separation program: 40 °C (5 min); 2500 °C (40 °C/min, 5 min). The temperature of the line connecting the chromatograph and the mass spectrometer is 2700 °C, the temperature of the ion source is 2300 °C, the temperature of the detector is 1500 °C. Registration was carried out by full ion current (SCAN mode).

The identification of methyl esters of FAs was carried out using a set of standards for methyl esters of LC from Supelco. 37-component mixture of FAME (cat. No. 18919-1MP) and using the NIST database. The concentration of LC methyl esters was determined by the areas of chromatographic peaks of the corresponding compounds by internal normalization [2]. The total area of the peaks of methyl esters of FAs was taken as 100 % and the percentage concentration of individual FAs in relation to the total content of FAs was calculated.

All analytical measurements were carried out three times. The results of the experiment are presented in the form of the arithmetic mean and its standard deviation. The calculation was carried out using the AnalystSoft package, StatPlus – statistical analysis program, v.2007. A Principal Component Analysis (PCA) method based on the use of a web server for the analysis of metabolic data called MetaboAnalyst was used.

Results and Discussion

One of the main features of the ecological and physiological mechanisms of adaptation of the organism of the indigenous inhabitants of the North is the protein-lipid type of metabolic processes, based on the use of lipids in bioenergy processes, which ensure the economical and most optimal functioning of the body under extreme conditions. At the same time, it is known that lipids can fulfill their bioenergy function, "only by burning in the flame of a candle of carbohydrates." Therefore, we studied the seasonal changes in glucose, cholesterol and fatty acid spectrum of blood serum MY. The lowest concentration of cholesterol in the blood was noted in winter (Fig. 1). The glucose level does not significantly change.

The dynamics of the content of individual fatty acids (FAs) in the blood plasma of men of the indigenous inhabitants of the North is presented in the table. By GC-MS method, 32 FAs were identified in the blood plasma of young men. The largest percentage of all FAs is occupied by palmitic saturated fatty acid (SFA, C16:0), which ranges from 38.23 % in winter to 44.37 % in autumn.

In winter, compared with the fall, the content of saturated fatty acids (SFA) in blood serum decreases by 11.4 %, the concentration of monounsaturated fatty acids (MUSFA) increases by 6.92 % and the content of polyunsaturated fatty acids increases by 4.5 % (PUSFA) (Fig. 2A).

The results obtained indicate that in winter, in comparison with other periods, the ratio of unsaturated fatty acids (USFA) to SFA increases by 1.8 times (Fig. 2B). And the proportion of PUSFA increases approximately as many times compared with MUSFA (Fig. 2B).

A decrease in the level of SFA in the blood during the winter period indicates an increase in their use as bioenergetic raw materials. A higher content

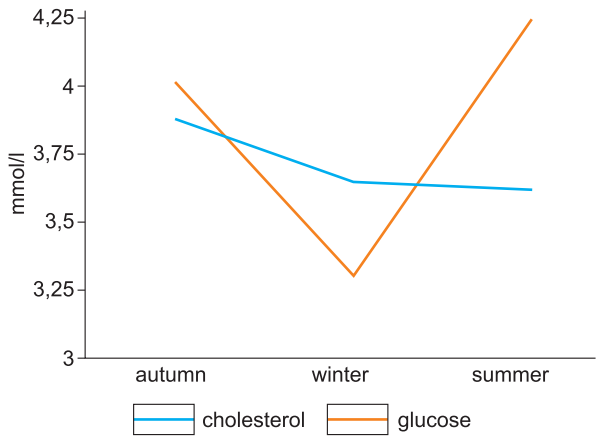

Fig. 1. Seasonal dynamics of glucose concentration and serum cholesterol level (mmol/ l)

Seasonal dynamics of the content of some fatty acids in the blood plasma of young Yakut men (% of the sum of fatty acids M±SD)

Chemical class	Fatty acids	Summer (1)	Autumn (2)	Winter (3)	
SFA	Hexanoic acid, C6:0	0.0083±0.003	0.0068±0.002	0.0031±0.001	$p_{1,3} \leq 0.0001$ $p_{1,2} \leq 0.001$
SFA	Caprylic acid, C8:0	0.0004±0.0001	0.0004± 0.0001	0.0005±0.0001	
SFA	Capric acid, C10:0	0.0013±0.0005	0.0006±0.002	0.0022±0.001	$p \leq 0.05$
SFA	Undecanoic acid, C11:0	0.0022±0.0009	0.0006±0.001	0.0032±0.001	$p_{1,2} \leq 0.001$ $p_{2,3} \leq 0.001$
SFA	Lauric acid, C12:0	0.0072±0.002	0.0117±0.003	0.0014±0.001	$p_{1,2} \leq 0.05$ $p_{1,3} \leq 0.0001$
SFA	Tridecanoic acid, C13:0	0.0038±0.001	0.0045±0.003	0.0032±0.001	
SFA	Myristic acid, C14:0	0.0052±0.002	0.0049±0.002	0.0021±0.002	$p \leq 0.05$
ω 5-MUSFA	Myristoleic acid, C14:1	0.0238±0.006	0.0188±0.006	0.1710±0.006	$p_{1,3} \leq 0.0001$ $p_{2,3} \leq 0.0001$
SFA	Pentadecanoic acid, C15:0	0.0339±0.004	0.0183±0.004	0.0066±0.001	$p = 0.001$
SFA	cis-10-Pentadecenoic acid, C15:1	0.0023±0.0001	0.0024±0.0001	0.0403±0.0001	$p_{1,3} \leq 0.0001$ $p_{2,3} \leq 0.0001$
SFA	Palmitic acid, C16:0	43.91±0.841	44.37±0.641	38.23±0.895	
ω 7-MUSFA	Palmitoleic acid, C16:1Δ7	0.0311±0.004	0.0290±0.0026	0.2512±0.062	$p_{1,3} \leq 0.0001$ $p_{2,3} \leq 0.0001$
SFA	Heptadecanoic acid, C 17:0	0.4359±0.034	0.4190±0.041	0.0088±0.003	$p_{1,3} \leq 0.0001$ $p_{2,3} \leq 0.0001$
ω 7-MUSFA	cis-10-Heptadecenoic, C 17:1Δ10	0.0016±0.0004	0.0021±0.006	0.1207±0.011	$p_{1,3} \leq 0.0001$ $p_{2,3} \leq 0.0001$
ω 6-PUSFA	γ-Linolenic acid, C 18:3Δ6,9,12	0.0077±0.001	0.0058±0.001	0.0087±0.001	
ω 6-PUSFA	Linoleic acid, C 18:2Δ9,12	13.1382±0.454	13.8441±0.231	12.1904±0.641	
ω 9-MUSFA	Oleic acid, C 18:1Δ9	4.7712±0.124	4.5493±0.655	8.8852±0.676	$p_{1,3} \leq 0.001$ $p_{2,3} \leq 0.001$
ω 3-PUSFA	Linolenic acid, C 18:3Δ3	0.1401±0.004	0.1617±0.008	0.0102±0.003	$p_{1,3} \leq 0.0001$ $p_{2,3} \leq 0.0001$
SFA	Stearic acid, C 18:0	29.9621±1.024	31.1415±1.008	26.3863±2.548	
ω 6-PUSFA	Arachidonic acid, C 20:4Δ5,8,11,14	3.7447±0.104	3.2598±0.023	3.6719±0.034	
ω 3-PUSFA	cis-5,8,11,14,17-Eicosapenoic acid, C 20:5Δ5,8,11,14,17	0.1186±0.004	0.1208±0.004	0.0365±0.001	$p_{1,3} \leq 0.0001$ $p_{2,3} \leq 0.0001$
ω 6-PUSFA	cis-8,11,14 -Eicosatrienic acid, C 22:3Δ11,14,17	0.7274±0.112	0.7026±0.104	0.0744±0.133	$p_{1,3} \leq 0.0001$ $p_{2,3} \leq 0.0001$
ω 6-PUSFA	cis-11-14-Eicosadienoic acid, C 20:2Δ11,14.	0.0596±0.008	0.0566±0.002	0.0240±0.003	$p_{1,3} \leq 0.001$ $p_{2,3} \leq 0.001$
ω 9-MUSFA	cis-11-Eicosenoic acid, C 20:1Δ11	0.0416±0.004	0.0410±0.003	0.0195±0.005	$p_{1,3} \leq 0.001$ $p_{2,3} \leq 0.001$
ω 3-PUSFA	cis-11-14-17-Eicosatrienic acid, C22:3Δ11,14,17	0.0121±0.004	0.0050±0.002	6.0082±0.001	$p_{1,3} \leq 0.0000$ $p_{2,3} \leq 0.0000$
SFA	Arachidic acid, C20:0	0.2729±0.041	0.2444±0.069	0.0071±0.0005	$p_{1,3} \leq 0.0001$ $p_{2,3} \leq 0.0001$
SFA	Heneicosanoic acid C21:0	0.4965±0.042	0.4020±0.012	0.1815±0.005	$p_{1,3} \leq 0.0001$ $p_{2,3} \leq 0.0001$
ω 6-PUSFA	cis-13,16-Docasadienoic acid, C 22:2Δ13,16	0.1466±0.004	0.0370±0.0031	0.0133±0.0024	$p_{1,3} \leq 0.0001$

Окончание таблицы

Chemical class	Fatty acids	Summer (1)	Autumn (2)	Winter (3)	
ω 9-MUSFA	Erucic acid, C 22:1Δ9	0.9796±0.098	0.2566±0.065	2.9959±0.042	$p_{1,2} \leq 0.001$ $p_{1,3} \leq 0.001$
SFA	Behenic acid, C22:0	0.7616±0.124	0.2059±0.062	0.4093±0.061	$p_{1,2} \leq 0.01$ $p_{2,3} \leq 0.05$
SFA	Tricosanoic acid, C23:0	0.0162±0.004	0.0126±0.003	0.0286±0.001	
SFA	Lignoceric acid, C24:0	0.1310±0.031	0.0624±0.002	0.2027±0.061	

* SFA – saturated fatty acid, MUFA – monounsaturated fatty acid, PUSFA – polyunsaturated fatty acid.

of PUFAs in blood plasma in winter indicates an increased need for their use as a structural component of cell membranes that provide optimal membrane viscosity for this season. A significantly higher percentage of PUSFA in winter probably provides the body of indigenous people with a sufficient amount of regulatory substances that determine the optimal functioning of the body in harsh environmental conditions.

A decrease in the "palmitic acid/oleic acid" ratio in winter indicates an increase in the involvement of oleic acid in the composition of biological membranes (Fig. 3). An increase in the concentration of oleic acid in the composition of biological membranes in the central nervous system is important for the course of nervous processes, as it provides greater conductivity, stability and strength of nervous processes.

The results of a study of seasonal changes in the content of FSA in blood plasma of young Yakut men, statistically processed by the method of principal components (PCA) are presented in Fig. 4. The first principal component (PC1) values are plotted on the abscissa axis, the variance of which reaches 44.8 % – these are fatty acids whose seasonal changes are most significant. A significant difference is visible between the content of the total AFs complex in winter and summer-autumn seasons.

For a detailed analysis of seasonal changes in the content of 32 specific FAs recorded in the blood serum of 20-21-year-old Yakut men an analysis of the results was carried out by the method of assessing the importance of variables (Fig. 5).

It was established which of the investigated FAs are included in PC1 (15 of 32; VIPscores> 1.3) and which in component 2 (4 of 32; VIPscores <0.3), which is in good agreement with the results shown in Fig. 1. It is seen that of the saturated FAs studied, the most significant role in seasonal changes is played by winter decreases (summer increases) in the contents of palmitic and stearic and tridecane FAs. Apparently, this is due to adaptive changes in the state of the lipid layer of cell membranes - a decrease in viscosity in the winter due to a decrease in the percentage of SFA and its increase in the summer due to an increase in the percentage of SFA.

Of the USFA, the most significant role is played by the winter increase in the content of 11, 14, 17-Eicosatrienoic acid and the winter decrease in the content of eicosatrienoic acid, its other deriva-

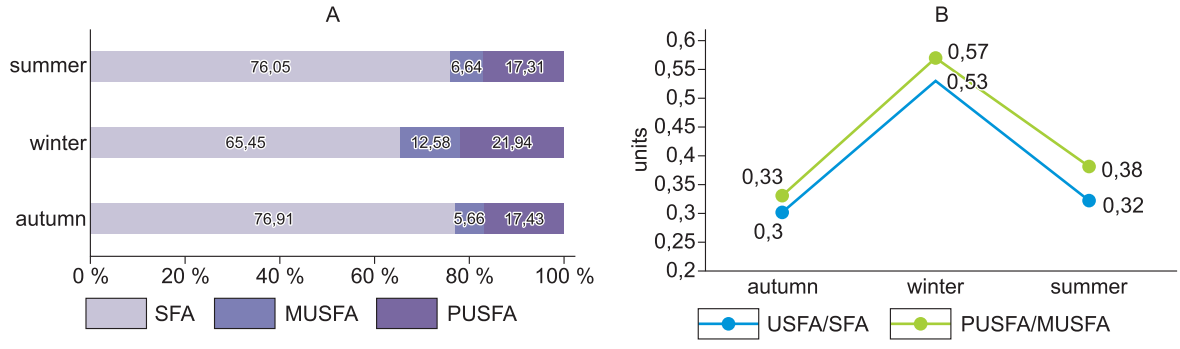

Fig. 2. (A) Seasonal dynamics of the concentration (%) of different classes of fatty acids the blood plasma of students: SFA – saturated fatty acid, MUSFA – monounsaturated fatty acid, PUSFA – polyunsaturated fatty acid; (B) The ratio of the total content of PUSFA / MUSFA in the blood plasma of the subjects in different seasons of the year (units)

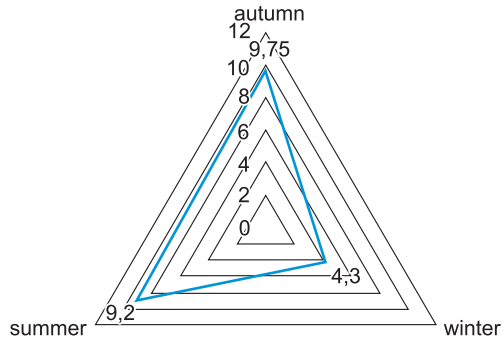

Fig. 3. The ratio of the content of palmitic acid C16:0 SFA to oleic acid C18:1 ω9-MUSFA in the studied groups of students in different seasons of the year (units)

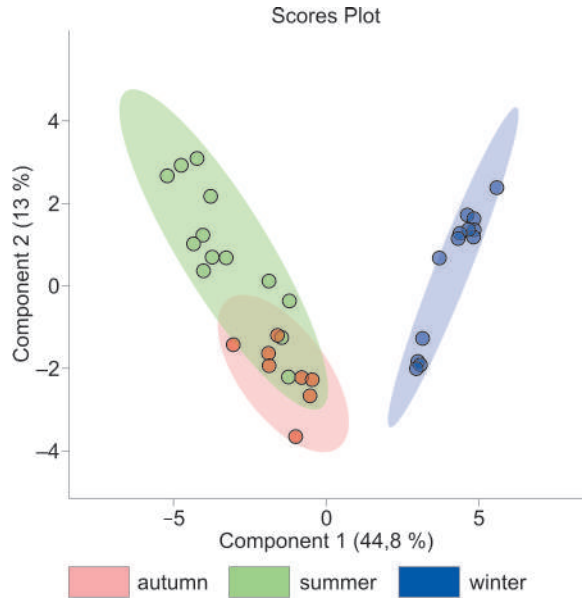

Fig. 4. Changes in the content of fatty acids in the blood serum of students depending on the seasons. The resulting image is a graphical representation of the statistical processing of the obtained data by the method of principal components (PCA) with the allocation of PC1 and PC2

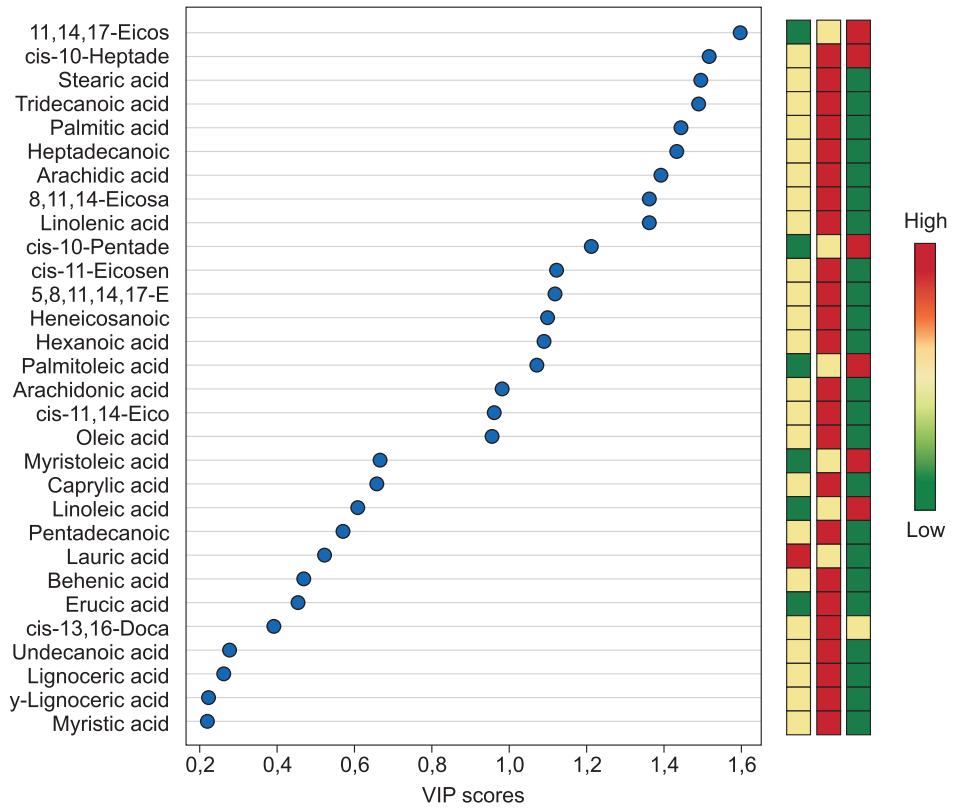

Fig. 5. The importance of projection variables for the studied plasma fatty acids of students depending on the season. Values Variable Importance in Projection (VIP) – The importance of the variable in the projection. Colored rectangles on the right indicate the trend of seasonal changes in the concentration of the corresponding component in each study group

tives (cis-11,14-Eicosadienoic acid, 8,11,14-Eicosatrienoic acid) and arachidonic acid (see fig. 5 and table). These FAs are the precursors of prostaglandins and their last group are inhibitors of the processes of elongation and saturation of other fatty acids, which is in good agreement with the winter decrease in the limiting FAs in the blood of young Yakut men.

For a more detailed assessment of the role of unsaturated FAs in the processes of seasonal adaptation of the human body in Central Yakutia, an analysis was made of the importance of the variables of various groups of unsaturated FAs. From the results presented in Fig. 3, it is seen that the winter increase in the content of omega-3 and omega-7 PUSFA and a decrease in the content of MUSFA play a leading role in adaptation to seasonal changes. This indicates the intensification of the processes of regulatory processes in the winter period, in which these polyunsaturated FAs take part.

Conclusion

Thus, there are statistically significant differences in the content of fatty acids in the blood plasma of young Yakut men in the winter and summer-autumn periods. The leading role in the adaptation of the human body to inter-seasonal changes among fatty acids is played by PUFSA, especially 11,14,17-eicosatrienic acid (omega-3), the concentration of which increases significantly in winter, which may indicate increased secretion of physiologically active substances in the human body.

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