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Metabolic Syndrome: Modification of the Fatty Acid Composition and Glucose-insulin Homeostasis

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Authors' contributions

All authors have made substantial contributions to this work. Authors YKD, TPN and MVA were responsible for the initial conception and design of the manuscript. Authors YKD and VVK wrote the protocol and first draft of the manuscript. Author NVZ performed fatty acid analysis and participated in discussion of the results. Author TAG participated in critical revision of the manuscript. Author VVK carried out a set of clinical groups and laboratory investigations. Author AVN supported literature research and final corrections of the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Objective: Metabolic syndrome is a widespread disease associated with cardiovascular pathologies and diabetes mellitus. The underlying mechanisms for development of the metabolic syndrome are currently being intensively discussed. Contradictory data regarding the role of lipid metabolism disorders in trigger mechanisms of metabolic syndrome necessitate thorough analysis

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of the content of fatty acids in plasma and blood cells. The aim of our study was to examine the content of free and esterified fatty acids and the levels of eicosanoids in metabolic syndrome with various insulin resistance. We evaluate the role of fatty acids and their metabolites in the development of metabolic syndrome.
Study Design: Cross-sectional study.
Place and Duration of Study: Vladivostok Branch of Far Eastern Scientific Center of Physiology and Pathology of Respiration – Research Institute of Medical Climatology and Rehabilitation Treatment, Russia, 2012-2013.
Materials/Methods: The study involved three groups of volunteers: 15 persons without components of the metabolic syndrome, 30 patients with metabolic syndrome and the normal insulin level, 31 patients with metabolic syndrome and diagnosed insulin resistance. We examined the levels of fasting glucose and glucose content after 2 hours of per oral glucose load, insulin, total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, eicosanoids. The content of fatty acids in plasma and erythrocytes was analyzed by gas chromatography. Statistica software was used for data analysis.
Results: We detect reciprocal changes in the content of plasma and erythrocytes fatty acids in metabolic syndrome with and without insulin resistance. Such fatty acids as 18:2n-6, 18:3n-3 are accumulating in plasma, while 18:2n-6, 18:3n-3, 20:4n-6 in erythrocytes are in deficiency. The levels of eicosanoids in metabolic syndrome are elevated.
Conclusion: Our results determined the role of fatty acids and their metabolites in the development of the metabolic syndrome. We propose the concept of metabolic syndrome according to which the trigger for the development of the metabolic syndrome is the modification of the fatty acid composition of blood cells as the result of disorders in their receptor-mediated transport.

Keywords: Metabolic syndrome; erythrocyte fatty acid composition; glucose-insulin homeostasis; eicosanoid.

ABBREVIATIONS

PUFA: polyunsaturated fatty acids; FFA: free fatty acids; HOMA-IR: homeostasis model assessment of insulin resistance; BP: blood pressure; apo B-100: apolipoprotein B-100; HDL: high-density lipoprotein; LDL: low-density lipoprotein; VLDL: very low-density lipoprotein; mLDL: modified low-density lipoprotein; TNFα: tumor necrosis factor – α; USI: unsaturation index.

1. INTRODUCTION

The metabolic syndrome has become a widely discussed scientific, medical and social problem worldwide. Indeed, definition of the metabolic syndrome is important for clinical practice and worth serious scientific and medical research. The syndrome is characterized by the following clinical criteria: abdominal obesity, reduced sensitivity of peripherical tissues to insulin and hyperinsulinemia that cause disorders of carbohydrate, lipid, and purine metabolism. This combination of metabolic abnormalities often occurs in one individual and so considerably increases a risk of cardiovascular disease and results in development of atherosclerosis and type II diabetes mellitus [1-3].

The concept of the metabolic syndrome is mainly important for detection of the patients with a high risk of cardiovascular diseases within the population. Modification of the life style and the use of the adequate medical treatment could significantly improve the health indices of such patients and to prevent the emergence or progression of atherosclerotic damage of vessels and the development of the type II diabetes mellitus [4-7].

The most researchers believe that hyperinsulinemia and/or insulin resistance are the first link in the chain of clinical-metabolic disturbances [3,8]. However, possible causes and mechanisms of the development of insulin resistance have not been so far properly studied; and moreover, not all the individual components of the metabolic syndrome can be directly associated with and explained by insulin resistance, and this, undoubtedly, raises the necessity of further research in the field. The development of the insulin resistance *per se* results from a long chain of pathological events [9]. According to many authors, insulin resistance is preceded by the deficiency of the essential

polyunsaturated fatty acids (PUFA) in cells [10-13]. Deficiency of PUFA in cells leads to alteration of the fatty acid composition of phospholipids and physical-chemical properties of plasma membranes, reduction of their fluidity, disturbance of the function of insulin receptors and transport systems of entry of glucose into the cell [14]. Disturbance in the expression of insulin receptors and decrease of their number on the membrane promote a change in the glucose-insulin homeostasis and lead to development of insulin resistance and cellular hypoglycemia. These disorders are recognized as trigger mechanisms in diabetes pathogenesis.

One of the causes of the deficiency of essential PUFA in cells can be a disturbance in their apolipoprotein B-100 (apo B-100) receptor-mediated active transport with low-density lipoproteins [15]. The main function of lipoproteins is lipid transport from the site of their synthesis (intestine, liver) to the cells of the peripheral organs. Lipoproteins exert also the so-called reverse transport of lipids (cholesterol in particular) from the cells of the peripheral organs back to the liver.

A natural consequence of the blockade of the receptor-mediated transfer of fatty acids is a compensatory increase of passive adsorption of nonesterified free fatty acids (FFA) in cells [12]. An adjustment of cells to this type of fatty acids transport activates lipolysis, enhances the insulin secretion and so enhances the potency of formation of hyperinsulinemia [10]. In its turn, hyperinsulinemia further enhances peripheral insulin resistance through violation of autoregulation of insulin receptors.

Thus, participation of saturated and PUFA in the development of widely known diseases of inner organs and their role as a physiologically necessary component is an acute problem of contemporary biomedicine. So far, there is vast debate around the particular properties of biological activity of individual fatty acids and eicosanoids that are synthesized from them within the cells.

However, we still face many open questions on the role of individual fatty acids in the development of the metabolic syndrome. Very likely, the development of insulin resistance and disorders of the fatty acid transfer and absorption occur as a result of some latent functional defects of proteins and enzymes and insufficient expression of genes. Further development of this direction in biochemistry and physiology of fatty acids will elucidate pathogenesis of many diseases.

Another negative side of depletion of the pool of physiologically important PUFAs in the cell membranes is a dysfunction of the synthesis of biologically active metabolites – eicosanoids (prostaglandins, leukotrienes, thromboxanes), which are the key regulators of the function of endothelium, immunocompetent cells, and thrombocytes [12]. Eicosanoids consitute a vast group of molecular mediators that are synthesized from arachidonic (20:4n-6), dihomo-γ-linolenic (20:3n-6) and eicosapentaenoic (20:5n-3) fatty acids under the action of the enzymes, cyclooxygenase and lipooxygenase, almost in all the cells of the body. Arachidonic acid servers as precursor of series-2 prostanoids, thromboxanes and series-4 leukotrienes. Eicosapentaenoic acid is metabolized into series-3 prostanoids and series-5 leukotrienes; and dihomo-γ-linolenic acid forms series-1 prostanoids [16].

Biological effects of eicosanoids are very diverse. They serve as secondary messengers of hydrophilic hormones, control the contraction of smooth muscles of the blood vessels, bronchs, and the uterus; influence the aggregation of platelets; stimulate chemotaxis of leukocytes and thus act as the strongest proinflammatory mediators [17]. In most cases, arachidonic acid is the primary precursor of eicosanoids, which have a higher biological activity than that of eicosanoids formed from dihomo-γ-linolenic or eicosapentaenoic acids. Consequently, the contribution of arachidonic acid metabolism products and violation of their synthesis in the pathogenesis of many diseases is obvious and significant [18].

Violation of the synthesis and misbalance of eicosanoids in the body can cause chronic inflammation, arterial hypertension, coronary heart disease, atherosclerosis, and diabetes mellitus [17]. However, we have so far no clear evidence on a pathogenic role of abnormal composition and transport of fatty acids, and on dysfunction in eicosanoids synthesis in the mechanisms of development of the metabolic syndrome. The mechanisms that trigger the cascade of pathological reactions, which lead to the development of the metabolic syndrome, have not yet been revealed.

The aim of our research is to analyze fatty acid composition of plasma and erythrocytes, as well

as eicosanoids in blood of patients with metabolic syndrome and different glucose-insuline homeostasis; to determine the relationship between metabolism of fatty acids, lipid, carbohydrate and anthropometric parameters; to determine the role of fatty acids in the development of the metabolic syndrome.

2. MATERIALS AND METHODS

All procedures undertaken in this study have been approved by The Committee on Biomedical Ethics of Vladivostok Branch of Far Eastern Scientific Center of Physiology and Pathology of Respiration – Research Institute of Medical Climatology and Rehabilitation Treatment. The study involved 76 well-informed volunteers (30 men, 46 women) aged 21–69 years. To diagnose metabolic syndrome, we used the criteria proposed by the American Association of Cardiology [19]. Taking into account the presence of the metabolic syndrome components and changes in glucose-insulin homeostasis, we divided the volunteers into the following groups: Group 1 (control) comprised 15 persons without components of the metabolic syndrome, Group 2 included 30 patients with metabolic syndrome and the normal insulin level, Group 3 consisted of 31 patients with metabolic syndrome and diagnosed insulin resistance.

The study of carbohydrate metabolism involved determination of the fasting glucose content of the blood serum and, after 2 hrs of *per oral* glucose load, measuring of the level of insulin with the use of immune-enzyme method (DRG Diagnostics Kits, Germany); calculation of the homeostasis model assessment of insulin resistance (HOMA-IR) (fasting insulin level, m Units/ml × fasting glucose level, mmol/l/22.5). The lipid spectrum of the blood serum was assessed by the blood serum contents of total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol (Lab System kits, Finnland). Low-density lipoprotein (LDL) cholesterol was calculated by Friedewald Equation and expressed in mmol/l [20]. Lipids were extracted from the blood plasma and membranes of the red blood cells using the solvent system chloroform – methanol, 1:2 (v/v), and then chloroform-methanol (1:1 v/v) and 0.9% sodium chloride were added until a complete phase separation is reached [21]. Fatty acid methyl esters were obtained by a sequential treatment of the total lipids with 1% sodium methylate/methanol and 5% HCl/methanol according to Carreau and Dubacq [22] and purified by preparative silica gel thin-layer chromatography, using the silica gel plates developed in benzene. Fatty acid methyl esters were analyzed on a Shimadzu GC-2010 (Japan) gas chromatograph equipped with a flame ionization detector, using a fused silica capillary column (Supelcowax-10, 30 m $\times$ 0.25 mm i. d. Supelco, Bellefonte, PA). Helium was used as a carrier gas at a linear velocity of 30 cm s^{-1}. The column temperature was 210ºC, injector and detector temperatures were 250ºC. Fatty acids were identified by a comparison with standard mixtures and equivalent chain length values [23]. The results were expressed in relative % of the total fatty acids. The level of eicosanoids (thromboxane B2, 6-keto-prostaglandin F1α, and leukotrien B4) in the blood was determined by an immunoenzyme analysis using the Amersham Biosciences kits (United Kingdom).

Statistica ver 6.1 (1203C for Windows) software was used for data analysis. Kolmogorov-Smirnov test was applied for testing normality of the distribution. All data were presented as mean ± standart error of the mean. The significance of a difference between two means was assessed by 'Student's' independent samples t-test. A *P*-value < 0.05 was considered statistically significant. Number and strength of correlation relationships between parameters were examined using Spearman statistics.

3. RESULTS AND DISCUSSION

In the examined patients with metabolic syndrome, we revealed apparent clinical and metabolic changes, characteristic of this syndrome complex: increased body mass index, increased waist-to-hips ratio, and elevated blood pressure (BP) (Table 1).

In Group 2 (patients with metabolic syndrome), the condition of carbohydrate metabolism was characterized by normal levels of glucose and insulin in the blood. Group 3 patients had elevated levels of glucose and insulin in the blood, the state indicating disturbances of glucose-insulin homeostasis and development of insulin resistance (Table 2).

The condition of the lipid exchange in the blood of the patients with metabolic syndrome, irrespective of the presence or absence of disturbance of the carbohydrate metabolism, was characterized by an increased content of atherogenic lipid fractions (total cholesterol, triglycerides, and LDL cholesterol (Table 3). With that, the level of antiatherogenic fraction of lipoproteins – HDL cholesterol was reduced.

Table 1. Anthropometric characteristics of patients with metabolic syndrome

Characteristics of the study participants	Group 1 (control, n = 15)	Patients with metabolic syndrome (n = 61)	
		Group 2 (without insulin resistance) n = 30	Group 3 (with insulin resistance) n = 31
Index of body weight, kg/m^2	21.97±0.65	*27.86±1.07	*31.55±1.79
Waist circumference, cm	70.45±1.55	*91.88±3.50	*95.76±3.98
Hip circumference, cm	98.63±1.88	108±2.08	*116.07±3.40
Waist-to-hip ratio	0.71±0.01	*0.84±0.02	*0.82±0.01
Systolic BP, mm Hg	105±2	*135±2	*142±2
Diastolic BP, mm Hg	65±2	*80±2	*88±2

Notes: Values are expressed as means ± SD. Statistical significance against the Group 1; P < .001*

Table 2. Characters of carbohydrate metabolism in patients with metabolic syndrome

Characteristics of the study participant	Group 1 (control) n = 15	Patients with metabolic syndrome, n=61	
		Group 2 (without insulin resistance) n = 30	Group 3 (with insulin resistance) n = 31
Fasting glucose, mmol/l	4.06±0.31	4.73±0.28	$^{\neq}$5.80±0.22
Glucose, mmol/l, after 2 hrs	4.32±0.39	5.13±0.40	*6.52±0.65
Insulin, μUnits/ml	8.08±1.04	9.48±0.93	$^{+}$18.50±2.19^{+}
HOMA-IR	1.50±0.21	1.99±0.17	$^{+}$4.50±0.66$^{\neq}$

*Notes: Values are expressed as means ±SD. On left statistical significance against the Group 1; on right statistical significance against Group 2; * P = .05; $^{\neq}$ P = .01; $^{+}$ P < .001*

Table 3. Content of lipids and lipoproteins in the blood serum in patients with metabolic syndrome

Content of lipid fractions	Group 1 (control) n = 15	Patients with metabolic syndrome, n = 61	
		Group 2 (without insulin resistance) n = 30	Group 3 (with insulin resistance) n = 31
Total cholesterol, mmol/l	4.44±0.21	$^{\neq}$5.80±0.55	$^{\neq}$5.80±0.29
Triglycerides, mmol/l	0.68±0.07	$^{\neq}$1.69±0.23	*1.79±0.14
HDL cholesterol, mmol/l	1.41±0.11	$^{\neq}$1.11±0.06	1.14±0.06$^{\neq}$
LDL cholesterol, mmol/l	2.72±0.22	$^{\neq}$4.15±0.58	*3.88±0.28

*Notes: Values are expressed as means ± SD. On left statistical significance against the Group 1; on right statistical significance against Group 2; * P = .05; $^{\neq}$ P = .01*

It is known that LDL concentration in the blood is determined by the activity of their formation from VLDL and the efficiency of the capture of LDL from the bloodstream by apo B-100 receptors [24,25]. Mechanisms facilitating the accumulation of LDL cholesterol in the blood and in the intracellular fluid can be triggered by the blockade of active accumulation of LDL in cells through apolipoprotein B-100 receptor endocytosis, as well as by inhibition of TG hydrolysis in VLDL [26]. Elevated concentrations of LDL in the blood are indicators of such disorders. There is known today at least one factor capable of inhibiting apolipoprotein B-100 ligand-receptor capture of lipoproteins: namely the acute-phase proteins that are synthesized by the liver in response to inflammation [10]. The presence of an inflammatory component in the development of the metabolic syndrome is indicated by an elevated content of proinflammatory cytokine, tumor necrosis factor-α (TNFα) in the blood: 1.6-fold increase (8,5±0,7 pg/ml, *P* < .001) in Group 2 patients and 1.4–fold increase in Group 3 patients (7,5±0,5 pg/ml, *P* < .001), compared to the control group.

Insufficient receptor activity of apolipoprotein B-100 is associated with disorders in the capture of LDL by cells and thus with lengthening of the time of their circulation in the blood and reduced income of PUFAs into the peripheral cells of organs. Disturbance in the time frame of LDL elimination from the bloodstream leads to formation of modified (oxidized) ligand-transport mLDLs, which play the role of autoantigenes and are bound by scavenger-receptors of the immune-competent cells [15]. The consequences of these disorders are accumulation of

cholesterol and cholesterol esters, triacylglycerols, and free fatty acids in the liver; deficiency of physiologically important polyunsaturated fatty acids in the cells of the peripheral organs; violation of the synthesis of vasoactive eicosanoids; permanent antigenic load supporting systemic inflammation in the body and determining the depletion of phagocytic activity of mononuclear. Slow transcytosis involving all energy substrates and deficiency of saturated fatty acids and glucose in the cells are major pathogenetic factors of metabolic syndrome development. An elevated content of lipids rich in unsaturated fatty acids in the blood stream at a simultaneous deficiency of the same unsaturated fatty acids in the cell membranes shall be recognized as a direct proof of PUFA transport disorder.

Taking into account that lipid metabolism disturbance was recorded in all the patients with the metabolic syndrome regardless of the state of carbohydrate metabolism; we can conclude that the primary link that triggers the development of insulin resistance and hyperinsulinemia is a change in the pattern of lipid metabolism. The cellular-molecular mechanism, determining at first disturbance of lipid homeostasis and later also carbohydrate metabolism, is probably a distortion of the normal fatty acid content of cell membranes. In order to confirm the above hypothesis and to establish the features of lipid metabolism in the development of the metabolic syndrome, we studied the fatty acid composition in the blood plasma and erythrocytes in patients with components of the metabolic syndrome.

Composition of FFA in the examined groups of patients included 31 individual fatty acids with the carbon chain length from C12 to C24, with both even and odd carbon atoms in the chain, of normal and branched structure, saturated, mono- and polyunsaturated. Composition of the main fatty acids of the blood plasma and the main fatty acids of erythrocyte lipids in the blood of metabolic syndrome patients is presented in Fig. 3, Table 5 and Fig. 4, Table 6 respectively.

An analysis of the composition of FFA has shown for metabolic syndrome patients of Group 2 a decline in the levels of individual saturated fatty acids: lauric (12:0), myristic (14:0, P = .05), palmitic (16:0, P = .01), and margarinic (17:0, P = .05) (Fig. 3). Among the branched acids (*iso*-), the relative content of 16:0-iso (P = .01) decreased almost thrice. On the background of the decline in the relative amounts of saturated FFAs, the content of PUFA increased. The percentages of linoleic (18:2n-6) and α-linolenic (18:3n-3) acids increased twice (P = .01), this increase was reflected also in the rise of the total content of n-6 fatty acids (Σ n-6) (Fig. 1, Table 4). The disagreement of results with another study [26] may be caused by the different length of observation and the duration of metabolic syndrome development.

The indicator of changes in the content and composition of fatty acids –the unsaturation index (USI) calculated as the sum of the products of the number of double bonds in each individual fatty acids and its relative percentage – increased with P = .05. In Group 3 of patients with metabolic syndrome and insulin resistance, the vector of changes in the composition of saturated fatty acids in the blood plasma was similar to that in Group 2. The results of the study have shown that changes in the composition of saturated fatty acids in blood plasma either in patients without disorders of glucose-insulin homeostasis or in patients with pronounced insulin resistance are rather strongly expressed, presumably, because of disorder of the transfer in the blood and absorption of saturated and PUFA by body cells. These processes can result in deficit of the essential PUFA in cells. The red blood cells are known to serve as a diagnostic criteria for assessment of different diseases [27].

Table 4. The total content of n-3 and n-6 in the plasma and the unsaturation index

Free fatty acids	Control group (Group 1), n = 11	Patients with metabolic syndrome	
		Group 2 (without insulin resistance)	Group 3 (with insulin resistance)
Σ n-6	7.77±1.71	*15.54±2.39	*11.42±2.27
Σ n-3	1.01±0.18	1.56±0.28	1.21±0.22
n-3/n-6	0.15±0.02	*0.104±0.009	0.11±0.01
USI	38.41±7.30	*65.94±9.08	49.08±8.83

*Notes: Values are expressed as means ±SD. Statistical significance against the control group; *P = .05*

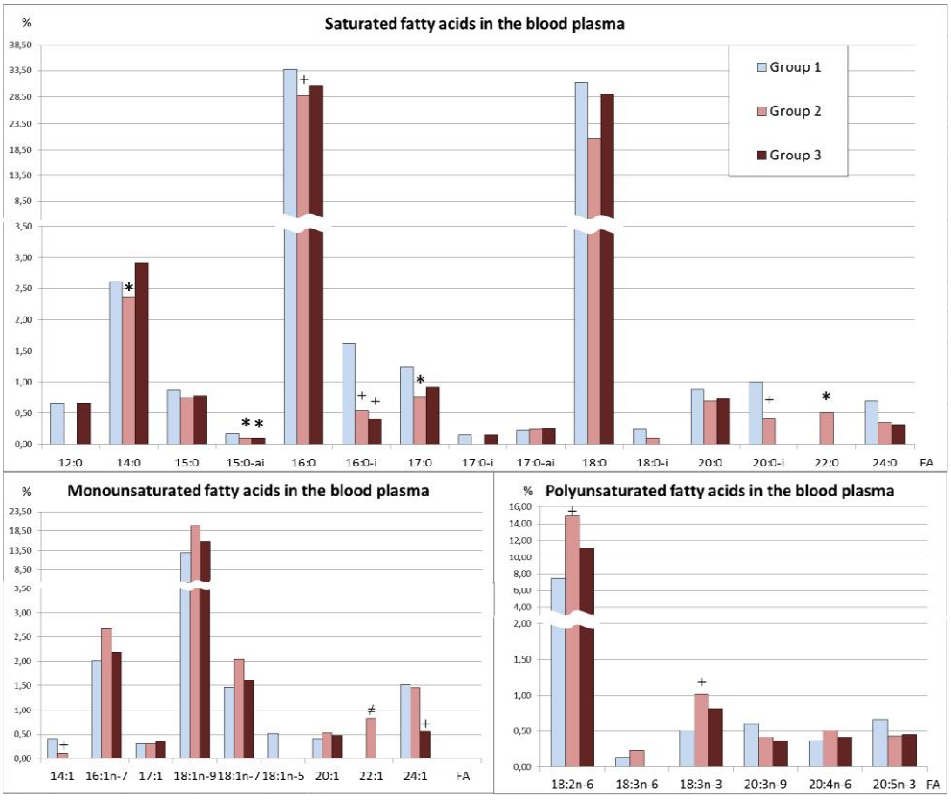

Fig. 1. Composition of free fatty acids in the blood plasma (% of the total fatty acids)
*Notes: statistical significance against the Group 1; * - P = .05; + - P = .01; ≠ - P < .001*

Table 5. The total content of n-3 and n-6 PUFA in erythrocytes and the unsaturation index

Fatty acids	Control group, n = 11	Patients with metabolic syndrome, n = 22	
		Group 2 (without insulin resistance)	Group 3 (with insulin resistance)
Σ n-6	30.1±0.6	*27.52±0.7	$^{+}$25.7±0.67
Σ n-3	9.98±0.78	9.92±0.38	9.94±0.47
USI	155.98±1.33	$^{\neq}$127.6±7.5	$^{\neq}$133.16±6.17

*Notes: Values are expressed as means ± SD. Statistical significance against the control group; * P = .05; $^{\neq}$ P = .01; $^{+}$ P < .001*

To proof the assumption above, we studied the fatty acid composition of erythrocyte lipids in patients with metabolic syndrome (Fig. 2, Table 5). In the fatty acid contents of erythrocyte lipids of patients with metabolic syndrome we revealed substantial alterations compared to the control group. In metabolic syndrome patients without insulin resistance elevated levels of arachidic (20:0). monoenoic (16:1n-7 and 17:1) and polyunsaturated (18:3n-3 and 20:4n-6) fatty acids were found.

The concentration of Mead acid (20:3n-9) was increased. It is known that compensatory synthesis of this fatty acid occurs at a lack of polyunsaturated acids of the n-6 и n-3 series [28]. In the patients of Group 3 we recorded accumulation of miristinic (14:0), arachidic (20:0) and stearic (18:0) acids on the background of a significant decrease of the contents of essential linoleic (18:2n-6), stearidonic 18:4n-3, arachidonic (20:4n-6) and 22:4n-6 PUFA, along with an increased percentage of eicosatrienoic acid (20:3n-6). Decreased values of the total Σ n-6 PUFA (*P* = .05) and USI (*P* = .01) were found in both groups of patients. This results are consistent with results obtained in other studies [12,14].

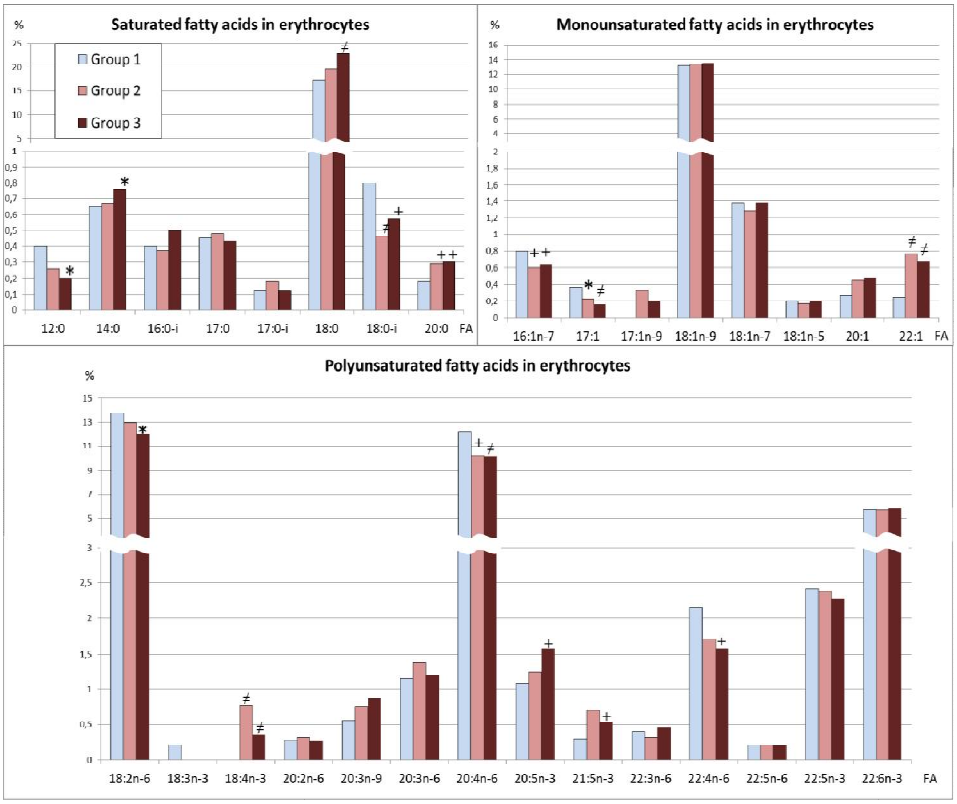

Fig. 2. Fatty acid composition of erythrocytes (% of the total fatty acids)

*Notes: Values are expressed as means ± SD. Statistical significance against the control group; * - P = .05; + - P = .01; ≠ - P < .001*

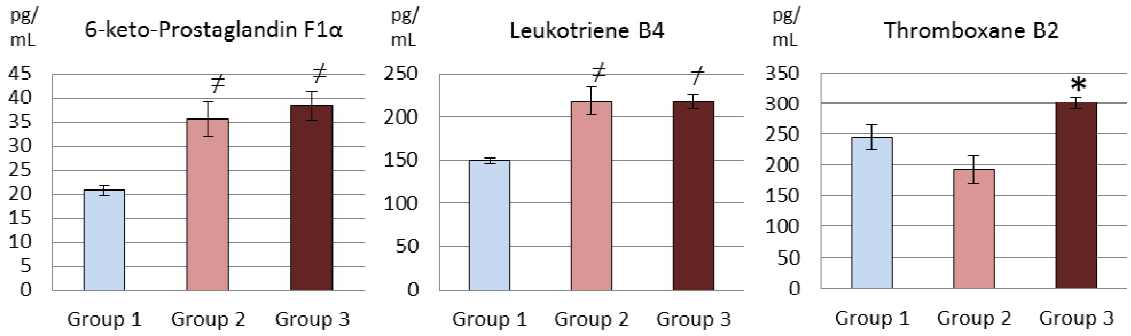

Fig. 3. Contents of eicosanoids in the blood serum

*Notes: Statistical significance against the control group; * - P = .05; ≠ - P < .001*

Our results demonstrate a modification of the fatty acid composition of the blood plasma and erythrocytes in patients with metabolic syndrome components. The reduction revealed in the concentration of arachidonic acid in the red blood cells indicates a disturbance of the eicosanoid cycle and an increased synthesis of eicosanoids with expressed vasoconstriction (thromboxane A2) and anti-inflammatory (leukotrien B4) properties.

Really, our study of the levels of eicosanoids in the blood serum of patients with metabolic syndrome has shown an increase in concentrations of 6-keto-prostaglandin F1α ($P < .001$) and leukotriene B4 ($P < .001$) in patients of Groups 2 and 3 compared to the Control group (Fig. 3). In Group 3, we detected increased levels of thromboxane B2 ($P < .001$) that were not recorded in Group 2. Elevated levels of leukotrienes, which are the most

powerful mediators of allergic and inflammatory processes, as well as high contents of TNFα in the blood of patients with metabolic syndrome, indicate an activation of inflammatory reactions. The 6-Keto-prostaglandin F1α is a powerful vasodilator in patients with metabolic syndrome; its excess level testifies a launch of compensatory mechanisms that support the preservation of the balance between the formations of pro- and anti-inflammatory eicosanoids [29].

Table 6. Correlation between fatty acids and parameters of carbohydrate and lipid metabolism and anthropometric parameters in metabolic syndrome with insulin resistance

Parameters	Coefficient of correlation
Plasma	
15:0/ LDL cholesterol	0.652; *P* = .016
15:0/ Triglyce-rides	0.651; *P* = .016
15:0/HDL cholesterol	0.560; *P* = .046
18:3n-3/Index of body weight	0.622; *P* = .023
18:3n-3/ Hip circumference	0.620; *P* = .024
20:1/ Triglycerides	-0.621; *P* = .023
20:1/ LDL cholesterol	-0.621; *P* = .023
20:3n-9/ Hip circumference	0.651; *P* = .016
20:3n-9/ Waist-to-hip ratio	0.603; *P* = .029
20:5n-3/ Triglycerides	0.565; *P* = .044
20:5n-3/ LDL cholesterol	0.565; *P* = .044
Erythrocytes	
12:0/ Fasting glucose	0.673; *P* = .023
14:0/ Insulin	0.654; *P* = .029
14:0/ HOMA-IR	0.628; *P* = .038
15:0/ Insulin	0.712; *P* = .014
15:0/ HOMA-IR	0.665; *P* = .026
16:1n-7/ Waist-to-hip ratio	0.797; *P* = .003
20:3n-9/ HDL cholesterol	-0.720; *P*= .012
20:4n-6/ HDL cholesterol	-0.604; *P* = .049
20:4n-6/ Triglycerides	-0.604; *P* = .049
20:5n-3/ Triglyceride	0.851; *P* = .034
22:4n-6/ HDL cholesterol	0.799; *P* = .003
22:5n-6/ Fasting glucose	0.612; *P* = .045

However, the attempt of the organism to maintain the state of dynamic balance between leukotrienes and prostaglandins has its limitations. These irregularities can become a crucial step in starting the mechanisms of cardiovascular diseases, diabetes mellitus, and other pathologies developing metabolic syndrome patients. It is confirmed by hyperproduction of thromboxane B2 in patients with metabolic syndrome complicated by insulin resistance, thus indicating the involvement of pathogenetic mechanisms of metabolic syndrome development, such as vasoconstriction and hypercoagulation aggravating disturbances in the function of vascular walls and enhancing cell resistance to insulin.

Table 7. Correlation between fatty acids and parameters of carbohydrate and lipid metabolism and anthropometric parameters in metabolic syndrome without insulin resistance

Parameters	Coefficient of correlation
Plasma	
18:0/ HOMA-IR	0.674; *P* = .046
18:1n-9/ HOMA- IR	-0.712; *P* = .031
18:3n-6/ Index of body weight	0.671; *P* = .048
18:3n-6/ Waist circumference	0.746; *P* = .021
18:3n-3/ Waist-to-hip ratio	0.745; *P* = .021
20:0/ Triglycerides	-0.719; *P* = .029
20:4n-6/ HOMA-IR	-0.871; *P* = .002
22:0/ Triglycerides	0.711; *P* = .032
22:0/ LDL cholesterol	0.781; *P* = .024
Erythrocytes	
12:0/ Waist circumference	-0.778; *P* = .013
12:0/ Hip circumference	-0.706; *P* = .033
15:0/ Insulin	0.840; *P* = .005
16:0/ Fasting glucose	-0.701; *P* = .035
18:0/ Insulin	-0.927; *P* = .0001
18:0/ HOMA-IR	-0.685; *P* = .042
18:0/ Index of body weight	-0.717; *P* = .029
18:1n-9/ Insulin	0.702; *P* = .035
18:1n-9/ Index of body weight	0.761; *P* = .017
18:1n-7/ Insulin	0.770; *P* = .015
18:1n-7/ HOMA- IR	0.769; *P* = .015
18:2n-6/ Fasting glucose	-0,799; *P* = 0,010
18:2n-6/ Index of body weight	0.806; *P* = .009
18:2n-6/ Waist circumference	0.763; *P* = .017
18:2n-6/ Hip circumference	0.885; *P* = .001
20:3n-9/ Triglycerides	0.842; *P* = .004
20:3n-9/ LDL cholesterol	0.842; *P* = .004
20:5n-3/ Total cholesterol	0.748; *P* = .020
20:5n-3/ LDL cholesterol	0.673; *P* = .047
22:4n-6/ Hip circumference	0.740; *P* = .023
22:6n-3/ Total cholesterol	0.677; *P* = .045

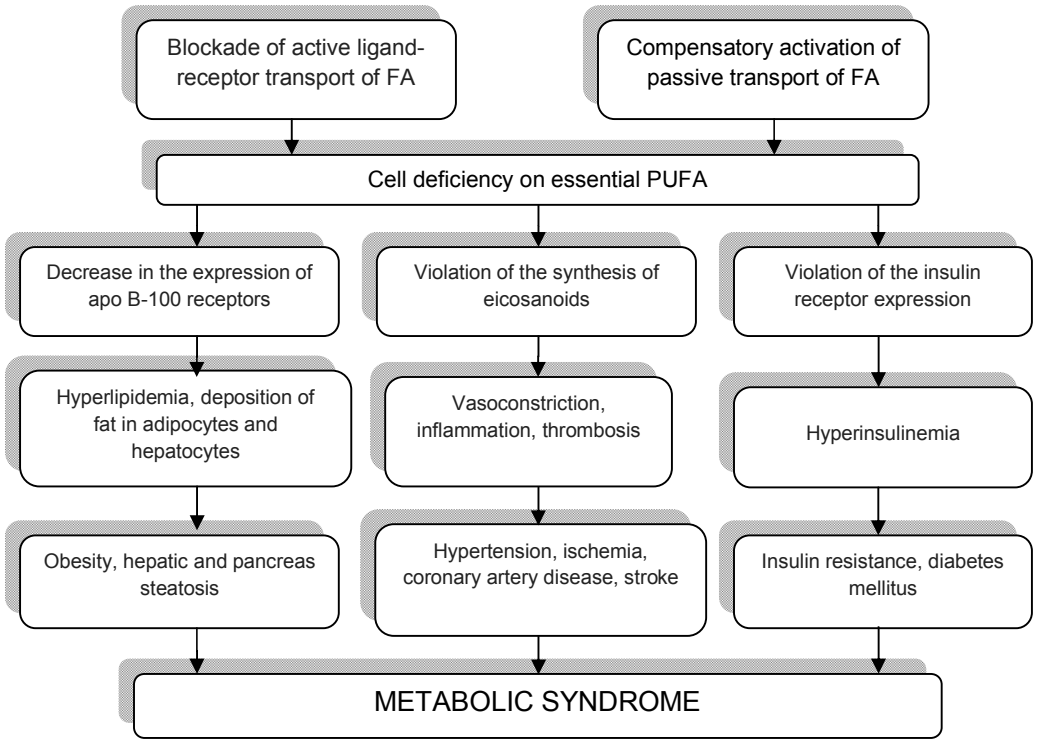

Fig. 4. Scheme of the development mechanisms of the metabolic syndrome

We examined the relationship between fatty acids and parameters of carbohydrate and lipid metabolism and anthropometric parameters to confirm the role of fatty acids and their metabolites in the development of metabolic syndrome (Tables 6, 7 above).

In metabolic syndrome without insulin resistance (Group 2) the maximum number of correlations was detected between fatty acids, parameters of carbohydrate metabolism and anthropometric parameters with priority for the fatty acids of erythrocytes. This suggests that there is a link between the fatty acids of cell membranes and the metabolic processes in metabolic syndrome. Thus, the lipid composition of the cell membrane determines the functions of membrane-embedded receptors including insulin receptors. The lack of membrane fluidity happens due to increased saturated fatty acids and decreased PUFA. This leads to impaired expression of insulin receptors and predicts insulin resistance.

In Group 3 the correlation between erythrocytes fatty acids and carbohydrate metabolism was shown (Table 7). The maximum number of connections was determined between parameters of lipid metabolism and fatty acids of plasma and erythrocytes. This confirms the changes in lipid-transport system as a factor in the development of metabolic disorders. Decreased apolipoprotein B-100 receptor activity leads to disorders in cellular uptake of LDL and, therefore, to reduced uptake of PUFA by cells in peripheral organs. Due to this, there occurs a lack of the physiologically important polyunsaturated fatty acids in the cells of the peripheral organs and impaired synthesis of eicosanoids. Therefore, the molecular mechanisms of development are linked to violations of fatty acid composition of the cell membranes and changes in fatty acids transport.

4. CONCLUSION

Thus, the development of the metabolic syndrome is associated with modification of the composition of FFA and esterified fatty acids in the blood plasma and blood cells. The proposed scheme of metabolic syndrome development mechanisms is shown in Fig. 4. The revealed modification of the fatty acid composition of plasma and blood cells can be caused by pathology of their transport resulting from the blockade of the receptor endocytosis and compensatory activation of passive fatty acid transport. Adaptation of cells to this type of fatty acid transport activates lipolysis, enhances

insulin secretion and potentiates development of hyperinsulinemia. Deficiency of endogenous PUFA in cells leads to changes in the fatty acid composition of phospholipids and physico-chemical properties of the plasma membranes, to their lower fluidity, to disturbances in the function of insulin receptors and the systems of glucose transport into the cell. Gradually, there develops mild intracellular hypoglycemia, and, by the feedback mechanism, β-cells of the Langerhans islets of the pancreas increase insulin secretion to start the development of compensatory hyperinsulinemia. Consequently, the development of insulin resistance is preceded by PUFA deficiency in cells resulting from disorders of their receptor endocytosis in LDL and activation of the passive transport of fatty acids.

The marker of the cell shift to passive absorption of PUFA resulting from the receptor blockade of lipoproteins, transporting polyunsaturated fatty acids, as well as the indicator of disturbance in transport of saturated fatty acids is hypercholesterolemia and hyperglyceridemia owing to a slow circulation rate of LDL and VLDL in the blood stream. Accumulation of VLDL in the blood enhances the lipolytic reactions, deposition of fatty acids in adipocytes, and so provokes obesity.

Disruption of the membrane structure owing to PUFA deficit leads to formation of sites with non-specific ionic channels, to reduction of the activity of membrane-bound Na, K-ATPase. The appearance of only a few channels is sufficient to start uncontrolled flow of sodium and calcium ions into and potassium ions from the cells. The resulting development of hypersodiemia and hypercaliemia of the cytosol contribute to the violation of the function of the loose connective tissues, to enhanced synthesis and secretion of collagen and elastin, to thickening of the artery walls and their reduced elasticity.

Changes in the fatty acid composition of cell membranes towards deficiency of n-3 PUFA contribute to distortion of the synthesis of eicosanoids with vasodilatation and anti-aggregation properties. These results in compensatory activation of the synthesis of eicosanoids with expressed vasoconstrictive, aggregative and pro-inflammatory properties, initiating so the development of hypertension, inflammation, and thromboses. One of the causes for changes in the fatty acid composition is disturbance of their active transport. This leads to changes in the structure of cell membranes, to reduction of functional activity of insulin-dependent glucose transporters, violation of the synthesis of eicosanoids, and to imbalance between eicosanoids with pro- and anti-inflammatory, vasoconstrictive and vasodilatation properties. A shift of the dynamic balance of the biosynthesis of cytoprotective and cytotoxic eicosanoids towards the predominance of the latter and disruption of the function of insulin receptors launch the pathogenetic mechanisms of the appearance and advance of metabolic complications, thus becoming one of the main components in the development of cardiovascular diseases, diabetes mellitus and others. A chain of consecutive disorders starting from pathology of the receptor transport of fatty acids leads to cell deficiency of the essential polyunsaturated fatty acids and to disturbance of the synthesis of eicosanoids and finally creates a vicious circle of metabolic syndrome development. The results obtained in the study distinctly demonstrate the important role of fatty acids and their metabolites in pathogenesis of the metabolic syndrome; they should be considered at elaboration and selection of appropriate preventive and therapeutic measures aimed at the prevention or elimination of the revealed disorders in the lipid transport, as well as in the cyclooxygenase and lipoxygenase enzyme systems.

CONSENT

All authors declare that written informed consent was obtained from the patient (or other approved parties) for publication of this manuscript.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by The Committee on Biomedical Ethics of Vladivostok Branch of Far Eastern Scientific Center of Physiology and Pathology of Respiration – Research Institute of Medical Climatology and Rehabilitation Treatment and have therefore been performed in accordance with the ethical standards laid down in the 2013 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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