Biomeditsinskaya Khimiya, 2023 vol. 69, issue 3, pp. 174–183.

CLINICAL-DIAGNOSTIC STUDIES

THE LIPIDOME ANALYSIS OF MOLECULAR SPECIES OF GLYCEROPHOSPHATIDYLETHANOLAMINES IN PATIENTS WITH BRONCHIAL ASTHMA COMPLICATED BY OBESITY

Yu.K. Denisenko[1], U.M. Omatova[1]*, T.P. Novgorodtseva[1], E.V. Ermolenko[2]

[1]Vladivostok Branch of the Far Eastern Scientific Center for Physiology and Pathology of Respiration - Research Institute of Medical Climatology and Rehabilitation Treatment,
73d Russkaya str., Vladivostok, 690105 Russia; *e-mail: omatova.um@inbox.ru
[2]A.V. Zhirmunsky National Scientific Center for Marine Biology,
Far Eastern Branch of the Russian Academy of Sciences, 17 Palchevsky str., Vladivostok, 690041 Russia

Bronchial asthma (BA) complicated by obesity is a progressive disease phenotype that hardly responds to standard therapy. In this regard, it is important to elucidate cellular and molecular mechanisms of development of this comorbid pathology. In recent years, lipidomics has become an active research tool, opening new opportunities not only for understanding cellular processes in health and disease, but also for providing a personalized approach to medicine. The aim of this study was to characterize the lipidome phenotype based on the study of molecular species of glycerophosphatidylethanolamines (GPEs) in blood plasma of patients with BA complicated by obesity. Molecular species of GPEs were studied in blood samples of 11 patients. Identification and quantification of GPEs was carried out using high resolution tandem mass spectrometry. For the first time in this pathology, a change in the lipidome profile of molecular species of diacyl, alkyl-acyl and alkenyl-acyl GPEs of blood plasma was shown. In BA complicated by obesity, acyl groups 18:2 and 20:4 were dominated in the sn2 position of the molecular composition of diacylphosphoethanolamines. Simultaneously with the increase in the level of GPE diacyls with the fatty acids (FA) 20:4, 22:4, and 18:2, there was a decrease in these FAs in alkyl and alkenyl molecular species of GPEs, thus indicating their redistribution between subclasses. The eicosapentaenoic acid (20:5) deficiency at the sn2 position of alkenyl GPEs in patients with BA complicated by obesity indicates a decrease in the substrate for the synthesis of anti-inflammatory mediators. The resulting imbalance in the distribution of GPE subclasses, due to a pronounced increase in the content of diacyl GPE under conditions of the deficiency of molecular species of ether forms, can probably cause chronic inflammation and the development of oxidative stress. The recognized lipidome profile characterized by the modification of the basic composition and the chemical structure of GPE molecular species in BA complicated by obesity indicates their involvement in the pathogenetic mechanisms underlying BA development. The elucidation of particular roles of individual subclasses of glycerophospholipids and their individual members may contribute to the identification of new therapeutic targets and biomarkers of bronchopulmonary pathology.

Key words: glycerophosphatidylethanolamines; ether lipids; bronchial asthma; obesity; chronic respiratory diseases; plasma

DOI: 10.18097/PBMC20236903174

INTRODUCTION

The phenotype of bronchial asthma (BA) complicated by obesity represents a global public health problem. It is known that many metabolic disorders, such as diabetes mellitus, cardiovascular diseases, metabolic syndrome, are associated with defects in lipid metabolism [1]. The phenotype of BA complicated by obesity belongs not only to the category of chronic inflammatory respiratory diseases, but also to the broader concept of a general metabolic disorder. Excessive accumulation of lipids in adipose tissue and liver impairs the balance between synthesis, degradation, oxidation, and transesterification of lipid molecules; this ultimately affects immune, antioxidant, and hormonal processes [2]. The exact cellular, molecular and genetic mechanisms for the development of the obesity-associated BA phenotype remain unclear and are likely multifactorial.

Lipidomics, including the determination of the molecular structure of complex and simple lipids, becomes a more and more popular research tool in deciphering the pathophysiological mechanisms of various diseases [3]. Identification of individual molecular types of lipids in BA can become a reliable tool for elucidating the pathogenesis of this disease, identification of biomarkers, and development of the personalized approach to therapy.

Currently, 8 main classes of lipids are known; among them glycerophospholipids (GPLs) perform structural, energy, and signal functions [3]. Glycerophosphatidylethanolamine (GPE) is the second

most abundant glycerophospholipid (after glycerophosphatidylcholine) in biological membranes. GPE is a multifunctional lipid required for many essential processes in the cell. It plays an essential role in autophagy, cell division, and protein folding. In addition, GPE is an important intermediate in the synthesis of other classes of glycerophospholipids in the Kennedy cycle [4, 5]. It has a significant impact on the membrane topology and promotes the fusion of cell membranes and organelles, oxidative phosphorylation, and mitochondrial biogenesis [6, 7]. Impaired GPE metabolism is associated with the development of Alzheimer's disease, Parkinson's disease, and non-alcoholic liver disease [8, 9].

In the human body, GPEs are characterized by a wide diversity of their molecular species, achieved by numerous combinations of fatty acid species and ways of fatty acid chain linkage to the glycerol backbone.

GPEs with an alkenyl bond are important structural and signaling components of the cell membrane and lung surfactant; they also have anti-inflammatory and antioxidant properties. It has been shown that alkenyl GPE containing a 20:4 (arachidonic) FA residue in the sn2 position reduces free radical processes, which protects the cell membrane from the oxidative damage [8].

Despite the high importance of GPE in the cellular processes of the body, there are no studies on the characterization of the molecular composition of GPE in BA complicated by obesity. The use of modern methods of lipidomics to identify the features of the composition and distribution of molecular species of certain classes of glycerophospholipids in BA complicated by obesity will help to decipher the molecular mechanisms of the pathogenesis of this disease.

The aim of this study was to investigate molecular types of plasma glycerophosphatidylethanolamines in patients with BA and with BA complicated by obesity, and to identify the molecular features of the lipidome of the BA phenotype complicated by obesity.

MATERIALS AND METHODS

The study was conducted on the basis of the Vladivostok branch of the Far Eastern Scientific Center for Physiology and Pathology of Respiration (FESC PPR), the Research Institute of Medical Climatology and Rehabilitation Treatment.

Blood plasma samples were obtained from 11 BA patients and 5 healthy volumeters. Among 11 patients with mild BA of controlled and partially controlled course (mean age 52.6±9.8 years), 5 patients had alimentary-constitutional obesity of I-II degree. Five volunteers (mean age 37.6±5.9 years) were conditionally healthy. BA was diagnosed in accordance with the criteria of the Global Strategy for the Treatment and Prevention of Bronchial Asthma (GINA, 2019) and ICD-10. Alimentary-constitutional obesity was diagnosed according to the WHO recommendations (2004). BA patients received drug inhalation therapy (budesonide/formoterol 160/4.5 μg, 2 doses per day). The criteria for inclusion in the control group of volunteers that fitted the definition of "conditionally healthy" were: the absence of respiratory pathology, chronic infectious, and non-infectious pathologies, decompensated conditions, a negative allergological anamnesis and heredity not complicated by BA and other allergic diseases.

During general clinical examination, patients' complaints, anamnesis of the disease and life, the results of a survey in order to identify risk factors for the development of respiratory pathology, and physical examination data were analyzed. We took into account the BA duration, the history and duration of obesity, the presence or absence of signs of atopy, triggers that cause deterioration of the condition, the fact and experience of smoking, and exposure to industrial pollutants.

The following anthropometric parameters were measured: height, weight, waist circumference (WC) and hip circumference (HC). The Quetelet index (QI) was calculated using the formula: QI (kg/m^2) = body weight (kg)/height (m^2). Normal body weight (NBM) corresponded to QI from 18.9 kg/m^2 to 24.9 kg/m^2, overweight — QI from 25 kg/m^2 to 29.9 kg/m^2, class I obesity — QI from 30 kg/m^2 to 34.9 kg/m^2, class II obesity — QI from 35 kg/m^2 to 39.9 kg/m^2.

National clinical guidelines and GINA 2019 were used to assess the current clinical control of BA. The Asthma Control Questionnaire (ACQ-5) was used to quantify the level of BA control. Values up to 0.75 corresponded to controlled and partially controlled course of BA, the values from 0.75 to 1.5 corresponded to the risk of exacerbations, and the values more than 1.5 corresponded to uncontrolled BA.

The molecular species of GPLs were analyzed after their preliminary extraction by the classical method of Bligh and Dyer [11] with modifications [12, 13]. Mass determination accuracy for lipid identification ranged from 2 ppm to 7 ppm. For extraction, 0.5 ml of a blood plasma sample was taken. The systems of chemically pure solvents chloroform-methanol 1:2 (v/v), then 1:1 (v/v) were used; the phases were separated by 0.9% sodium chloride solution. Separation of the lipid extract was carried out on a Shim-Pack UC-X Diol column (2.1×150 mm, particle size 3 μm) (Shimadzu, Japan) in a gradient elution system. The system A contained: *n*-hexane/2-propanol/H_2O/HCOOH/NH_4OH/Et_3N (72:28:1.5:0.1:0.05:0.02; v/v); the system B contained: 2-propanol/H_2O/HCOOH/NH_4OH/Et_3N (100:1.5:0.1:0.05:0.02; v/v). The following elution program was used: 0% B (8 min), 0% to 20% B (7 min), 20% to 100% B (5 min), 100% B (15 min),

100% to 0% B (0.1 min), 0% B (10 min). The flow rate was 0.2 ml/min. Samples were injected in a volume of 2 μl. The GPE elution time of phosphatidylethanolamines was 4–6 min.

The GPE molecular species were analyzed on a high-resolution LCMS-IT-TOF tandem mass spectrometer (Shimadzu, Japan). Registration of ions was carried out in the range of 200-1500 *m*/*z* in the electrospray ionization mode with simultaneous registration of positive and negative ions. The temperature of the ion source was 200°C, the pressure of the drying gas (nitrogen) was 140 kPa, and the flow of the spray gas (nitrogen) was 1.5 l/min. Fragmentation in tandem mass spectrometry was carried out in automatic mode with a precursor ion isolation window of 400-1000 *m*/*z*. Argon was used as the collision gas at 0.003 Pa (50% of volume). Characteristic ions for molecular species of the phosphatidylethanolamine classes were given by Imbs et al. [14]. The identification of molecular species was carried out on the basis of the mass spectra obtained during the fragmentation of phospholipid molecules. The chromatogram of total lipids and GPE mass spectra are shown in Figure 1.

Statistical processing of the results was carried out using Statistica 6.1. Quantitative values are shown as a median (Me) and an interquartile range (Q1 and Q3), where Q1 is the 25th percentile and Q3 is the 75th percentile. Data were evaluated using the Kruskal-Wallis test. Differences were considered as statistically significant at $p<0.05$. In the case of statistically significant differences, pairwise comparisons were made to identify new critical levels of significance. For a posteriori comparison, the Mann-Whitney test was used. In the case of comparing more than two groups, the Bonferroni correction was used, where the significance level was p/k (k is the number of comparisons). The critical significance level for comparison of three groups was $p=0.017$.

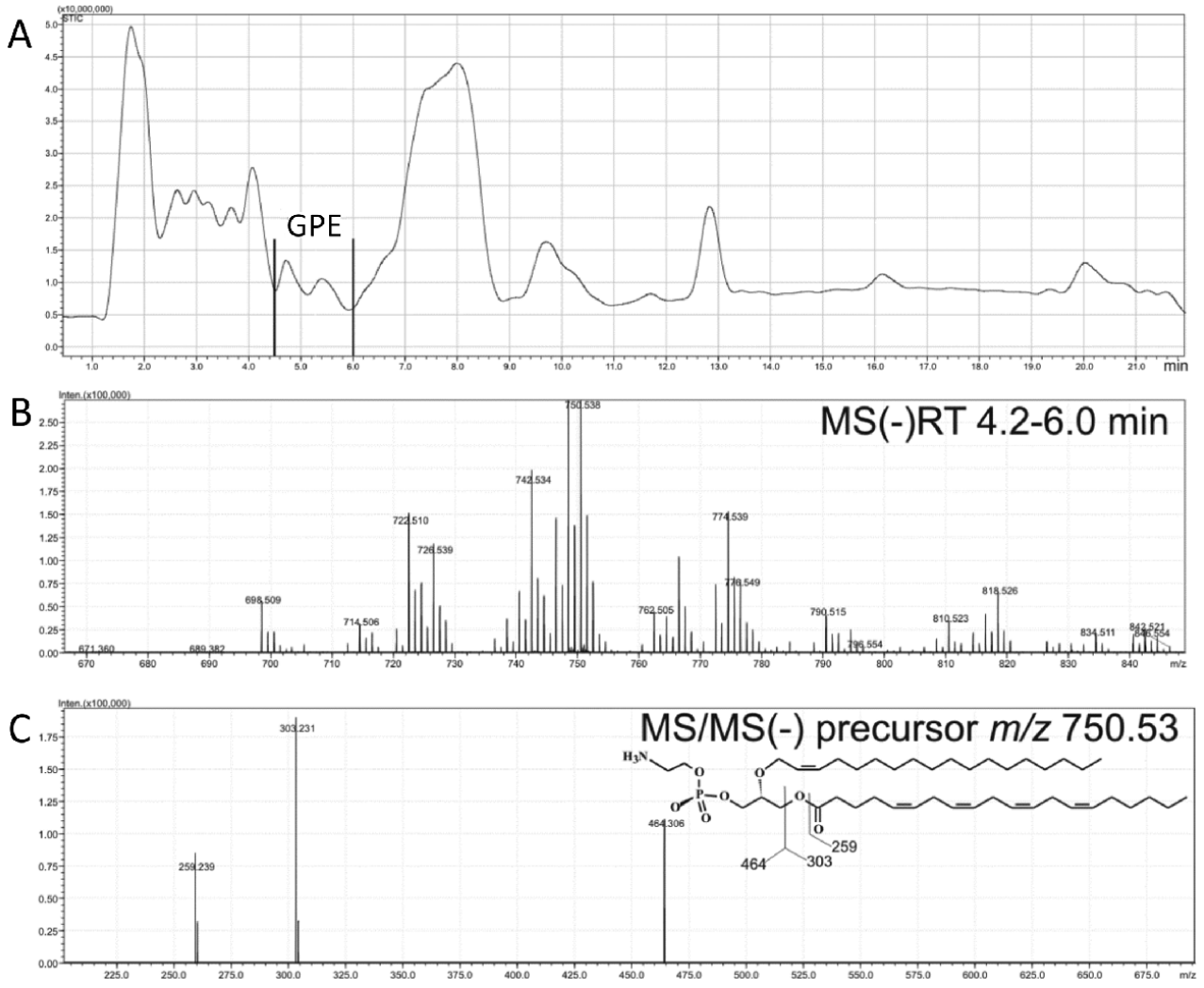

Figure 1. The chromatogram of total lipids and GPE mass spectra. (**A**) Total plasma lipids (analyzed using normal phase liquid chromatography-high resolution tandem mass spectrometry in electric field spray ionization mode. (**B**) Mass spectra for lipids eluted between 4.2-6.0 min. (**C**) The MS/MS spectrum of the 750.53 *m*/*z* precursor and the scheme of GPE P-18:0_20:4 fragmentation.

RESULTS

Forty five molecular species GPEs were identified for the first time by chromatography-mass spectrometry in the blood plasma of patients with BA complicated by obesity (Figure 2). Molecular formulas and the number of double bonds in the GPE molecules were determined on the basis of the determination of the *m*/*z* ratio of deprotonated ions with the accuracy from 2 ppm to 7 ppm.

The lipidomic profile of diacyl forms of phosphatidylethanolamines and their percentage in the blood plasma of the studied groups is shown in Table 1. Fifteen molecular species of 1,2-diacyl-sn-glycero-3-phosphoethanolamines have been identified. In BA patients, the following molecular species of diacyl GPE were increased regardless of the body weight: 16:0_18:1, 16:0_20:4, 18:0_18:2, 18:0_20:3, 18:0_20:4, 18:0_22:4. In patients with BA, the level of 18:0_20:4 diacyl GPE (Figure 2) was higher by 49% as compared with the control group; in patients with BA complicated by obesity; this GPE species was higher by 74%. Other GPE species were also increase 18:0_18:2 diacyl GPE by 71% and 90% (in BA and BA complicated by obesity, respectively); 16:0_20:4 diacyl GPE by 40% and 43%; 16:0_18:1 diacyl GPE by 66% and 76%; 18:0_20:3 diacyl GPE by 30% and 40%; 18:0_22:4 diacyl GPE by 43% and 66%, respectively. In the group of patients with BA complicated by obesity, the content of 16:0_22:6 diacyl GPE increased by 35%, 18:1_18:2 diacyl GPE by 65%, 18:0_22:4 diacyl GPE by 92% as compared with the group of BA patients with normal body weight.

The analysis showed that the blood plasma of patients with BA contained an increased level of the molecular species diacyl GPE containing 20:4 in the sn2 position. The total composition of these diacyl GPEs was higher by 49% and 60%, respectively, in patients with BA and BA complicated by obesity, as compared with the control group, while no significant differences were found between the BA phenotypes. The results obtained indicate the predominance of molecular species of phospholipids rich in arachidonic acid (20:4) in the blood plasma of patients with BA. The total parameter of molecular species of diacyl GPE esterified with the 18:2 acyl fragment at position sn2 also exceeded the values of the control group: by 69% in the group of patients with BA and by 92% in the group of patients with BA complicated by obesity.

Analysis of the chemical structure and the content of molecular species of 1-*O*-alkyl-2-acyl-sn-glycero-3-phosphoethanolamines (O-GPE, Plasmanyl GPE or alkyl GPE) in blood plasma sample of the studied groups revealed a decrease in the level of 18:1_20:5 and 18:2_20:5 alkyl GPE in BA, regardless of the body weight (Table 2). The blood plasma samples of all patients with BA contained a reduced level of molecular species carrying a fragment of eicosapentaenoic acid (20:5) in the sn2 position. The set of presented molecular species of alkyl phosphatidylethanolamines was significantly lower

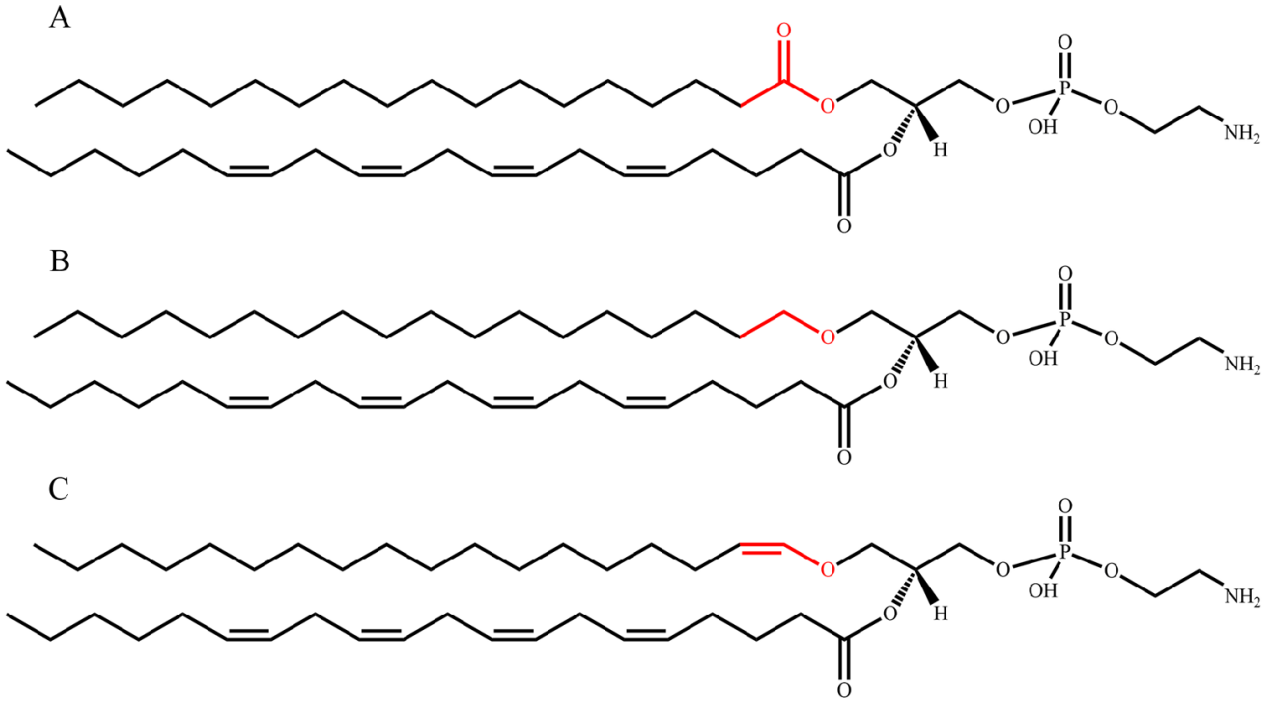

Figure 2. Molecular species of the diacyl, alkyl-acyl, and alkenyl-acyl subclasses using 18:0_20:4 GPE as an example. (**A**) 1-octadecanoyl-2-(5Z,8Z,11Z,14Z-eicosatetranoyl)-glycero-3-phosphoethanolamine or GPE (18:0_20:4(5Z,8Z,11Z,14Z)). (**B**) 1-octadecyl-2-(5Z,8Z,11Z,14Z-eicosatetranoyl)-sn-glycero-3-phosphoethanolamine or GPE (O-18:0_20:4(5Z,8Z,11Z,14Z)). (**C**) 1-(1Z-octadecenyl)-2-(5Z,8Z,11Z,14Z-eicosatetranoyl)-sn-glycero-3-phosphoethanolamine or GPE (P-18:0_20:4(5Z,8Z,11Z,14Z)).

Table 1. The profile of molecular species of diacyl phosphatidylethanolamines in blood plasma of patients with bronchial asthma

sn1_sn2	Control, %	Patients with BA and normal body weight , %	Patients with BA complicated by obesity , %
16:0_18:1	0.81 (0.79–0.81)	*1.14 (1.05–1.17)	*1.09 (1.06–1.15)
16:0_18:2	0.97 (0.75–0.99)	1.24 (0.98–1.40)	*1.54 (1.28–1.69)
16:0_20:4	1.41 (1.28–1.42)	*2.34 (2.22–2.88)	*2.49 (2.22–2.71)
16:0_22:6	1.79 (1.74–1.80)	1.80 (1.71–1.84)	* 2.43 (2.36–2.45)
18:0_18:1	0.90 (0.84–0.91)	1.00 (0.87–1.27)	0.98 (0.92–1.02)
18:0_18:2	3.71 (3.45–3.99)	*6.35 (5.62–7.18)	*7.04 (6.92–7.27)
18:0_20:3	0.29 (0.28–0.31)	*0.42 (0.36–0.48)	*0.48 (0.45–0.48)
18:0_20:4	5.06 (5.04–5.64)	*7.52 (7.09–8.24)	*8.82 (7.58–8.98)
18:0_20:5	0.31 (0.29–0.31)	0.23 (0.21–0.28)	0.29 (0.28–0.30)
18:0_22:4	0.10 (0.09–0.11)	*0.13 (0.12–0.15)	*0.25 (0.21–0.26)*
18:0_22:5	0.16 (0.15–0.17)	0.19 (0.18–0.21)	*0.21 (0.20–0.21)
18:0_22:6	1.05 (1.05–1.19)	*1.49 (1.41–1.59)	1.26 (1.14–1.29)
18:1_18:2	0.67 (0.65–0.68)	0.62 (0.58–0.67)	*1.02 (0.92–1.05)*
18:1_20:4	0.69 (0.68–0.71)	0.77 (0.71–0.85)	*0.98 (0.92–1.01)
18:1_22:6	0.15 (0.14–0.15)	*0.19 (0.18–0.24)	*0.17 (0.16–0.17)
sum sn2_20:4	7.16 (6.87–7.63)	*10.67 (10.01–12.49)	*11.42 (10.90–12.37)
sum sn2_18:2	5.07 (4.85–5.63)	*8.58 (7.79–8.78)	*9.73 (9.18–9.85)
sum diacyl GPE	18.09 (17.93–19.02)	*26.49 (25.46–27.50)	*28.95 (28.35–29.18)*

Quantitative values are shown as a median (Me) and an interquartile range (Q25–Q75). The relative content of each molecular species is given in % of the sum of all molecular species of phosphatidylethanolamines; sn1 is an acyl substituent in the first position, sn2 is an acyl substituent in the second position. Asterisks on the right indicate statistically significant differences between the phenotypes of bronchial asthma, on asterisks the left indicate statistically significant differences versus control. (*) – $p<0.017$.

by 16% and 21%, respectively, in the groups of patients with BA and BA complicated by obesity, as compared with the control group. A decrease in the level of GPE, carrying 20:5 in the position sn2, and a general increase in esterification with arachidonic acid (20:4) suggests redistribution of fatty acids towards a decrease in anti-inflammatory mediators and an increase in pro-inflammatory ones.

In both groups of patients with BA, molecular species of alkyl GPE containing acyl fragments 20:4 (16:1_20:4, 18:0_20:4, 18:1_20:4, 18:2_20:4, 20:1_20:4) and 22:6 in position sn2 (16:1_22:6, 18:1_22:6, 18:2_22:6) prevailed over other types of acyl residues. The total value of the relative content of the acyl residue 20:4 in the molecular species of alkyl GPE was 18.83% in the control group, 17.58% in the group of patients with BA and normal body weight, and 16.95% in the group of patients with BA complicated by obesity. The sum of molecular species of 1-*O*-alkyl-2-acyl-sn-glycero-3-phosphoethanolamines containing the 22:6 fragment in the sn2 position was 7.11%, 7.00%, and 6.68% in the studied groups, respectively.

The use of tandem mass spectrometry made it possible to identify 11 molecular species of 1-*O*-alkenyl-2-acyl-sn-glycero-3-phosphoethanolamines (P-GPE, alkenyl GPE, plasmenyl GPE) in the blood plasma of the studied groups (Table 3). Alkenyl phosphatidylethanolamines in the sn1 position were represented by 18:0, 16:0, and 18:1 fragments. The relative content of molecular species of the alkenyl form of phosphatidylethanolamines containing 18:0 in the sn1 position (18:0_20:4, 18:0_18:2, 18:0_22:6, 18:0_18:1, 18:0_20:5) prevailed over other molecular species and was characterized by a wide diversity of plasmenyl GPEs. This pattern was observed both in the control group and among BA patients.

In both groups of patients with BA, molecular species of alkenyl GPE containing acyl groups 20:4 (16:0_20:4, 18:1_20:4, 18:0_20:4) and 18:2 in the position sn2 (18:0_18:2, 16:0_18:2, 18:1_18:2), prevailed over other types of acyl residues. The total value of the relative content of molecular species of alkenyl ethanolamines with the acyl residue of 20:4 was 27.31% in the control group, 23.40% in the group of patients with BA and normal body weight, and 20.95% in the group of patients with BA complicated by obesity. The sum of this molecular species was lower by 14% and 23%, respectively, in the group of patients with BA and BA complicated by obesity, as compared with the control group. The sum of molecular species of alkenyl GPE containing the 18:2 fragment in the sn2 position was 8.22%, 6.86% and 6.86% in the control group, patients with BA and BA complicated by obesity, respectively.

Table 2. Profile of molecular species of alkyl-acyl phosphatidylethanolamines in blood plasma of patients with bronchial asthma

sn1_sn2	Control, %	Patients with BA and normal body weight , %	Patients with BA complicated by obesity, %
O-16:1_18:1	0.68 (0.67–0.72)	0.64 (0.62–0.78)	0.85 (0.85–0.89)
O-16:1_18:2	1.49 (1.36–1.60)	1.54 (1.41–1.75)	1.54 (1.40–1.61)
O-16:1_20:4	5.22 (4.65–5.79)	4.65 (4.52–5.05)	4.36 (4.01–4.57)
O-16:1_20:5	0.30 (0.25–0.35)	0.26 (0.24–0.29)	0.31 (0.26–0.31)
O-16:1_22:6	2.52 (2.32–2.87)	2.68 (1.96–2.94)	2.51 (2.50–2.73)
O-18:0_20:4	0.55 (0.49–0.56)	0.42 (0.39–0.46)	0.69 (0.64–0.75)*
O-18:1_18:1	0.74 (0.74–0.79)	0.80 (0.70–0.81)	0.72 (0.65–0.81)
O-18:1_18:2	3.13 (3.03–3.93)	3.15 (2.48–3.43)	2.99 (2.95–3.47)
O-18:1_20:3	1.01 (0.99–1.25)	1.03 (0.95–1.19)	1.04 (1.01–1.15)
O-18:1_20:4	6.53 (6.50–7.04)	6.13 (5.92–6.88)	5.40 (5.24–5.99)
O-18:1_20:5	0.78 (0.63–0.80)	*0.56 (0.46–0.62)	*0.52 (0.49–0.53)
O-18:1_22:5	1.39 (1.27–1.43)	1.29 (1.25–1.33)	1.37 (1.18–1.40)
O-18:1_22:6	2.12 (2.07–2.34)	2.19 (1.94–2.64)	2.14 (2.01–2.36)
O-18:2_18:2	2.00 (1.80–2.05)	1.80 (1.46–1.85)	1.62 (1.61–1.64)
O-18:2_20:4	5.94 (5.87–6.30)	5.58 (5.23–5.62)	5.65 (5.13–5.78)
O-18:2_20:5	0.80 (0.78–0.81)	*0.64 (0.62–0.67)	*0.62 (0.60–0.63)
O-18:2_22:6	2.38 (2.36–2.39)	2.04 (1.56–2.60)	1.85 (1.72–2.54)
O-20:1_18:2	0.28 (0.25–0.31)	0.32 (0.30–0.32)	0.30 (0.30–0.31)
O-20:1_20:4	0.30 (0.29–0.31)	0.26 (0.25–0.29)	0.27 (0.26–0.35)
sum alkyl GPE	38.92 (36.97–39.35)	36.46 (34.34–38.61)	*35.46 (34.72–36.36)
sum sn2_20:4	18.83 (15.86–20.13)	17.58 (16.27–18.46)	16.95 (15.35–16.96)
sum sn2_20:5	1.77 (1.51–2.05)	*1.48 (1.39–1.52)	*1.40 (1.34–1.61)
sum sn2_22:6	7.11 (6.58–7.70)	7.00 (5.26–8.25)	6.68 (6.40–7.72)

Quantitative values are shown as a median (Me) and an interquartile range (Q25–Q75). The relative content of each molecular species is given in % of the sum of all molecular species of phosphatidylethanolamines; sn1 is an alkyl substituent in the first position, sn2 is an acyl substituent in the second position. Asterisks on the right indicate statistically significant differences between the phenotypes of bronchial asthma, on asterisks the left indicate statistically significant differences versus control. (*) – $p<0.017$.

The decrease in the total content of alkyl GPE and alkenyl GPE in the groups of patients with BA and BA complicated by obesity, found under conditions of the increased level of diacyl GPE, suggests redistribution of fatty acids between GPE subclasses and a deficiency of ether forms of GPL.

The results obtained in this study are presented in Figure 3, which reflects the heatmap of the distribution of all identified molecular types of GPE in healthy individuals and patients with BA. The phenotype of BA complicated by obesity is characterized by redistribution of fatty acids between diacyl and ether subclasses of GPE.

DISCUSSION

The presented study shows changes in the lipidomic profile of the GPA molecular species in the phenotype of BA complicated by obesity. In BA complicated by obesity, the level of the following molecular types of diacyl forms of GPE significantly increased: 16:0_20:4, 18:0_18:2, 18:0_20:4, 18:1_20:4, 18:0_22:4. The molecular composition of diacylphosphoethanolamines is characterized by the predominance of acyl groups 20:4 and 18:2 in the sn2 position. The prevalence of esterification of arachidonic acid (20:4) and its precursors suggests an increase in the synthesis of pro-inflammatory eicosanoids (e.g. leukotrienes). It involves lipoxygenase (LOX) and leads to worsening of symptoms. The increased content of linoleic acid (18:2), a substrate for 12/15-lipoxygenase (12/15LOX), contributes to the active synthesis of 13-S-hydroxyoctadecadienoic acid (HODE), a powerful pro-inflammatory mediator that causes mitochondrial dysfunction, neutrophilic inflammation, provoking severe airway obstruction [15]. It is known that an increased formation of HODE contributes to an increase in the severity of BA due to a decrease in sensitivity to glucocorticoids [14].

Table 3. Profile of molecular species of alkenyl-acyl phosphatidylethanolamines in blood plasma of patients with bronchial asthma

sn1_sn2	Control, %	Patients with BA and normal body weight, %	Patients with BA complicated by obesity, %
P-16:0_18:1	0.99 (0.97–1.02)	0.97 (0.95–1.00)	0.85 (0.76–0.85)
P-16:0_18:2	2.65 (2.12–2.72)	1.68 (1.60–2.01)	2.16 (2.09–2.55)
P-16:0_20:4	8.32 (8.11–9.06)	8.19 (8.02–8.65)	7.99 (7.20–8.15)
P-16:0_22:6	3.21 (3.21–3.95)	3.02 (2.99–3.22)	3.26 (3.25–3.69)
P-18:0_18:1	0.71 (0.65–0.73)	0.68 (0.60–0.80)	0.67 (0.65–0.69)
P-18:0_18:2	3.26 (2.98–3.96)	3.00 (2.85–3.15)	2.68 (2.67–3.18)
P-18:0_20:4	8.27 (7.81–8.78)	*7.15 (6.35–7.21)	*6.21 (5.95–6.58)
P-18:0_20:5	0.65 (0.59–0.74)	0.62 (0.58–0.65)	*0.53 (0.49–0.53)*
P-18:0_22:6	2.35 (2.34–2.89)	2.32 (2.17–2.55)	2.23 (2.09–2.85)
P-18:1_18:2	2.34 (2.05–2.69)	2.14 (1.96–2.54)	1.96 (1.95–2.06)
P-18:1_20:4	9.98 (9.54–10.42)	*8.11 (7.59–8.26)	*6.85 (6.36–6.91)
sum alkenyl GPE	42.56 (42.08–45.12)	*38.14 (37.43–38.25)	*35.35 (34.93–35.99)
sum sn2_18:2	8.22 (8.05–8.76)	6.86 (6.59–7.73)	6.86 (6.72–7.55)
sum sn2_20:4	27.31 (26.04–27.44)	*23.40 (22.29–23.73)	*20.95 (19.70–21.78)
sum sn2_22:6	5.97 (5.56–6.45)	5.34 (5.18–5.54)	6.08 (5.34–6.27)

Quantitative values are shown as a median (Me) and an interquartile range (Q25–Q75). The relative content of each molecular species is given in % of the sum of all molecular species of phosphatidylethanolamines; sn1 is an alkenyl substituent in the first position, sn2 is an acyl substituent in the second position. Asterisks on the right indicate statistically significant differences between the phenotypes of bronchial asthma, on asterisks the left indicate statistically significant differences versus control. (*) – $p<0.017$.

It should be noted that a significant increase in the level of diacyl GPE from 20:4, 22:4, and 18:2 FA, is accompanied by a decrease of these FAs in alkyl and alkenyl molecular types of GPE. In BA patients, there is also a deficiency of 18:0_20:5, 18:1_20:5, 18:2_20:5 GPE ethers. GPLs with the alkenyl bond (plasmalogens) are important cellular components that act as the primary scavenger of free radicals, thereby preventing oxidation of the membrane bilayer and subsequent triggering of free radical and inflammatory reactions [16, 17]. The recognized deficiency of 1-*O*-alkenyl-2-acyl-sn-glycero-3-phosphoethanolamines can provoke chronic inflammation and the development of oxidative stress in this comorbid phenotype.

Transetherification of fatty acids between diacyl, alkyl-acyl, and alkenyl-acyl GPE in BA promotes the formation of a new pool of molecular species of phospholipids and changes in the main balance of fatty acids. Under physiological conditions, GPLs are constantly remodeled in the Kennedy and Lands cycles [18, 19]. The reactions of the Kennedy cycle regulate the balance between polar and neutral lipids, between the GPL subclasses, while the successive reactions of the Lands cycle result in transetherification of fatty acids between phospholipids and the formation of new molecular types of GPLs [20].

The molecular structure of GPEs plays a decisive role in cell signaling and mitochondrial functioning, as well as the regulation of mitochondrial dynamics in general and protein biogenesis in the outer mitochondrial membrane [21, 22]. GPE is a precursor for the synthesis of N-acylphosphatidylethanolamine with further formation of anandamide (N-arachidonoylethanolamine). It is a donor of ethanolamine phosphate in the synthesis of glycosylphosphatidylinositol anchors, which attach many signaling proteins to the plasma membrane surface [23, 24]. In addition, GPE is an important substrate for the liver enzyme phosphatidylethanolamine-N-methyltransferase, responsible for the formation of about a third of liver phosphatidylcholine. The transformation of the main composition of GPL molecular species found in our study may be the result of a rearrangement or disturbance of the enzymatic reactions of GPL metabolism in the main synthesis cycles mentioned above thus provoking changes in the blood plasma lipidome.

Thus, modification of the basic composition and chemical structure of GPE molecular species can be considered as one of the mechanisms of lipid metabolism disorders, oxidation processes, and regulation of immune responses in BA. Further study of the molecular types of GPE on larger groups of patients, as well as the study of the activity of the main enzymes of synthesis and remodeling of phospholipids, will reveal the particular role of individual subclasses of phospholipids and their molecular species in the pathogenetic mechanisms of the development of the BA phenotype complicated by obesity.

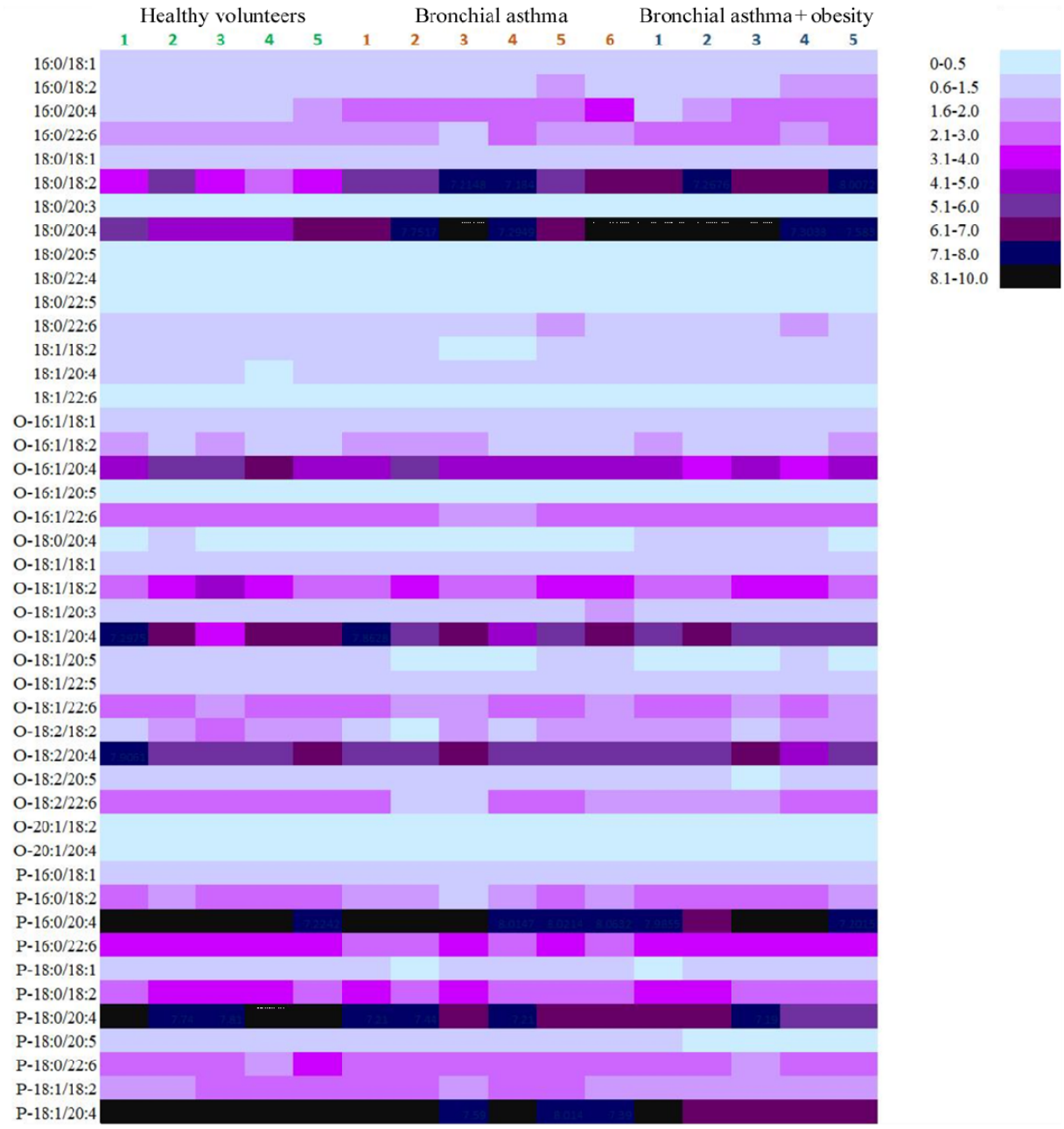

Figure 3. The heatmap of molecular species of glycerophosphatidylethanolamines in blood plasma of patients with BA and BA complicated by obesity. The heatmap shows the plasma levels of diacyl, alkyl-acyl and alkenyl-acyl glycerophosphatidylethanolamines in 5 healthy volunteers, 6 BA patients with and normal weight, 5 BA patients with obesity. The color intensity indicates the percentage of a particular molecular species in an examined person.

FUNDING

The study was performed using the Federal Budget Funds of the Russian Federation.

COMPLIANCE WITH ETHICAL STANDARDS

All procedures performed in human studies were in accordance with the ethical standards of the Institutional and/or National Research Committee, as well as the Declaration of Helsinki (1964) and its later amendments, or comparable ethical standards. The study was conducted in accordance with the requirements of the Declaration of Helsinki of the World Medical Association (2013) with the approval of the Local Ethics Committee (protocol No. 2/2021 of January 19, 2021). Informed consent was obtained from patients and volunteers participated in this study.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.

REFERENCES

1. *Nelson D.L., Cox M.M.* (2008) Lehninger Principles of Biochemistry. 5th Edition, W.H. Freeman Macmillan, New York.
2. *Denisenko Y.K., Kytikova O.Y., Novgorodtseva T.P., Antonyuk M.V., Gvozdenko T.A., Kantur T.A.* (2020) Lipid-induced mechanisms of metabolic syndrome. J. Obesity, **2020**, 5762395. DOI: 10.1155/2020/5762395
3. *Liebisch G., Fahy E., Aoki J., Dennis E.A., Durand T., Ejsing C.S., Fedorova M., Feussner I., Griffiths W.J., Köfeler H., Merrill A.H. Jr, Murphy R.C., O'Donnell V.B., Oskolkova O., Subramaniam S., Wakelam M.J.O., Spener F.* (2020) Update on LIPID MAPS classification, nomenclature, and shorthand notation for MS-derived lipid structures. J. Lipid Res., **61**(12), 1539-1555. DOI: 10.1194/JLR.S120001025
4. *Farine L., Niemann M., Schneider A., Bütikofer P.* (2015) Phosphatidylethanolamine and phosphatidylcholine biosynthesis by the Kennedy pathway occurs at different sites in *Trypanosoma brucei*. Sci. Rep., **5**(1), 16787. DOI: 10.1038/SREP16787
5. *Patel D., Witt S.N.* (2017) Ethanolamine and phosphatidylethanolamine: Partners in health and disease. Oxid. Med. Cell. Longev., **2017**, 4829180. DOI: 10.1155/2017/4829180
6. *Calzada E., Onguka O., Claypool S.M.* (2016) Phosphatidylethanolamine metabolism in health and disease. Int. Rev. Cell Mol. Biol., **321**, 29-88. DOI: 10.1016/BS.IRCMB.2015.10.001
7. *Kytikova O., Novgorodtseva T., Denisenko Y., Antonyuk M., Gvozdenko T.* (2019) Pro-resolving lipid mediators in the pathophysiology of asthma. Medicina (Kaunas, Lithuania), **55**(6), 284. DOI: 10.3390/MEDICINA55060284
8. *Bozelli J.C. Jr, Azher S., Epand R.M.* (2021) Plasmalogens and chronic inflammatory diseases. Front. Physiol., **12**, 730829. DOI: 10.3389/FPHYS.2021.730829
9. *Hatton S.L., Pandey M.K.* (2022) Fat and protein combat triggers immunological weapons of innate and adaptive immune systems to launch neuroinflammation in Parkinson's disease. Int. J. Mol. Sci., **23**(3), 1089. DOI: 10.3390/IJMS23031089
10. *Valentine W.J., Hashidate-Yoshida T., Yamamoto S., Shindou H.* (2020) Biosynthetic enzymes of membrane glycerophospholipid diversity as therapeutic targets for drug development. Adv. Exp. Med. Biol, **1274**, 5-27. DOI: 10.1007/978-3-030-50621-6_2
11. *Bligh E.G., Dyer W.J.* (1959) A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., **37**(8), 911-917. DOI: 10.1139/O59-099
12. *Carreau J.P., Dubacq J.P.* (1978) Adaptation of a macro-scale method to the micro-scale for fatty acid methyl transesterification of biological lipid extracts. J. Chromatography A, **151**(3), 384-390. DOI: 10.1016/S0021-9673(00)88356-9
13. *Christie W.W.* (1988) Equivalent chain-lengths of methyl ester derivatives of fatty acids on gas chromatography: A reappraisal. J. Chromatography A, **447**, 305-314. DOI: 10.1016/0021-9673(88)90040-4
14. *Imbs A.B., Dang L.P., Rybin V.G., Svetashev V.I.* (2015) Fatty acid, lipid class, and phospholipid molecular species composition of the soft coral *Xenia* sp.(Nha Trang Bay, the South China Sea, Vietnam). Lipids, **50**, 575-589. DOI: 10.1007/s11745-015-4021-0
15. *Panda L., Gheware A., Rehman R., Yadav M.K., Jayaraj B.S., Madhunapantula S.V., Mahesh P.A., Ghosh B., Agrawal A., Mabalirajan U.* (2017) Linoleic acid metabolite leads to steroid resistant asthma features partially through NF-κB. Sci. Rep., **7**(1), 9565. DOI: 10.1038/S41598-017-09869-9
16. *Almsherqi Z.A.* (2021) Potential role of plasmalogens in the modulation of biomembrane morphology. Front. Cell Dev. Biol., **9**, 673917. DOI: 10.3389/FCELL.2021.673917
17. *Astudillo A.M., Balboa M.A., Balsinde J.* (2022) Compartmentalized regulation of lipid signaling in oxidative stress and inflammation: Plasmalogens, oxidized lipids and ferroptosis as new paradigms of bioactive lipid research. Prog. Lipid. Res., **89**, 101207. DOI: 10.1016/j.plipres.2022.101207
18. *Gibellini F., Smith T.K.* (2010) The Kennedy pathway — *de novo* synthesis of phosphatidylethanolamine and phosphatidylcholine. IUBMB Life, **62**(6), 414-428. DOI: 10.1002/IUB.337
19. *O'Donnell V.B.* (2022) New appreciation for an old pathway: The Lands cycle moves into new arenas in health and disease. Biochem. Soc. Trans., **50**(1), 1-11. DOI: 10.1042/BST20210579
20. *Moessinger C., Klizaite K., Steinhagen A., Philippou-Massier J., Shevchenko A., Hoch M., Ejsing C.S., Thiele C.* (2014) Two different pathways of phosphatidylcholine synthesis, the Kennedy Pathway and the Lands Cycle, differentially regulate cellular triacylglycerol storage. BMC Cell Biology, **15**(1), 1-17. DOI: 10.1186/S12860-014-0043-3
21. *Annibal A., Riemer T., Jovanovic O., Westphal D., Griesser E., Pohl E.E., Schiller J., Hoffmann R., Fedorova M.* (2016) Structural, biological and biophysical properties of glycated and glycoxidized phosphatidylethanolamines. Free Rad. Biol. Med., **95**, 293-307. DOI: 10.1016/J.FREERADBIOMED.2016.03.011
22. *Basu Ball W., Neff J.K., Gohil V.M.* (2018) The role of nonbilayer phospholipids in mitochondrial structure and function. FEBS Lett., **592**(8), 1273-1290. DOI: 10.1002/1873-3468.12887
23. *Denisenko Yu.K., Bocharova N.V., Kovalenko I.S., Novgorodtseva T.P.* (2022) Influence of N-acyl-ethanolamine of arachidonic acid on the synthesis of cytokines and oxylipins by the blood leukocytes of patients with asthma under *in vitro* conditions. Bulletin of Physiology and Pathology of Respiration, **83**, 15-21. DOI: 10.36604/1998-5029-2022-83-15-21
24. *Dawaliby R., Trubbia C., Delporte C., Noyon C., Ruysschaert J.M., van Antwerpen P., Govaerts C.* (2016) Phosphatidylethanolamine is a key regulator of membrane fluidity in eukaryotic cells. J. Biol. Chem., **291**(7), 3658-3667. DOI: 10.1074/JBC.M115.706523

Received: 13. 02. 2023.
Revised: 11. 04. 2023.
Accepted: 26. 04. 2023.

ЛИПИДОМНЫЙ АНАЛИЗ МОЛЕКУЛЯРНЫХ ВИДОВ ГЛИЦЕРОФОСФАТИДИЛЭТАНОЛАМИНОВ У ПАЦИЕНТОВ С БРОНХИАЛЬНОЙ АСТМОЙ, ОТЯГОЩЁННОЙ ОЖИРЕНИЕМ

Ю.К. Денисенко[1], У.М. Оматова[1]*, Т.П. Новгородцева[1], Е.В. Ермоленко[2]

[1]Владивостокский филиал Дальневосточного научного центра физиологии и патологии дыхания — Научно-исследовательский институт медицинской климатологии и восстановительного лечения, 690105, Владивосток, ул. Русская, 73г; *эл. почта: omatova.um@inbox.ru
[2]Национальный научный центр морской биологии им. А.В. Жирмунского Дальневосточного отделения РАН, 690041, Владивосток, ул. Пальчевского 17

Бронхиальная астма (БА), отягощённая ожирением, представляет собой фенотип прогрессирующего заболевания, который трудно поддаётся стандартной терапии. В связи с этим детализация клеточно-молекулярных механизмов развития данной коморбидной патологии является актуальным направлением современных исследований. В последние годы активным исследовательским инструментом стала липидомика, открывающая ряд возможностей не только для понимания клеточных процессов в норме и патологии, но и для обеспечения персонализированного подхода в медицине. Цель исследования — охарактеризовать особенности липидома фенотипа БА, отягощённой ожирением, на основе исследования молекулярных видов глицерофосфатидилэтаноламинов (ГФЭ) плазмы крови у пациентов с данной коморбидной патологией. В образцах крови 11 пациентов исследованы молекулярные виды ГФЭ. Идентификацию и количественное определение ГФЭ проводили с использованием тандемной масс-спектрометрии высокого разрешения. Впервые при данной патологии показано изменение липидомного профиля молекулярных видов диацильных, алкил-ацильных и алкенил-ацильных ГФЭ плазмы крови. При БА, отягощённой ожирением, в молекулярном составе диацилфосфоэтаноламинов преобладают ацильные группы 18:2 и 20:4 в позиции sn2. Одновременно с повышением уровня диацилов ГФЭ с 20:4, 22:4 и 18:2 ЖК происходит уменьшение данных ЖК в алкильных и алкенильных молекулярных видах ГФЭ, что свидетельствует об их перераспределении между субклассами. Дефицит эйкозапентаеновой кислоты (20:5) в положении sn2 алкенил ГФЭ при БА, отягощённой ожирением, указывает на снижение субстрата для синтеза противовоспалительных медиаторов. Итоговый дисбаланс в процентном распределении субклассов ГФЭ, обусловленный выраженным увеличением содержания диацил ГФЭ на фоне дефицита молекулярных видов этерных форм, вероятно, может вызывать хронизацию воспаления и развитие окислительного стресса. Установленный липидомный профиль в виде модификации основного состава и химической структуры молекулярных видов ГФЭ при БА, отягощённой ожирением, свидетельствует об их важном участии в патогенетических механизмах развития БА. Раскрытие конкретной роли отдельных субклассов глицерофосфолипидов и их индивидуальных представителей может способствовать выявлению новых терапевтических мишеней и биомаркеров бронхолёгочной патологии.

Полный текст статьи на русском языке доступен на сайте журнала (http://pbmc.ibmc.msk.ru).

Ключевые слова: глицерофосфатидилэтаноламины; этерные липиды; бронхиальная астма; ожирение; хронические респираторные заболевания; плазма

Финансирование. Исследование проводено за счёт средств федерального бюджета Российской Федерации.

Поступила в редакцию: 13.02.2023; после доработки: 11.04.2023; принята к печати: 26.04.2023.