Redox Biology 76 (2024) 103309

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox

Free radical fragmentation and oxidation in the polar part of lysophospholipids: Results of the study of blood serum of healthy donors and patients with acute surgical pathology

Alexey Fedoruk [a,d], Oleg Shadyro [b,c,1], Irina Edimecheva [b,*], Dmitry Fedoruk [a], Valery Khrutskin [b,c], Leanid Kirkovsky [a], Viktor Sorokin [b,c], Halina Talkachova [a]

[a] Department of Hepatology and Minimally Invasive Surgery, Minsk Scientific and Practical Center for Surgery, Transplantology and Hematology, 220087, Semashko st., 8, Minsk, Belarus
[b] Laboratory of Chemistry of Free Radical Processes, Research Institute for Physical Chemical Problems of the Belarusian State University, 220006, Leningradskaya st., 14, Minsk, Belarus
[c] Department of Radiation Chemistry and Chemical Pharmaceutical Technologies, Belarusian State University, 220030, Nezavisimosti av., 4, Minsk, Belarus
[d] Department of Transplantology, Institute of Advanced Studies and retraining of Healthcare Personnel in Educational Institutions «Belarusian State Medical University», 220083, Dzerzhinsky Ave., 83, Minsk, Belarus

ARTICLE INFO

Keywords:
Free radical fragmentation
Lysophosphatidylcholine
Palmitoxyacetone
Stearoxyacetone
Blood serum
Septic and aseptic inflammation

ABSTRACT

The interaction of reactive oxygen species with cell membrane lipids is usually considered in the context of lipid peroxidation in the nonpolar component of the membrane. In this work, for the first time, data were obtained indicating that damage to human cell membranes can occur in the polar part of lysophospholipids at the interface with the aqueous environment due to free radical fragmentation (FRF) processes. FRF products, namely 1-hexadecanoyloxyacetone (PAc) and 1-octadecanoyloxyacetone (SAc), were identified in human serum, and a GC-MS method was developed to quantify PAc and SAc.

The content of FRF products in serum samples of 52 healthy donors was found to be in the range of 1.98–4.75 μmol/L. A linear regression equation, $C_{PAc\&SAc}$ (μmol/L) = 0.51 + 0.064 × years, was derived to describe the relationship between age and content of FRF products. In 70 patients with acute surgical pathology in comparison with the control group of healthy donors, two distinct clusters with different concentration levels of FRF products were revealed. The first cluster: groups of 43 patients with various localized inflammatory-destructive lesions of hollow organ walls and bacterial translocation (septic inflammation) of abdominal cavity organs. These patients showed a 1.5–1.9-fold ($p = 0.012$) decrease in the total concentration of PAc and SAc in serum. In the second cluster: groups of 27 patients with ischemia-reperfusion tissue damage (aseptic inflammation), – a statistically significant increase in the concentration of FRF products was observed: in 2.2–4.0 times ($p = 0.0001$).

The obtained data allow us to further understand the role of free-radical processes in the damage of lipid molecules. FRF products can potentially be used as markers of the degree of free-radical damage of hydroxyl containing phospholipids.

1. Introduction

The evolution of humans in an oxygen-rich environment has had a significant impact on redox regulation, which is crucial for cellular processes. Autoregulation systems in the human body are designed to maintain redox homeostasis, but oxidative stress can easily disrupt this balance. Oxidative stress is characterized by an imbalance between oxidants and antioxidants, which disrupts redox signaling and leads to molecular damage [1–3]. Interestingly, in various physiological and pathophysiological contexts, reactive oxygen and nitrogen species (ROS and RNS), which are commonly seen as cellular disruptors, also act as signaling molecules. Due to their production intensity and location, their roles can be varied, affecting target proteins and cellular components [4,

* Corresponding author. Research Institute for Physical Chemical Problems, Belarusian State University, 220006, Leningradskaya str. 14, Minsk, Belarus
E-mail addresses: doctorfam@mail.ru (A. Fedoruk), irina.edimecheva@gmail.com (I. Edimecheva), tetrafed@yandex.ru (D. Fedoruk), rv.khruckin@gmail.com (V. Khrutskin), kirkovsky@mail.ru (L. Kirkovsky), Sorokin@bsu.by (V. Sorokin), galina11575@gmail.com (H. Talkachova).
[1] Passed away.

https://doi.org/10.1016/j.redox.2024.103309
Received 12 July 2024; Received in revised form 3 August 2024; Accepted 11 August 2024
Available online 11 August 2024

Abbreviations

EI	Electron ionization
FRF	Free-radical fragmentation
GC-MS	Gas chromatography-linked mass spectrometry
LPLs	Lysoglycerophospholipids
LPC	Lysophosphatidylcholine
LPO	Lipid peroxidation
MAc	Margaroxyacetone
ME	Matrix effect
•OH	Hydroxyl radical
PAc/MAc/SAc	Palmitoxyacetone/Margaroxyacetone/ Stearoxyacetone
PChol	Phosphocholine
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SIM	Selected ion monitoring
SStd	Surrogate standard
TIC	Total ion charge

5]. The complex antioxidant system of the body is responsible for the regulation of ROS and RNS, with key endogenous ROS like superoxide anion, hydrogen peroxide, and hydroxyl radical, that are mainly generated in the mitochondrial respiratory chain, by NADPH oxidase (EC 1.6.3.1), and in peroxisomes. In case the cellular antioxidant defense systems are not functioning properly, the unregulated production of ROS and RNS is accompanied by an accumulation of damaged biomolecules, which often leads to the development of various pathologies [6–8].

Lipids are significant targets of ROS. Typically, lipid-ROS interactions are studied with the focus on lipid peroxidation (LPO) in hydrophobic regions of the phospholipid bilayer, where ROS attack unsaturated fatty acyl chains [9–13]. However, it is also known that free radical reactions occur in the polar part of lipid membranes. For instance, phospholipids with a free hydroxyl group near an amide or ester bond undergo fragmentation upon the exposure to ROS-producing ionizing radiation [14]. It has been shown that the polar parts of fully esterified lipids, such as phosphatidylcholine, phosphatidylethanolamine, or phosphatidylserine, are relatively resistant to radical agents, whereas hydroxyl-containing lipids (phosphatidylglycerol, phosphatidylinositol, lysolipids, and cardiolipin) are susceptible to •OH-induced degradation, forming various molecular products without oxygen involvement [15]. The pathways of free radical transformation in glycerophospholipids and sphingolipids, that involve initial lipid radical formation and subsequent decomposition, have been investigated using

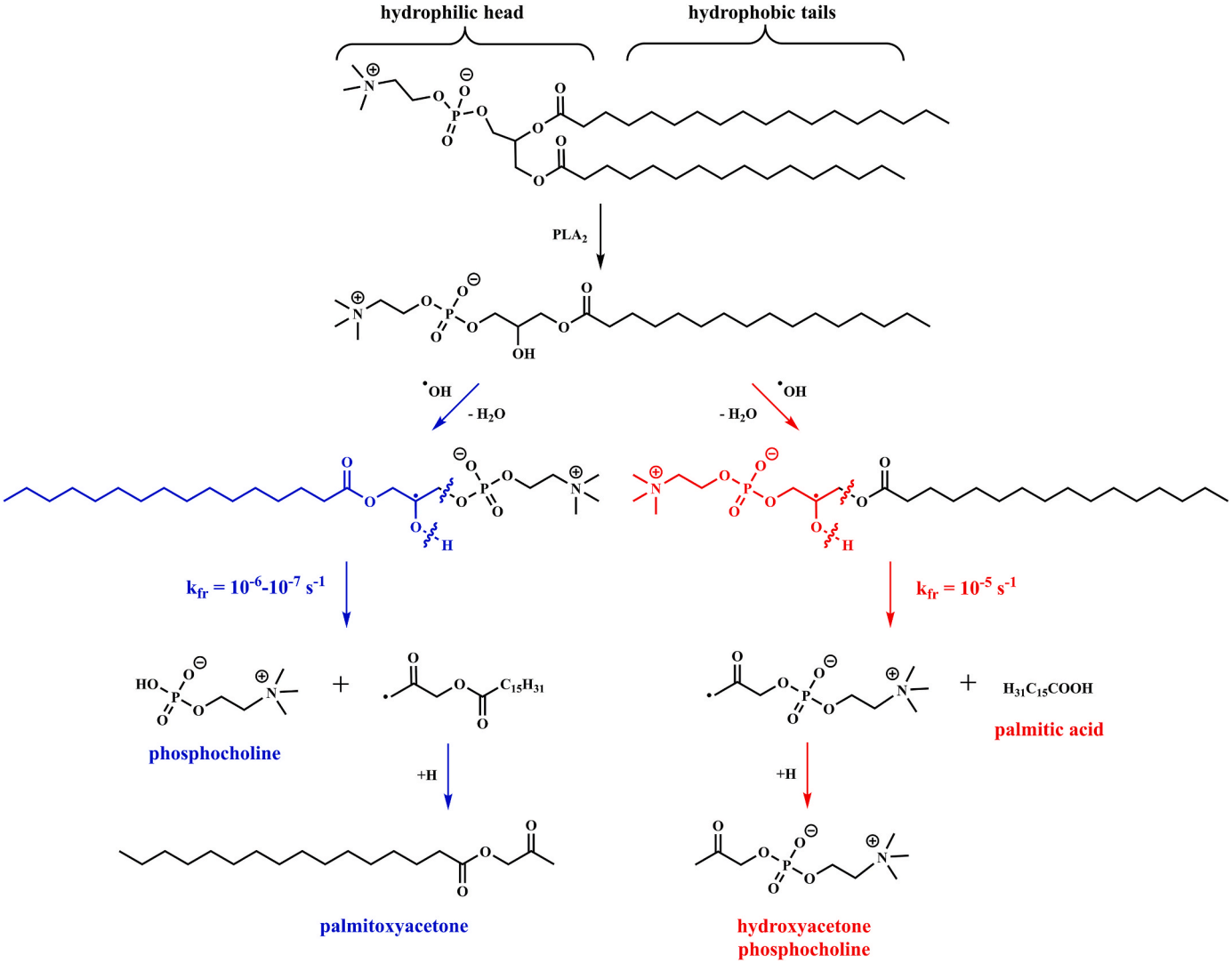

Fig. 1. Scheme of homogeneous FRF of lysoglycerophospholipids with the formation of acyloxyacetones.

chemical models and *in vitro* studies [16–21]. For instance, sphingolipids that have a free amino group form aminyl radicals, when they come into contact with ROS or chlorine species, which results in the accumulation of 2-hexadecenal [20–23]. Therefore, ROS interactions with membrane lipids can occur in both their hydrophobic (LPO) and polar parts (free radical lipid fragmentation, FRF).

Although there is an extensive research on LPO, the possibility and significance of FRF *in vivo* has not been considered. One reason for this oversight is the fact that, unlike LPO, the accumulation of FRF products is not facilitated but rather inhibited by molecular oxygen, making it a predominant process only in oxygen-deprived environments [1,14].

The FRF process can be viewed as a continuous chain of events [15,24,25]. All isoforms of the phospholipase A_2 (EC 3.1.1.4) family release a free fatty acid from the *sn*-2 position and form lysophospholipids, such as lysophosphatidylcholine [8,26], which are substrates for FRF.

The interaction between the $^{\bullet}$OH radical and the lysolipid molecule involves the removal of the most mobile hydrogen atom at the *sn*-2 position, creating a lysolipid radical at this position (Fig. 1). This lysolipid radical fragments homogeneously in two directions depending on whether the hydrogen atom of the hydroxyl group forms a hydrogen bond with the oxygen atom, either at the *sn*-1 or *sn*-3 position [19,25,27]. These processes lead to the formation of specific products such as acyloxyacetones, hydroxyacetone phosphocholine, which we observed in the study of model solutions of 2-lysophosphatidylcholine [15].

These studies focused on detecting palmitoxyacetone (PAc; 1-hexadecanoyloxyacetone) and stearoxyacetone (SAc; 1-octadecanoyloxyacetone) in human serum from healthy donors and patients with acute pathological conditions. Our results suggest that these lipid derivatives are produced *in vivo* by FRF, because there are no known enzymatic pathways for their formation. The realization of this process occurs during the functioning of cell membranes in normal conditions and also in the presence of acute surgical pathologies.

2. Materials and methods

2.1. Patient selection and characteristics

All patients were examined and treated at the Minsk Scientific and Practical Centre for Surgery, Transplantology and Hematology (MSPC STaH) from 2021 to 2022. The diagnosis and treatment of patients with acute surgical diseases were conducted in accordance with the clinical protocols [28] Methods for the quantitative determination of PAc and SAc in blood serum and the synthesis of the compounds PAc, SAc and surrogate standard (SStd) were carried out in the Research Institute for Physical Chemical Problems of the Belarusian State University. Blood samples were collected from the cubital vein of all patients before initiating treatment, following informed consent in accordance with Article 44 of the Law of the Republic of Belarus “On Health Care” as amended on June 16, 2014 No. 164-3. The ethics committee of MSPC STaH approved protocol No. 6 dated July 13, 2017 for the research. The research was conducted in accordance with the World Medical Association’s Code of Ethics for Human Subjects (Declaration of Helsinki). All patients were hospitalized for emergency surgical care for identified diseases for the first time, and there were no medical records regarding any undergoing treatment related to these diseases. Upon admission, the clarification of the medical history for patients included in the study revealed no previous use of radiation and isotope therapy, hyperbaric oxygen therapy, ozonizing solutions, and/or phospholipid-based drugs treatment.

The main criterion for the formation of research groups was the diagnosis of the patients’ diseases. 70 patients with acute surgical pathology were divided into groups based on nosology as follows: acute phlegmonous appendicitis, aged 40–50 years (N = 10); acute calculous cholecystitis, aged 43–83 years (N = 10); acute intestinal obstruction, aged 41–90 years (N = 15); acute perforation of gastric and duodenal ulcers, aged 41–70 years (N = 8); acute necrotizing pancreatitis, aged 28–59 years (N = 13); acute subdural and intracerebral hematomas, aged 42–85 years (N = 14). Table 1 describes the groups of patients with acute surgical pathologies included in the study. Serum samples were collected according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI).

All patients with acute surgical pathology, except those with acute necrotizing pancreatitis, underwent surgery within 6–24 h. In four patients with acute necrotizing pancreatitis, surgery was performed in the fifth week of illness due to the development of purulent-septic complications.

The control group was formed only from people who are in the database of regular blood donors and who undergo an annual medical examination. According to the Law of the Republic of Belarus “On donation of blood and its components” dated June 09, 2011, the upper age limit for blood donors is set at 60 years. A control group of healthy blood donors was formed after providing informed consent. This group included 52 participants aged 24–59 years. The research was performed in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

2.2. Chemicals and reagents

To conduct quantitative analysis by GC-MS, samples were prepared using methanol (CAS 67-56-1, Fisher Scientific, USA), hexane (CAS 110-54-3, Honeywell, USA), HPLC grade propan-2-ol (CAS 67-63-0, Fisher Scientific, USA), sodium chloride (CAS:7647-14-5), and nitrogen gas (CAS 7727-37-9, Eurogroup, Belarus).

Calibration solutions of PAc, SAc, and margaroxyacetone (MAc; 1-heptadecanoyloxyacetone) were prepared from synthesized compounds, with a purity of ≥98 % according to the GC analysis results. MAc was used as a surrogate standard.

2.3. Synthesis of PAc, MAc and SAc. Development of the GC-MS analytical method

Lysoglycerophospholipids constitute over 90 % of total lysolipid levels in human plasma and up to 6 % in cell membranes, with two-thirds being saturated lysophosphatidylcholine (LPC) species (C16:0 or C18:0) [48]. The reaction of LPC fragmentation is most likely to result in PAc and SAc as the products. (Fig. 1). To determine and measure FRF products in serum, we synthesized endogenous compounds and a surrogate standard. Standard and calibration solutions of PAc, MAc and SAc were prepared from the synthesized compounds, with a purity of ≥98 % confirmed by GC analysis.

2.3.1. Synthesis of palmitoxyacetone (1-hexadecanoyloxyacetone), stearoxyacetone (1-octadecanoyloxyacetone), margaroxyacetone (1-heptadecanoyloxyacetone)

PAc, SAc and MAc were prepared in good yields by DMAP-catalyzed acylation of 1-hydroxyacetone with the corresponding saturated fatty acids (Fig. 2).

PAc was prepared with palmitic acid according to the following procedure. Palmitic acid (2.56 g, 10 mmol) was dissolved in dichloromethane (DCM) (50 ml). 1-hydroxyacetone (1.74 g, 10 mmol) and N,N-dimethyl-4-amino-pyridine (DMAP) (0.15 g, 1.2 mmol) were added to this solution. N,N′-dicyclohexylcarbodiimide (DCC) (2.26 g, 11 mmol) was then added to the resulting solution for 30 min. The solution was stirred overnight at room temperature, then the precipitate was filtered and the solvent was removed under reduced pressure. The solid residue was then recrystallized from heptane. PAc was obtained (1.78 g, 57 % as white crystals), m.p. 50–51 °C. M.w. 312.48 $C_{19}H_{36}O_3$. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.92 t (3H, CH_3, *J* 7.3 Hz), 1.20–1.43 m (26H, 13CH_2), 1.65–1.76 m (2H, CH_2), 2.21 s (3H, CH_3), 2.47 t (2H, CH_2, *J* 7.2 Hz), 4.69 s (2H, CH_2).

MAc was prepared according to the procedure described above using heptadecanoic acid instead of palmitic acid. The crude product was

Table 1
Patient groups with acute surgical pathologies included in the study.

Surgical state	Group description	Pathology description	Refs.
Acutephlegmonous appendicitis	Two patients are under 40 years old, while eight patients are over 41 years old.	Acute appendicitis is an acute inflammatory-necrotic disease of the cecal process of the cecum, proceeding with the participation of microbiota (facultative and obligate anaerobes) living in the lumen of the appendix. The successive stages of the pathogenetic process include lymphoid hyperplasia, lumen obstruction, intraluminal pressure build-up, mucosal edema and ulceration, and bacterial translocation into the submucosal layer.	[29]
Acute calculous cholecystitis	Ten patients aged 43–83 years, seven of whom were aged 65–83 years.	Acute calculous cholecystitis is thought to be an acute inflammation of the gallbladder caused by obstruction of the gallbladder neck or cystic duct by a stone. The inflammatory process of the gallbladder wall is accompanied by impaired blood supply, necrosis, secondary bacterial infection, chemical stimulation and suppuration of the gallbladder contents.	[30]
Acute intestinal obstruction (Ileus)	Fifteen patients aged 41–90 years, including ten patients aged 65–90 years.	The pathogenesis of acute intestinal obstruction is based on a blockage and violation of the passage through the lumen of the intestine, an increase in intraluminal pressure and stretching of the proximal section, edema and necrotic changes in the mucosa, translocation of bacteria through the wall, activation of a local and systemic inflammatory response.	[31]
Acute perforation of gastric and duodenal ulcers	Eight patients aged 41–70 years, two patients were over 65 years old.	The basis of the pathogenesis of gastric and duodenal ulcer formation is an imbalance between the factors of aggression and protection of the mucosa. The development of an ulcer defect that penetrates beyond the muscular plate is caused by excessive production of hydrogen ions and enzymes, *H. pylori* infection, taking non-steroidal anti-inflammatory drugs, on the one hand, and a decrease in the mucous barrier, active regeneration and blood supply, on the other. The deepening of the process reaches a critical point, which is the rupture of the serous membrane and the ingress of contents into the peritoneum.	[32]
Acute necrotizing pancreatitis	Thirteen patients aged between 28 and 59 years. Male/female – 7/6, alcoholic/biliary – 12/1, moderately severe acute pancreatitis – 7, severe acute pancreatitis – 6 (Marshal 4–10), the volume of acute peri-pancreatic fluid collections from 67 to 347 cm^3, the volume of acute necrotic collections from 49 to 783 cm^3.	There is currently no clear theory of the pathogenesis of acute pancreatitis. One group of researchers supports the hypothesis of co-localization, in which intracellular lysosomes and zymogenic granules with internally ordered enzymes merge, leading to self-activation of the enzymes. According to the second hypothesis, the pathogenesis of acute pancreatitis is based on oxidative stress, where the excessive generation of ROS leads to the accumulation of LPO products, accompanied by changes in the structure and properties of cell biomembranes and subcellular structures, proteins, DNA, oxidative phosphorylation, $Ca^{2+}Na^{+}$ work ATP pumps and NF-kB translocation into the nucleus. All this occurs within 72 h and is accompanied by swelling of the gland and surrounding cellular tissue, disintegration of acini, impaired microcirculation and development of ischemia. Oxidative stress is considered to be a key mediator not only of the early local changes in the pancreatic tissue itself, but also of the associated systemic inflammatory response syndrome.	[33–43]
Acute intracranial hematomas	Fourteen patients ranging from 42 to 85 years old. Intracerebral and subdural hematomas were caused by trauma in six cases and spontaneous hemorrhage in eight cases. The volume of the hematomas ranged from 24.3 cm^3 to 126 cm^3. Cerebral ventricular rupture was found in six patients; all patients had a median brain shift of 3.8–14.3 mm.	In case of the development of intracranial hematomas, tissue damage and the formation of a compartment syndrome cause and increase edema, ischemia, hypoxia and ischemia-reperfusion injury of the brain, oxidative stress develops, accompanied by excessive production of various forms of ROS and RNS, which can cause brain tissue damage through various cellular and molecular mechanisms.	[44–47]

recrystallized from heptane, yielding white crystals, m.p. 54-55 °C M.w. 326.51 $C_{20}H_{38}O_3$. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.85 t (3H, CH_3, *J* 7.3 Hz), 1.18–1.37 m (28H, $14CH_2$), 1.59–1.68 m (2H, CH_2), 2.11 s (3H, CH_3), 2.40 t (2H, CH_2, *J* 7.2 Hz), 4.63 s (2H, CH_2).

SAc was prepared according to the procedure described above using octadecanoic acid instead of palmitic acid. The crude product was recrystallized from heptane, yielding white crystals, m.p. 58-60°C. M.w. 340.55 $C_{21}H_{40}O_3$. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.88 t (3H, CH_3, *J* 7.3 Hz), 1.16–1.38 m (30H, $15CH_2$), 1.60–1.69 m (2H,CH_2), 2.16 s (3H, CH_3), 2.41 t (2H, CH_2, *J* 7.2 Hz), 4.60 s (2H, CH_2).

2.3.2. Development of the GC-MS analytical method

2.3.2.1. Extraction of FRF from blood serum samples. The storage time for blood serum samples before the analysis was limited to 6 months at minus 60 °C. Serum samples (1.0–1.5 mL) were incubated for 30 min at room temperature. For the analysis, the samples were weighed using analytical balance (AUW120D, Shimadzu), and the sample volume was calculated using a density value of 1.024 g/mL for blood serum. To each serum sample (1.0–1.5 mL), 25 μL of 0.4 mmol/L MAc solution in propan-2-ol was added, and the molar concentration of the SStd was recalculated. To enhance interphase separation during extraction, sodium chloride was added to approximately 10 % by weight of the sample. The proteins from blood serum [49] were precipitated by adding 3 mL of methanol to the sample and thoroughly mixing the contents of the tube on a vortex for 1 min (universal vortex WiseMix VM-10, DaiHan Scientific). Samples were kept in the dark at room temperature for 2 h to complete protein precipitation. The resulting solution was centrifuged for 5 min at 5000 rpm (small centrifuge Z 206 A, Hermle Labortechnik).

The supernatant was transferred to a new tube and 1.0 mL of distilled water and then 3 mL of hexane were added to it. The extraction of acyloxyacetones was conducted for 10 min with constant stirring on a vortex. The sample was centrifuged at 5000 rpm for 1 min, and then the

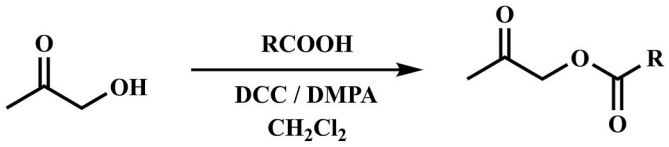

Fig. 2. General chemical synthesis scheme for acyloxyacetones (R = $C_{15}H_{31}$ for PAc, $C_{17}H_{35}$ for SAc); DCC, N,N′-dicyclohexylcarbodiimide; DMAP, 4-dimethylaminopyridine.

hexane layer was transferred to a vial. Afterwards, it was dried in a stream of nitrogen gas at 40 °C using a digital magnetic stirrer with heating (MSH-20D, DaiHan Scientific) until a lipid film was formed. The extraction with hexane was performed twice. Hexane, chosen as the extractant, yielded the most optimal results in terms of acceptable extraction of FRF products and low co-compound extraction during the method development. The lipid film was dissolved in 0.5 mL of propan-2-ol and analyzed by GC-MS.

PAc, MAc, and SAc peaks were identified by comparing the retention time and mass spectra with those of the PAc, MAc, and SAc calibration solutions. When the match factor of the spectra exceeded 800, it was concluded that PAc and SAc were present in the sample.

2.3.2.2. GC-MS with EI. Mass spectral analysis for the synthesized compounds was performed in the scan mode (scan) in the range *m/z* 50–900. The mass spectra for PAc, MAc and SAc are shown in Fig. 3.

The obtained mass spectra were utilized to identify PAc, SAc, and MAc in serum samples. The selected ion monitoring (SIM) mode was used for the quantification of FRF products. The characteristic ions were *m/z* 98, 116 and 239, 253, 267, respectively (Fig. 3). The chromatographic peak areas for PAc, MAc and SAc were calculated using the total ion current (TIC) signal and the most characteristic ion with *m/z* 116 (the determinant ion).

A GCMS-QP2010 Plus system (Shimadzu, Japan) was utilized for conducting the GC-MS analysis with an electron ionization (EI). Chromatographic separation of FRF products was performed using an RTX-65TG column (30 m × 0.25 mm × 0.10 μm: carrier gas, helium; flow rate, 0.96 mL/min; thermostat temperature from 100°C to 350°C at a rate of 10°C/min; injector temperature, 330°C; ion source temperature, 200°C; volume of injected sample - 1 μL; type of ionization - electron impact, electron energy 70 eV; detector voltage, 1.5 kV. Release time 21.9 ± 0.1 min for PAc, 23.4 ± 0.1 min for MAc (SStd) and 24.9 ± 0.1 min for SAc (Fig. 4A). The data was acquired through scan mode (*m/z* 50–900) and SIM mode. The calibration solution in propan-2-ol chromatogram is shown in Fig. 4A. To assess whether the previous sample affected the detector response, a solution of SStd in propan-2-ol was chromatographed after each serum sample to account for sample preparation losses and control of the baseline drift. This was taken into account when calculating the concentration of FRF products in the serum sample.

A blood serum sample was processed as described in 2.3.2.1. Fig. 4B shows the authentic matrix chromatogram without the addition of SStd. All serum samples had PAc and SAc present. This made quantification difficult due to the lack of a blank matrix. MAc was used as the SStd to solve this problem. (Fig. 4C). This compound is a homologue of PAc and SAc. MAc undergoes the same matrix effect as the target FRF products, has similar physicochemical properties and is not present in the authentic matrix. To use the surrogate standard method, a calibration curve must be created with the authentic matrix. It's important to verify if the matrix effects experienced by the authentic and surrogate analytes are identical. To ensure that this is the case, the parallelism of the calibration curves can be checked.

2.3.2.3. Preparation of calibration standards, authentic matrix, and relative response factor calculation. To prepare basic calibration solutions, weighed portions of PAc, MAc, and SAc were dissolved in propan-2-ol (surrogate matrix). Linearity was evaluated near the endogenous concentrations in the samples. In our case, the concentration of FRF products in samples ranged from 1.0 to 10.0 μmol/L. Calibration solutions of PAc, MAc, and SAc for concentrations of 1.0, 1.5, 2.0, 4.0, 8.0, and 10.0 μmol/L were prepared by serial dilutions. Authentic calibration matrix solutions were prepared in propan-2-ol from lipid extract of healthy human serum obtained as described in section 2.3.2.1. PAc and SAc were added to the matrix in equal concentrations. The GC-MS signal was recorded in SIM mode with *m/z* 98, 116, 239, 253, 267.

By subtracting the peak area of the authentic matrix from the peak area of the authentic matrix with added analyte, the concentration of PAc, SAc was determined (Fig. 5B).

As can be seen from the data obtained (Table 2), the percentage differences in ME [50,51] show equivalence for all analytes and increase the detector response by an average of 4 %.

The use of SStd to quantify endogenous compounds in biological samples requires a single-point internal standardization. Single-point internal standardization has the same limitations as normal calibration. To construct a calibration curve of the internal surrogate standard, a series of standards were prepared, each containing the same concentration of MAc and different concentrations of PAc and SAc (Fig. 5C). The dependence of the area ratio on the concentration of the analyte is described by linear regression equations as follows $S_{PAc}/S_{MAc} = -0.07 + 1.56E5 \times C_{PAc}$; $S_{SAc}/S_{MAc} = -0.08 + 1.09E \times C_{SAc}$. The square of the correlation is $r^2 = 0.998$ for PAc and $r^2 = 0.995$ for SAc. Based on the data obtained (Fig. 5A–C; Table 1) within the analyzed concentration range of the analytes, we can be conclude that a developed method allows a reliable determination of FRF products concentration in serum. The concentrations can be calculated using the following formula:

$$C_{PAc\&SAc} = S_{Ac} \times \rho_x \times C_{MAc} \times {}^{103} \times RRF / (S_{MAc} \times m \times E_{MAc})$$

Where S_{Ac} is area of chromatographic peaks PAc or SAc in a sample, units of peak area.

S_{MAc} is area of chromatographic peak MAc in a sample, units of peak area;
ρ_x is value of the density of blood serum in a sample, taken as 1.024 for all analyzed samples, g/mL;
C_{MAc} – final concentration of margaroxyacetone in samples after extraction, μmol/L;
m – sample weight, g;
10^3 – coefficient for converting milliliters to liters;
E_{MAc} – percent extraction of margaroxyacetone, relative units;
RRF – correction factor for PAc or SAc, set according to the calibration schedule, relative units.

The developed method, utilizing MAc as SStd, makes it possible to evaluate the following: the content of FRF products in blood serum, the efficiency of extraction, the detector performance and method accuracy.

2.4. Statistical analysis

Statistical analysis was performed using StatSoft Statistica 12 software. All data analyzed in this study were expressed as mean ± standard deviation. The total content of FRF products most fully reflects the intensity of FRF processes of lysoglycerophospholipids, with PAc and SAc being the most probable products, in accordance with the percentage composition of fatty acids in blood plasma lipids [52]. One-way analysis of variance (ANOVA) with Tukey's post hoc test and Mann-Whitney rank test were used for comparison of multiple groups. $P < 0.05$ was considered statistically significant.

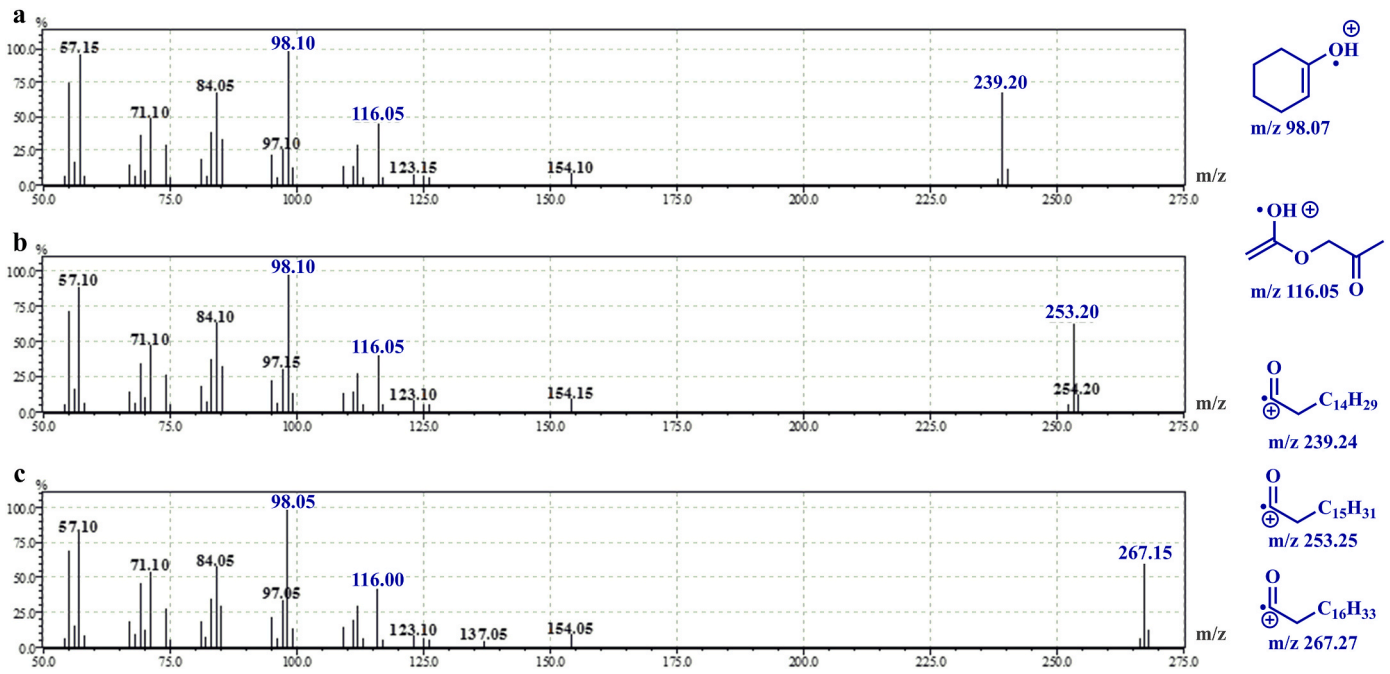

Fig. 3. The mass spectra of PAc (a), MAc (b) and SAc (c) in a calibration solution with a FRF products concentration of 10.0 μmol/L in the scan mode and the structures of the characteristic ionic fragments.

3. Results

3.1. Concentration of palmitoxyacetone and stearoxyacetone in blood serum of healthy donors

The results of determining the total concentration of PAc and SAc in the blood serum in relation to a group of healthy blood donors aged 24–59 years (N = 52) are presented in the histogram (Fig. 6) and a Gaussian Kernel density estimation curve. The histogram has two modes in the concentration ranges of 1.61–1.89 μmol/L and 3.55–3.88 μmol/L, indicating the absence of a normal distribution of data in the sample (Shapiro–Wilk test, p-value < 0.05).

The reason for the non-parametric distribution of the data was determined using known indicators from the blood donor medical records. The data were sorted according to the following indicators: age, assigned sex, and blood group (AB0, Rh factor).

The data, sorted by assigned sex and blood group, were compared between the groups and showed no significant differences. Significant differences in the total concentration of PAc and SAc were observed only between the groups sorted by age, specifically between those aged 24–40 years and those aged 41–59 years.

Fig. 6B shows histograms of data distribution frequencies for the total concentration of PAc and SAc and an estimation of the distribution density using the Gaussian smoothing method for two age groups: 24–40 years and 41–59 years (Table 3). The groups exhibit a normal distribution (Shapiro-Wilk test p-value > 0.05) and equal variances (Levene's test p-value > 0.05), which makes it possible to use both rank tests and tests based on analysis of variance (ANOVA) to establish significant differences between selections.

The relationship between the sum of FRF products concentrations and age was analyzed using Pearson's and Spearman's linear correlation coefficients. The standard residual and Cook's distance methods were used to identify the outliers. The data with a significant influence on the sample model was excluded. In this case, two values were excluded.

The Pearson correlation coefficient is 0.397, with p-value = 0.004, and the Spearman correlation coefficient is 0.351, with p-value = 0.012. Thus, the relationship between serum total PAc and SAc concentrations and age in healthy donors (Fig. 7A) is statistically significant (p = 0.004), positive, and moderate (r = 0.397). The equation $C_{PAc\&SAc}$ (μmol/L) = 0.51 + 0.064 × years old describes this linear relationship.

For each of FRF products, the Pearson and Spearman correlation coefficients between their concentration and patient age were similar to those calculated for the sum of FRF products, with r = 0.397 (p value = 0.004). Specifically, for PAc we found r = 0.399 (p value = 0.004), and for SAc we found r = 0.366 (p value = 0.008) (Fig. 7B).

Based on the calculations performed, it can be confidently stated that the total concentration of PAc and SAc is a reliable marker for the assessment of probability of the fragmentation reaction of lysophospholipid molecules. The total concentration of PAc and SAc, products of FRF, was found to be between 1.98 and 4.75 μmol/L in the serum of healthy donors, and a correlation with age was established. FRF is a potential mechanism for remodeling the membrane phospholipid bilayer, which becomes more important with age.

3.2. Concentration of palmitoxyacetone and stearoxyacetone in blood serum of patients with acute surgical pathology

Acute surgical pathology represents one of the most significant disturbance of homeostasis in the human body. Without surgical intervention in the body, there is a high probability of death for either of these conditions. Tissues exposed to hypoxia may experience an increase in the production of free radicals and the development of oxidative stress [53], which can in turn affect the concentration of FRF products. Therefore, the total concentration of PAc and SAc in blood serum was selected and assessed during acute surgical conditions of various pathogenetic origins and localizations in 70 patients. The ratio of PAc and SAc concentrations remained the same as in the group of healthy donors. The descriptive statistical parameters of the studied groups are presented in Table 4.

Since the total concentration of PAc and SAc in the blood serum of healthy donors is dependent on age and can be described by a linear regression equation, it is important to consider this factor when establishing differences between the compared groups. The majority of patients with the pathologies acute calculous cholecystitis, ileus, and organ perforation were aged 60–90 years (Table 1); the control group consisted of donors under the age of 60 years. To increase the reliability of

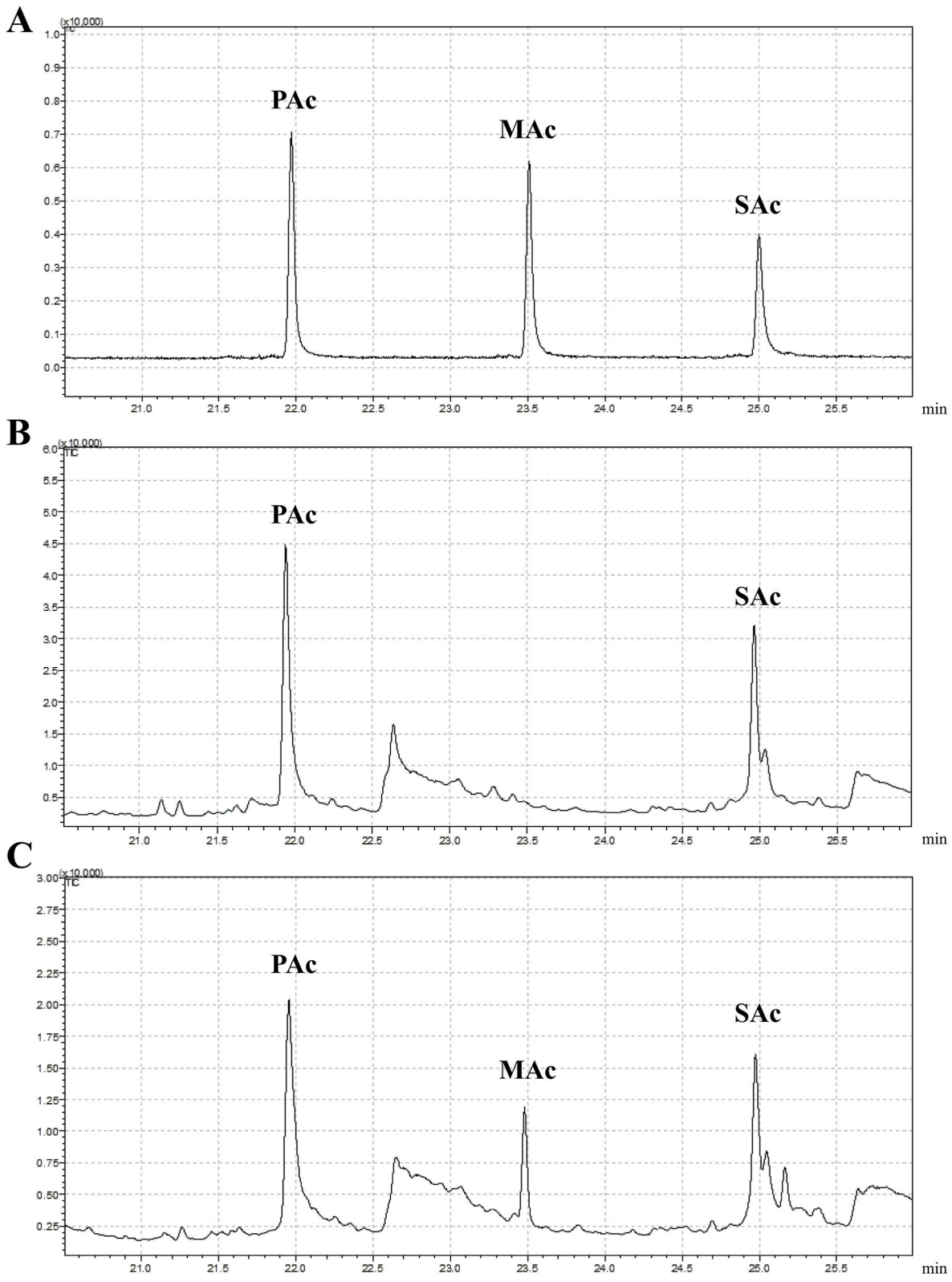

Fig. 4. A – The chromatogram of a solution of PAc, MAc and SAc in SIM mode;
B – The chromatogram of an authentic matrix sample in SIM mode;
C – The chromatogram of an authentic matrix sample with the addition of MAc in SIM mode.

between-group comparisons, an extrapolation method was used to calculate the expected mean FRF product concentrations for individuals aged 65, 70, 75, and 80 years as "presumed healthy individuals" and included these calculated concentrations in the "healthy" group sample. As determined using mathematical statistical methods, the relationship between the two variables is unaffected by the absence of FRF product concentrations at ages 65, 70, 75, and 80 years. The relationship remains linear even outside the observed range. It should be noted that the

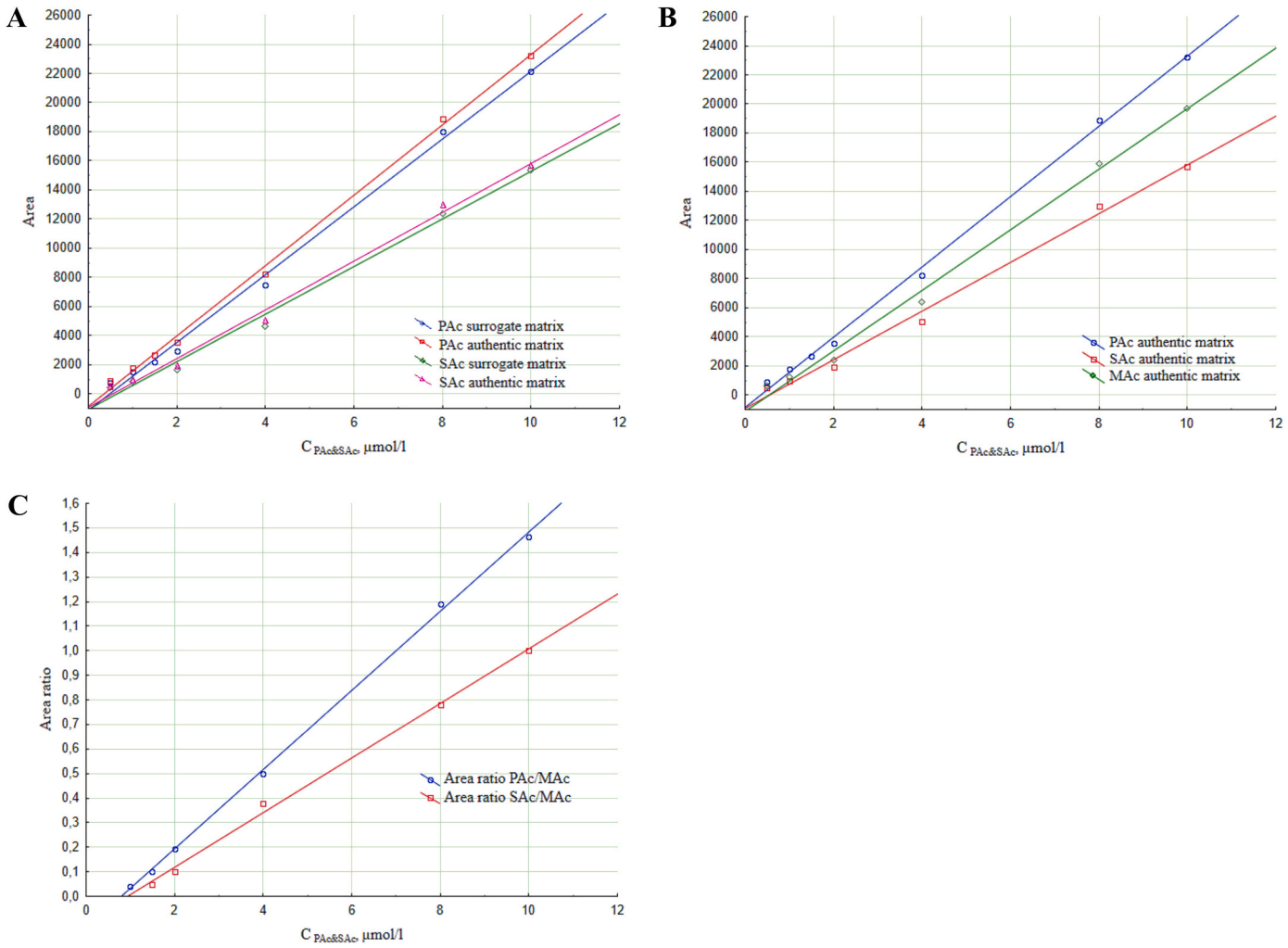

Fig. 5. Calibration curves of PAc, SAc, MAc solutions in the concentration range of 1–10 µmol/L
A – Calibration curve for PAc and SAc in surrogate and authentic matrix;
B – Calibration curve for PAc, SAc and MAc in authentic matrix;
C – Calibration curve for ratio of peak arears PAc to MAc and SAc to MAc.

Table 2
Assessment of the parallelism between the surrogate matrix and the authentic matrix.

Analyte (matrix)	Regression equation	R^2	ME, %	RRF	LLOQ, µmol/L	LOQ, µmol/L	CV,%	RE,%
PAc (surrogate)	Y = −1125 + 2.33E9X;	0.996		1.17	0.43	0.70	0.77–4.07	−4.33–11.10
PAc (authentic)	Y = −1037 + 2.43E9X	0.997	104	1.16	0.49	0.69	4.70–16.66	1.70–9.52
SAc (surrogate)	Y = −1454 + 1.65E9X	0.992		0.83	0.96	1.00	2.09–15.80	−10.20–3.90
SAc (authentic)	Y = −1266 + 1.71E9X	0.994	102	0.80	0.74	1.00	2.05–13.67	−1.04–6.48
MAc (surrogate)	Y = −888 + 1.97E9X	0.999		1.00	0.27	0.50	0.71–6.93	0.06–14.18
MAc (authentic)	Y = −1128 + 2.08E9X	1.000	106	1.00	0.36	0.50	0.22–13.47	1.02–7.86

ME – matrix effect, estimated by comparing slope of curve with addition of analytes in authentic matrix with standard curve in surrogate matrix.
RRF – relative response factor calculated by the formula $RF_{PAc(SAc)}/RF_{MAc}$, where RF is the ratio of peak area to concentration.
LLOQ – lower limit of quantitation.
LOQ – limit of quantitation, lowest concentration level of the calibration curve.
CV – coefficient of variation was calculated as a standard deviation expressed as a percentage of a mean.
RE – relative error, calculated by the formula as the ratio of difference between calculated and nominal concentration to nominal concentration, expressed as a percentage.

concentration of FRF products in conditionally healthy people over 60 years old can be influenced by decreased antioxidant defenses, decreased plasma lysophospholypids levels and other factors.

Fig. 8B shows the dependence of the total FRF products concentrations on patient age under normal conditions and in the presence of the considered pathologies. The course of the curves indicates a decrease in FRF products concentrations in diseases caused by septic inflammation.

In order to compare the level of concentrations of PAc and SAc in the groups with acute surgical pathology and the group of healthy donors, the type of data distribution in each group was determined. The Shapiro-Wilk's test was used to check the normal distribution of the data in the groups. All study groups had a normal distribution and equal variances

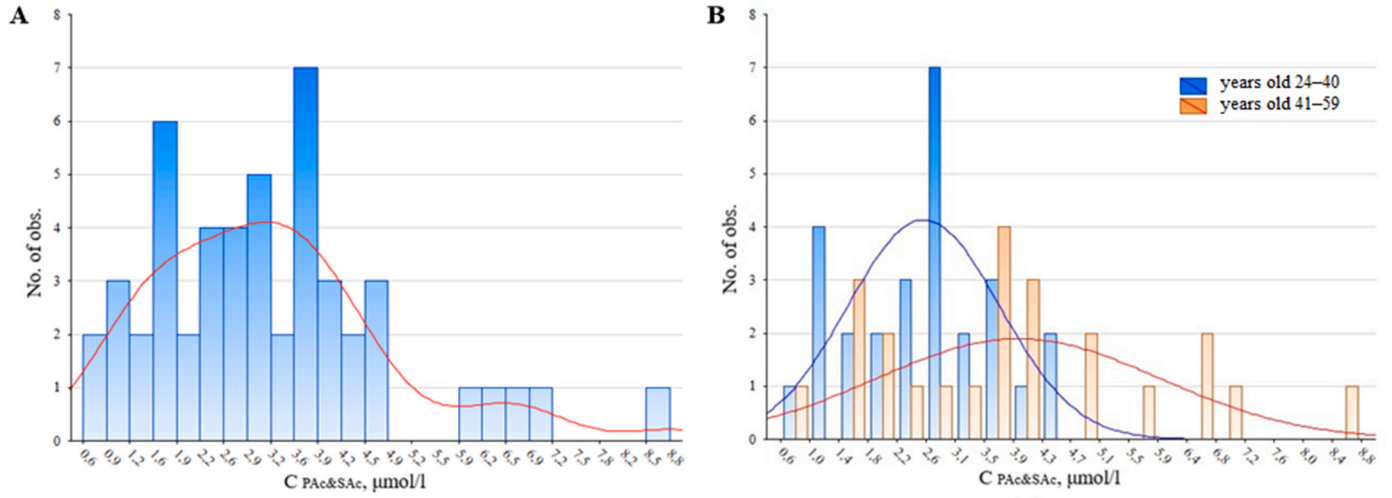

Fig. 6. A – Histogram of the distribution of the total concentration of PAc and SAc and Gaussian Kernel density estimation curve for the entire group of healthy donors aged 24–59 years (N = 52);
B – Histograms of the data distribution frequencies for the total concentration of PAc and SAc and an estimation of the distribution density using the Gaussian smoothing method for two age groups: 24–40 years and 41–59 years.

Table 3
Descriptive statistical parameters for two groups of healthy donors, categorized by age 24–40 years and 41–59 years. The total concentration of FRF products is reported in µmol/L.

Years old	Valid N	Mean	Confidence −95 %	Confidence 95 %	Median	Lower quartile	Upper quartile	Std.D	Std.E
24–40	28	2.42	1.98	2.87	2.62	1.45	3.06	1.14	0.22
41–59	24	3.93	3.10	4.75	3.77	2.29	4.79	1.95	0.19

Differences between age groups of healthy donors were analyzed using one-way ANOVA with Tukey's post hoc multiple comparisons (p-value = 0.0038) and Mann-Whitne's test (p-value = 0.009). Intergroup comparisons indicate statistically significant differences between age groups. The total concentration of PAc and SAc is in the range of 1.98–2.87 µmol/L for 24–40-year-olds and 3.10–4.75 µmol/L for 41–59-year-olds. The ratio of PAc and SAc concentrations in the samples was constant and amounted to 3:1.

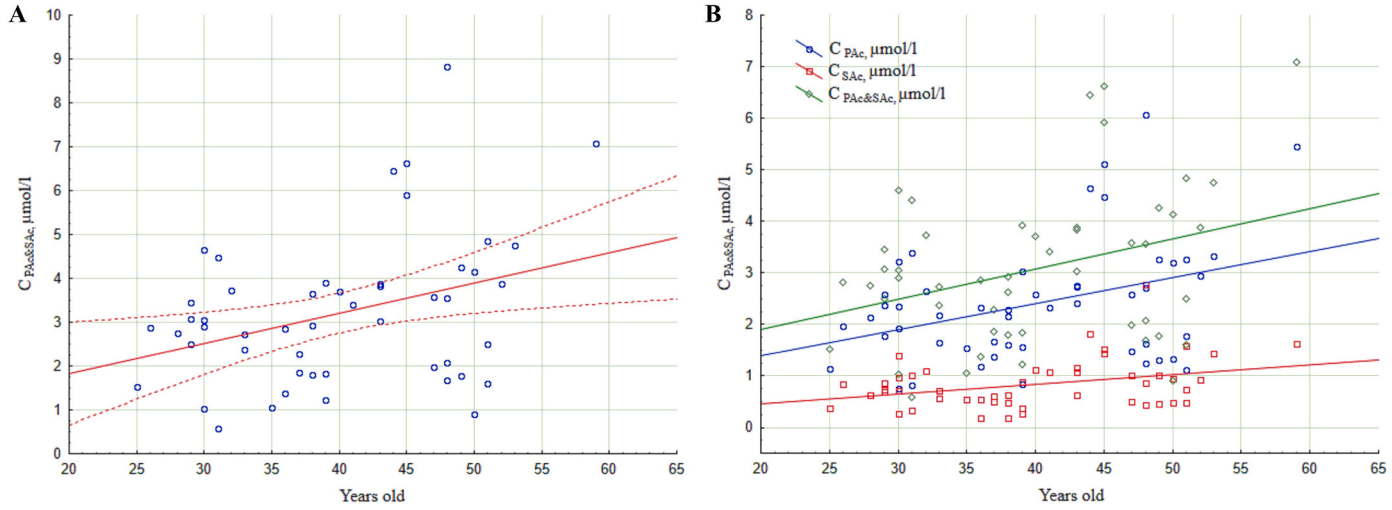

Fig. 7. A – Scatter plot of total PAc and SAc concentration data and regression lines for the group of healthy donors (N = 50);
B – Dependence of the concentration of PAc, SAc and the sum of these FRF products on the age of patients C_{PAc} (µmol/L) = 0.208 + 0.054 × years old, C_{SAc} (µmol/L) = 0.011 + 0.021 × years old; $C_{PAc\&SAc}$ (µmol/L) = 0.51 + 0.064 × years old.

(Levene's test, p-value > 0.05). This allowed the use of variance and rank tests for the comparison between groups (Table 5).

The Tukey's test and the Mann-Whitney's test were used to compare the groups of healthy donors (age 41–60 years) and patients with acute surgical pathology, because all patients were in this age category. Levels of statistical significance p-value are given in Table 6.

A statistically significant decrease in the intensity of FRF and its product formation is indicated by the data distribution density using the Gaussian smoothing method for the groups of patients with acute surgical pathology of abdominal organs (Fig. 8A) compared to healthy blood donors. The dependence of the sum of FRF products concentrations on the age of patients also confirms the trend towards a decrease in

the content of PAs and SAs in the blood serum of patients with pathologies (Fig. 8B).

A localized inflammatory-destructive abnormality of the hollow viscus wall with bacterial translocation (septic inflammation) is characterized by a decrease in the intensity of FRF and in the summed concentration of PAc and SAc in blood serum. Thus, in the group of patients with acute phlegmonous appendicitis aged 41–60 years, a decrease in these concentrations by 1.9 times (p-values = 0.015) was observed in comparison with a group of healthy blood donors. In groups of patients with acute calculous cholecystitis, acute intestinal obstruction, acute perforation of a gastric or duodenal ulcer, a statistically significant decrease in the concentration of PAc and SAc in blood serum by 1.5 times (p-value = 0.046) was observed in comparison with the group of healthy donors of the corresponding age categories.

Patients diagnosed with acute necrotizing pancreatitis and intracranial have ischemic-reperfusion tissue damage (microcirculatory blockage, hypoxia, ischemia, edema and development of compartment syndrome, reperfusion), i.e. aseptic inflammation (N = 27) in common. A scatter plot of the data on the total concentration of PAc and SAc in healthy blood donors and in groups of patients with pancreatic necrosis and intracranial hematomas as a function of patient age is shown in Fig. 9. The presented data show a statistically significant increase in the intensity of FRF and its products in patients with acute necrotizing pancreatitis and intracranial hematomas compared to healthy blood donors.

Ischemia-reperfusion tissue damage (aseptic inflammation) was associated with an increase in the intensity of FRF and the summed concentration of its products in blood serum. Thus, the summed concentration of PAc and SAc in blood serum increased 2.2 times (p-value = 0.005) with the development of intracerebral hematoma and 4 times (p-value = 0.0001) with acute necrotizing pancreatitis compared to healthy blood donors. The Tukey's HSD test and the Mann-Whitney's U rank test show the same level of significance (Table 6).

4. Discussion

Our study is based on the hypothesis that damage to cell membranes can occur in the polar part of phospholipids at the interface with the aqueous phase, both inside and outside the cell, due to FRF. Considering the high reactivity and short lifetime of $^{\bullet}$OH radicals, it can be assumed that the main damage will occur in the polar part of the membrane in contact with the aqueous phase [53,54].

Our study was based on the results of studies using chemical models which are founded on the ability of lipids to form stable supramolecular complexes that mimic the structure of cell membranes. In such chemical models, ionizing radiation was used as a source of $^{\bullet}$OH radicals. This approach allows for the following: the determination of the probability of homolytic decomposition reactions of carbon- and nitrogen-containing radicals of lipids in the polar part of the molecule; the identification of the dependency of this process on the structure of the lipid and the reaction conditions; and the identification of resulting lipid products [14–23,25].

The varying probabilities of free radical reactions occurring in the polar part of the phospholipid molecule under the conditions of normoxia, and/or oxygenation, as well as hypoxia, predetermined the choice of a comparison group (healthy blood donors) and subgroups of patients with different acute surgical pathologies for our study. Subgroups of patients with acute phlegmonous appendicitis, acute calculous cholecystitis, acute intestinal obstruction, and acute perforation of gastric and duodenal ulcers were characterized by the development of various localized inflammatory and destructive lesions of the wall of a hollow organ under the conditions of normoxia and bacterial translocation, leading to a septic type of inflammation. Subgroups of patients with acute pancreatic necrosis and intracranial brain injuries (acute subdural and intracerebral hematoma) were characterized by the development of ischemia-reperfusion tissue damage, leading to an aseptic type of inflammation [55].

After statistical processing of the data on the total concentrations of PAc and SAc in each studied subgroup and after intergroup comparison with the comparison group, cluster analysis was performed using the method of centroid-weighted pair groups. This method was considered most suitable for our study as it accounts for the differences in cluster sizes (weighting). Following the clustering of the studied subgroups, two clusters were identified in relation to the compared group (healthy blood donors). Groups with diagnoses of "Acute calculous cholecystitis", "Acute phlegmonous appendicitis", "Ileus" and "Organ perforation" were combined into one cluster, exhibiting a decrease in the content of FRF products compared to the norm. The second cluster consisted of the subgroups "Intracranial hematoma" and "Acute necrotizing pancreatitis," showing an increase in the concentration of PAc and SAc in the blood serum (Fig. 10).

In groups of patients with acute calculous cholecystitis, acute phlegmonous appendicitis, ileus, and organ perforation, the development of septic inflammation was accompanied by the initiation of uncontrolled oxidative stress under the conditions of oxygenation, where up to 1 % of oxygen is converted into ROS due to activation of redox pathways [53]. ROS react actively and indiscriminately with surrounding molecules. Within the cell, this can lead to swelling of mitochondria, rupture of their outer membranes, damage to cell membranes, increased permeability, and disruption of the activity of respiratory enzymes in both the internal and external membranes. These effects are characteristic of septic inflammation [53,56].

According to the data obtained (Table 4), localized inflammatory-destructive damage to the wall of a hollow abdominal organ with bacterial translocation (septic inflammation) is characterized by a decrease in the total concentration of PAc and SAc in the blood serum.

Under normoxia and oxygenation conditions, molecular oxygen inhibits the FRF process by oxidizing lysolipid radicals (Fig. 11). At the *sn*-2 position of the radical, the formation of a carbonyl group takes place. This leads to the modification of the molecular structure without its destruction. The rate of lysolipid radical - oxygen interaction is $\geq 10^9$ L $\times$ mol^{-1} $\times$ s^{-1} [14,57]. Modification of the molecular structure of membranes without breaking chemical bonds can lead to a change in the properties of the membrane layer, which are important for the

Table 4
Descriptive statistic parameters of the study groups in relation to the summed concentration of PAc and SAc (μmol/L) for healthy donors and patients with acute surgical pathology.

Study group	Years old	Valid N	Mean	Std.D	Confidence −95 %	Confidence +95 %	Median	Lower quartile	Upper quartile	Coef. Var., %
Healthy 1	25–40	26	2.47	1.14	2.02	2.92	2.73	1.52	3.07	45.99
Healthy 2	41–59	24	3.96	1.91	3.16	4.77	3.78	2.29	4.80	48.06
Acute calculous cholecystitis	43–83	10	2.57	0.85	1.97	3.18	2.54	2.27	3.01	32.86
Appendicitis	40–60	10	2.36	1.15	1.27	3.44	2.15	0.78	3.25	48.83
Ileus	41–90	15	2.81	1.28	2.10	3.52	2.39	2.06	3.90	45.49
Organ perforation	41–70	8	2.47	0.54	1.90	3.04	2.63	1.97	2.94	22.08
Intracranial hematoma	42–85	14	7.19	2.34	5.84	8.54	6.99	4.88	10.11	32.53
Acute necrotizing pancreatitis	28–59	13	12.65	6.31	8.21	17.09	11.40	8.09	20.50	49.89

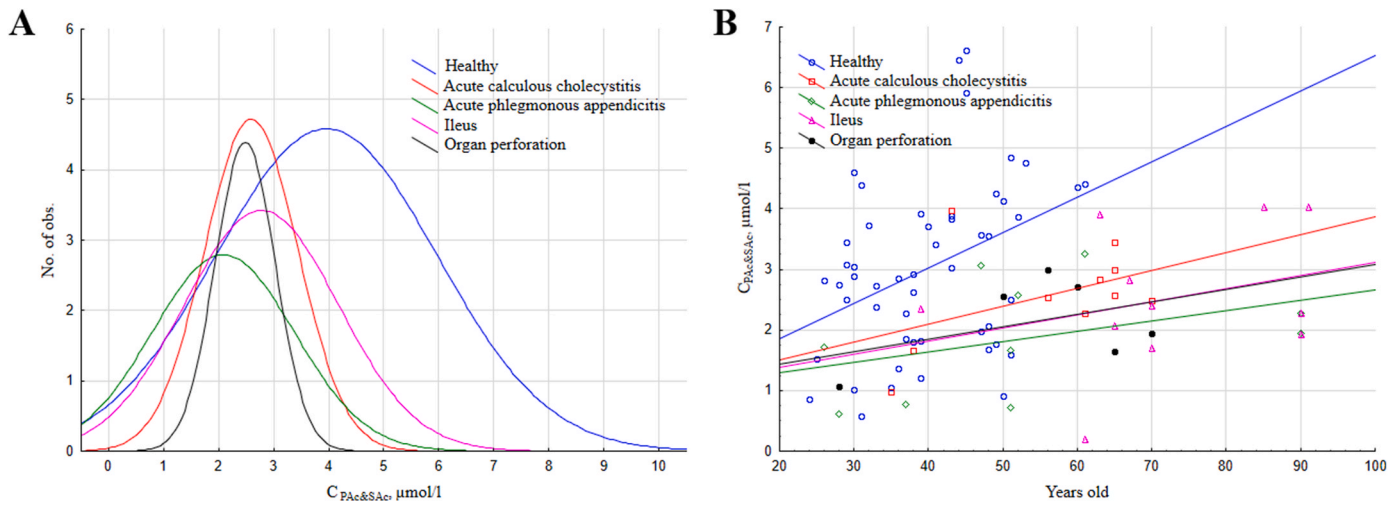

Fig. 8. A – The density distribution of data using the Gaussian smoothing method for the compared groups of patients with acute abdominal surgical pathology compared to healthy blood donors;
B – Dependence of the sum of FRF products concentrations on the age of patients.

Table 5
The p-value for both Shapiro-Wilk's and Levene's tests.

Test	Healthy	Acute appendicitis	Acute calculous cholecystitis	Ileus	Organ perforation	Intracranial hematoma	Acute necrotizing pancreatitis
Shapiro-Wilk's	0.066	0.420	0.413	0.915	0.482	0.327	0.093
Levene's	0.063	0.092	0.068	0.057	0.187	0.050	0.263

Table 6
The p-value for both Tukey's and Mann-Whitney's tests.

Test	Groups	Acute appendicitis	Acute calculous cholecystitis	Ileus	Organ perforation	Intracranial hematoma	Acute necrotizing pancreatitis
Tukey's HSD	Healthy	**0.0150**	**0.0455**	**0.0463**	**0.0120**	**0.0052**	**0.0001**
Mann-Whitney's U	Healthy	**0.0119**	**0.0394**	0.1371	**0.0457**	**0.0001**	**0.0002**

Statistically significant values (p-value < 0.05) are in bold.

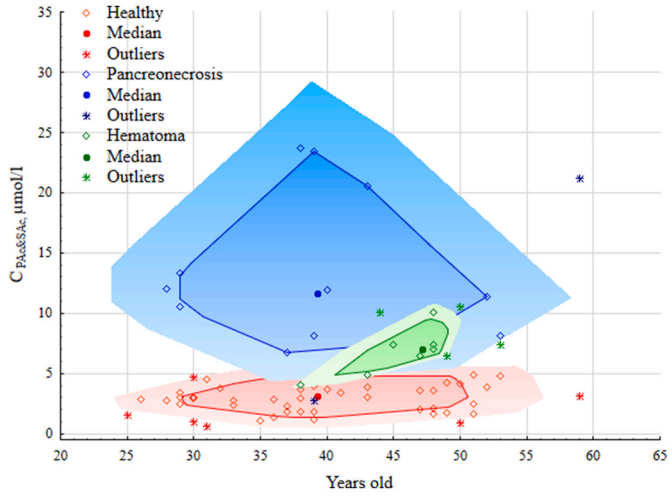

Fig. 9. Scatter plot of total PAc and SAc concentration data for healthy donors, groups of patients with pancreonecrosis and intracranial hematomas against the age of patients.

functioning of the cell. The influence and role of these modified molecules on biomembrane homeostasis *in vivo* is currently unknown. Further studies are nedeed to establish the role of lipids oxidized in their polar heads in human homeostasis, both in health and disease. Oxidation of cell membrane lipids in their polar parts, which predominates under conditions of normoxia and/or oxygenation, may be one of the reasons for the decrease in the concentration of PAc and SAc fragmentation products in the blood serum of patients with pathology, specifically based on septic inflammation.

In groups of patients with acute necrotizing pancreatitis and intracranial brain injuries, the formation of ischemia-reperfusion tissue damage occurs as a result of a sudden cessation of oxygen supply. During cellular ischemia and/or hypoxia, O_2 decreases from 21 % to 1–3 % [58]. The hypoxic phase is accompanied by the changes in cellular metabolism, characterized by the suppression of mitochondrial energy production caused by oxygen deprivation. The main process of tissue damage at this stage of sterile inflammation may be the fragmentation reaction of lipid molecules (Fig. 1), leading to an increase in the concentration of PAc and SAc in the blood serum in patients diagnosed with intracranial hematoma and acute necrotizing pancreatitis. It should be noted that the triggers of sterile inflammation have not yet been identified, and the signaling pathways of sterile inflammation are not fully understood.

The concentration of stable end products of $^{\bullet}$OH-induced

fragmentation of 1-acyl-2-lyso-*sn*-glycero-3-phospholipids is influenced by both the intensity of OH generation and the concentrations of lysoglycerophospholipid molecules that it can interact with (Fig. 1). Currently unknown are other pathways that lead to the formation of the investigated markers, which includes the enzymatic ones, as well as the pathways for their subsequent metabolism.

Regarding substrates for FRF reactions, it should be noted that in cells, lysoglycerophospholipids (LPLs) are precursors for the biosynthesis of other lipids and their intracellular concentration is 0.5–6.0 % [59]. Endogenous LPLs are important for the formation and remodeling of membrane phospholipids. They are involved in maintaining the proper functioning of the bilayer. Activation of PLA_2 leads to an increase in the concentration of LPLs, resulting in irregular membrane curvature and destabilization. In addition to their structural role, intracellular LPLs are lipid mediators in signal transduction [59]. In our opinion, the ordered structure of membrane lipids increases the probability of the lysophospholipid fragmentation reaction to occur.

LPLs are also distributed in the extracellular environment, such as plasma and interstitial fluids, where LPLs bind to carrier proteins. For example, in plasma, LPC is bound to albumin. A small proportion of LPC is present in lipoprotein particles, in erythrocytes and in platelets. Unbound forms of LPC tend to be less abundant and less stable. Thus, the concentration of LPC, the most abundant lysophospholipid, in human blood plasma can vary from 150 to 500 μmol/L [60,61]. A concentration of 120–150 μmol/L represents LPC acylated with a palmitic acid residue. The concentration of LPC with an acyl chain at the *sn*-1 position is nine times higher than the concentration of *sn*-2 isomers [62].

LPC, through G protein-coupled receptor signaling, has negative effects on various cells, including enhancing inflammatory responses, disrupting mitochondrial integrity and inducing apoptosis. However, the optimal plasma level of LPC has not been established and the mechanisms underlying the effects of LPC are not well understood [62].

Hypoxia regulates glycolysis and overproduction of lysophospholipids. Under hypoxic conditions, most cells can switch their primary metabolic strategy from predominantly mitochondrial respiration to increased glycolysis to maintain ATP levels. This hypoxia-induced metabolic reprogramming is key to meeting cellular energy demands during acute hypoxic stress [63]. Free radical production increases during cellular hypoxia. ROS activate phospholipase A_2, resulting in increased lysolipid content in cell membranes. ROS react with cellular lysolipids, causing their oxidation and fragmentation [64].

We have considered the fragmentation pathway of the molecule LPC, where the degradation products are PAc/SAc and phosphocholine (PChol). PAc and SAc are stable compounds that are biologically inert

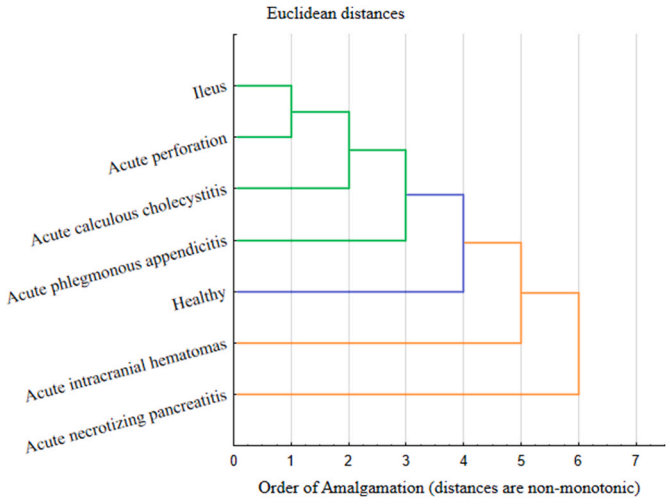

Fig. 10. Dendrogram of clustering of the studied groups using the method of centroid-weighted pair groups.

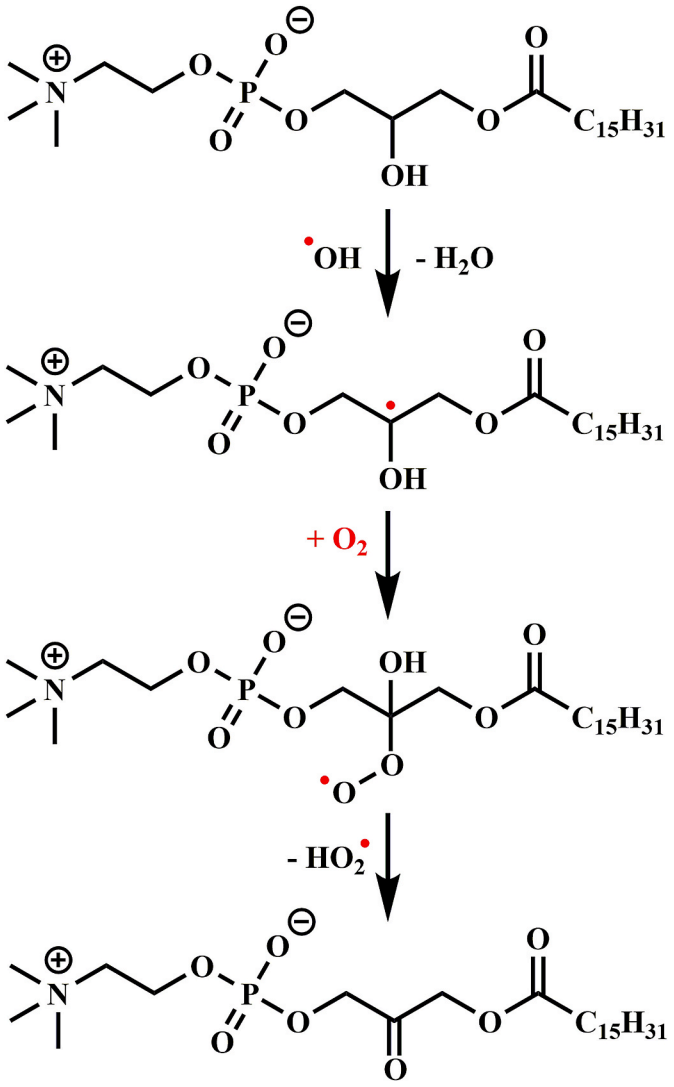

Fig. 11. Suppression of the fragmentation reaction of alpha hydroxyl radicals of lysolipids by oxygen due to their oxidation.

and can serve as reliable markers of the FRF process itself. PChol is a molecule formed together with PAc and SAc during the fragmentation of the lysolipid. PChol is known to have immunomodulatory properties. PChol and PChol-modified macromolecules interact with C-reactive protein, which has a strong anti-inflammatory effect and induces immune evasion. The mechanisms by which PChol-modified macromolecules induce immune evasion are not fully understood [65]. Consequently, PChol is in a bound state in blood serum due to its high chemical activity and cannot be a marker for FRF of lysophosphatidylcholine.

Regarding the second pathway of FRF with the formation of palmitic acid and hydroxyacetone phosphocholine (Fig. 1), hydroxyacetone phosphocholine, like PChol, also has activity and binds to G proteins [65]. Palmitic acid is already present in blood serum in a rather high concentration (0.3–4.1 mmol/L), which makes it impossible to consider this compound as a marker of this process [66].

5. Conclusions and outlook

Ensuring homeostasis in the human body as a whole is achieved through the formation in cell membranes of a certain pool of highly active, multifunctional molecules – lysophospholipids. These molecules are generated from three sources: 1) damage/remodeling of cell

membranes; 2) synthesis in the liver; 3) intake from food.

The phospholipid bilayer creates a barrier between the inside and outside of the cell because polar or charged substances cannot easily penetrate the hydrophobic core of the membrane. The hydrophilic heads of phospholipids in the membrane bilayer face outward, coming into contact with aqueous fluid both inside and outside the cell. ROS are essential for normal physiological functioning, but elevated ROS levels can lead to oxidative stress. PLA_2 is one of the key players activated by increased levels of ROS, which leads to the hydrolysis of membrane phospholipids. The relationship of PLA_2s to ROS is also traced through the production of arachidonic and other polyene fatty acids, which in turn are involved in cascades of signaling pathways for the translocation, assembly and/or activation of NADPH oxidase in monocytes and neutrophils, leading to stimulation of their activity and ROS production [67].

The presence of a hydroxyl group at the *sn*-2 position in the polar part of lysophospholipids makes the molecule sensitive to the action of the OH radical, leading to the formation of a lipid radical at this position and its subsequent fragmentation into palmitoxyacetone or stearoxyacetone and phosphocholine (Fig. 1). To quantify PAc and SAc in serum samples, a GC-MS method was developed using MAc as a surrogate standard to assess the intensity of the free radical fragmentation reaction of lysoglycerophospholipids.

For the first time, it has been shown that FRF products are present in the blood serum of healthy donors aged 24–59 years (N = 52). The total concentration of PAc and SAc falls within the range of 1.98–4.75 μmol/L, indicating the levels of normal biological processes in the human body overall, and specifically in cell membranes. The inclusion of FRF processes seems to be crucial for maintaining redox homeostasis and restoring cell membranes. As the requirement for membrane phospholipid bilayer remodeling increases with age, the concentration of FRF products also changes. This dependence is represented by a linear regression equation: $C_{PAc\&SAc}$ (μmol/L) = 0.51 + 0.068 × years.

Statistically significant differences were found in the total concentration of PAc and SAc in the blood serum of healthy donors and patients with acute surgical pathology. Acute surgical pathology leads the body to a state where maintaining homeostasis without surgical intervention is impossible. Changes in the pool of lysophospholipids and their FRF products in the body are determined by the predominant type of tissue damage that occurs as a result of the immune system's reaction to a pathogen (sepsis) or as a result of non-infectious processes (ischemia-reperfusion).

The first cluster included groups of 43 patients with various localized inflammatory-destructive lesions of the wall of a hollow organ and bacterial translocation (septic inflammation) of the abdominal organs (such as acute phlegmonous appendicitis, acute calculous cholecystitis, acute intestinal obstruction, acute perforation, and peptic ulcer of the stomach and duodenum). In the first cluster, there was a significant decrease in the total concentration of PAc and SAc in the blood serum by 1.5–1.9 times (p-value = 0.012–0.046) compared to the comparison group (healthy blood donors) of the corresponding age category, which may be associated with a decrease in the intensity of FRF reactions. In the first cluster of patients, food intake (intake of lysophospholipids) is physically impossible. Synthetic processes in the liver were not affected. The inhibitory effect of oxygen on the fragmentation process due to the oxidation of hydroxyalkyl radicals of the starting substances leads to gross disturbances in the formation of lysophospholipids, the ability to remodel cell membranes, increasing the depletion of ROS, which programs a further increase in cellular dysfunction and damage.

In the second cluster, comprising groups of 27 patients with ischemia-reperfusion tissue damage (microcirculation blockage, hypoxia, ischemia, edema, and the development of compartment syndrome upon reperfusion) in the retroperitoneal space (acute necrotizing pancreatitis) and those with intracranial brain injuries (acute subdural and intracerebral hematoma), there was a statistically significant increase in the total concentration of FRF products (PAc and SAc) compared to healthy blood donors. The increase was by 2.2 times (p-value = 0.0052) with the development of intracerebral hematoma and by 4.0 times (p-value = 0.0001) with acute necrotizing pancreatitis (pancreatic necrosis). The second cluster of patients, like the first, experienced the inability to eat, leading to no intake of lysophospholipids from food. Synthetic processes in the liver remained unaffected. However, the development of ischemia-reperfusion tissue damage resulted in a significant disruption of redox homeostasis, shifting FRF reactions from the category of regulatory to damaging due to their predominance under ischemic conditions. This further exacerbates cellular dysfunction and tissue damage.

The findings of this study are significant for future studies of PAc and SAc as potential markers of the process of fragmentation of membrane phospholipids and potential signaling molecules in human homeostasis. PAc and SAc reflect the intensity of free radical-induced lipid damage under the conditions of oxygenation and hypoxia. The data obtained in this study are essential for elucidating the role of such processes in both sterile and infection-induced inflammatory reactions within the body. Phosphocholine and its derivatives, which are formed along with PAc and SAc during fragmentation reaction, are recognized as biologically active molecules involved in a number of crucial processes, indicating the potential importance of FRF in human homeostasis.

Source of support

This work was funded by The Ministry of Health, Republic of Belarus [research number 20220706].

All authors read the published version of the manuscript and agreed with it.

CRediT authorship contribution statement

Alexey Fedoruk: Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization. **Oleg Shadyro:** Resources, Project administration, Investigation, Data curation, Conceptualization. **Irina Edimecheva:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Data curation, Conceptualization. **Dmitry Fedoruk:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Valery Khrutskin:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Leanid Kirkovsky:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition. **Viktor Sorokin:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Halina Talkachova:** Writing – review & editing, Formal analysis, Data curation.

Declaration of competing interest

We are sending the manuscript (original research article) by Alexey Fedoruk, Oleg Shadyro. Irina Edimecheva, Dmitry Fedoruk, Valery Khrutskin, Leanid Kirkovsky, Viktor Sorokin, Halina Talkachova entitled « Free radical fragmentation and oxidation in the polar part of lysophospholipids: results of the study of blood serum of healthy donors and patients with acute surgical pathology » to be evaluated for publication in «Redox Biology».

The authors declare that there are **no conflicts of interest**. The authors alone are responsible for the content and writing of the paper. All authors have read the manuscript and concur with the submission, the work has not been published elsewhere, either completely, in part, or in another form, the manuscript has not been submitted to another journal and will not be published elsewhere.

Data availability

Data will be made available on request.

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