DIET-INDUCED AND AGE-RELATED CHANGES IN RATS: THE IMPACT OF N-STEAROYLETHANOLAMINE INTAKE ON PLASMA LIPOPROTEINS, ADIPONECTIN, AND ADIPOCYTE CHOLESTEROL-PHOSPHOLIPID COMPOSITION

O. S. TKACHENKO✉, H. V. KOSIAKOVA

Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kyiv, Ukraine;
✉e-mail: 888oksana.tkachenko@gmail.com

Adiponectin is secreted by adipose tissue, associated with lipoprotein (LP) metabolism, down-regulated in insulin resistance states, and reduced in individuals suffering from obesity and cardiovascular diseases. Phospholipids and cholesterol are the main components of cell membranes and play a critical role in storage and secretory adipocyte functions. N-stearoylethanolamine (NSE) is a minor lipid affecting cell membrane lipids' composition. Our study aimed to investigate plasma levels of adiponectin and cholesterol of low- and high-density LP (LDL and HDL) and adipocyte cholesterol-phospholipid (Chol-PL) composition of different age rats with high-fat diet (HFD)-induced obesity and insulin resistance and their changes under NSE administration. Our study demonstrated that chronic dietary fat overloading leads to obesity accompanied by impairment of glucose tolerance, a manifestation of dyslipidemia, and changes in plasma adiponectin levels in rats from two age groups (10-month-old and and 24-month-old). Prolonged HFD led to a reduction in plasma adiponectin levels and the growth of adipocyte cholesterol content in rats of different ages. A significant increase in plasma LDL-Chol level and main adipocyte PLs (phosphatidylcholine, phosphatidylethanolamine, sphingomyelin, and lysophosphatidylcholine) was observed in younger rats, whereas not detected in elder animals after dietary fats overloading. The decrease in the content of anionic phospholipids (phosphatidylinositol + phosphatidylserine) was also detected in 10-month-old HFD rats compared to the control animals. NSE administration positively affected the normalization of adiponectin levels in both age HFD groups. It significantly impacted the reduction of LDL-Chol levels and the growth of HDL-Chol concentration in the blood plasma of 10-month-old rats as well as PL-composition of young HFD rats and anionic PL restoring in 24-month-old rats. The positive effect on investigated parameters makes NSE a prospective agent for treating diet-induced and age-related metabolic disorders threatening cardiovascular diseases.

K e y w o r d s: N-stearoylethanolamine, adipose tissue, dyslipidemia, aging, cholesterol, phospholipids, adiponectin, lipoproteins.

Obesity is accompanied by dyslipidemia and is one of the significant risk factors for cardiometabolic and cardiovascular diseases in different age groups of the population [1]. Research in recent years shows that obesity radically changes the metabolism of lipoproteins, which leads to a change in the ratio of their classes and, accordingly, the implementation of their functions, and an imbalance of cholesterol in the composition of lipoproteins of different densities usually characterizes dyslipidemia in obesity [2, 3]. Our previous studies showed that rats with obesity induced by a high-fat diet (HFD) had increased blood coagulability, development of low-grade inflammation, and changes in aorta morphology, which we assume indicated early atherosclerosis [4].

The primary structural units of adipose tissue are adipocytes - large cells, the predominant volume inside which is occupied by lipid droplets. However, adipose tissue is more than just adipocytes. It also includes other cells such as preadipocytes, macrophages, stromal, stem and endothelial cells [5], as well as neutrophils [6] and lymphocytes [7]. While many research results have been published on the

changes in the lipid composition of adipose tissue in healthy individuals and various pathologies, it is crucial to focus on the lipidome of adipocytes themselves. It underlines the urgency and importance of our study, as we analyzed not the whole adipose tissue, which is a complex composition of different types of cells, but adipocytes isolated from it.

Adipocytes are not just storage units for lipids, but they play a pivotal role in lipid metabolism. They regulate the balance of lipids in the body and are involved in producing hormones that control appetite and energy expenditure. Adiponectin is one of the main adipokines specifically expressed by adipose tissues, which regulate glucose and lipid metabolism and obtain insulin-sensitizing and anti-inflammatory properties [8, 9]. Low plasma adiponectin level correlates with obesity, type 2 diabetes, lipoprotein metabolism disorders, and cardiovascular diseases [8-11]. It is also known that adiponectin level changes during aging and it has been clinically established that long-lived people and some of their descendants have high adiponectin levels [12].

Cardiovascular disease, with atherosclerosis as a critical cofactor, remains the leading cause of death worldwide. It is well known that the presence of a large number of foam cells with accumulated cholesterol esters is a characteristic marker of atherosclerotic plaques. Extraction of cholesterol from foam cells with the direct participation of high-density lipoprotein (HDL) particles is the first step of reverse cholesterol transport (RCT). RCT is a process by which excess cholesterol is removed from cells and transported back to the liver for excretion [13]. Therefore, the influence on the intensification of this process is a promising antiatherogenic strategy.

It is noteworthy that adipose tissue contains a significant amount of cholesterol, and in obese adults, over 50% of the total cholesterol in the body is contained in adipocytes. It is a consequence of cholesterol deposition in adipose tissue that buffers excess cholesterol synthesized by the liver [14]. Interestingly, with obesity, the activity of receptors to low-density lipoproteins (LDL) in adipose tissue increases, and the outflow of cholesterol to HDL decreases [14]. Thus, plasma LDL- and HDL-cholesterol levels at the condition of different cholesterol production in diet-induced or age-related metabolic disorders may indicate an adipose tissue role in cholesterol homeostasis.

The cholesterol balance in the fat cell is an essential regulator of metabolic activity and the ability to accumulate triglycerides [15]. At the same time, modern studies demonstrate that the imbalance of the phospholipid component plays a vital role in the pathological process of metabolic disorders [16].

A deeper study of the mechanisms underlying these described above processes is crucial for a better understanding of the effects of obesity and aging on lipid metabolism, particularly lipoprotein metabolism, and adipose tissue role in these complex metabolic passways for developing appropriate therapeutic approaches for reducing age-related and diet-induced cardio-metabolic complications.

It was previously shown that saturated NAE - N-stearoylethanolamine (NSE) can regulate the lipid content of biological membranes under different pathological conditions [17]. NSE's membrane-stabilizing and anti-inflammatory action was shown on different tissues in various pathological conditions in animal models [18-20]. The main effect of NSE has been speculated to be the improvement of lipid imbalance. The results of previous studies demonstrated that NSE positively influences insulin-sensitive tissues' lipid composition. This effect is also associated with improving insulin sensitivity in rats with obesity-induced insulin resistance (IR) [21-24]. We also previously showed that NSE helped reduce inflammation manifestations accompanying atherogenesis in a spontaneously hypertensive rat model [25]. That is why our study aimed to investigate diet-induced and age-related changes in plasma lipoprotein profile, adiponectin level, and adipocyte cholesterol-phospholipid composition and the effect of NSE administration on these parameters.

Materials and Methods

Animal model. The animal model was conducted on male Wistar rats of two age groups during 24 weeks and in the end of experiment younger animals were 10-month-old (10-m.o.) and older were 24-month-old (24-m.o.). The started average rat weight was 150.50 ± 1.76 g and 491 ± 13.52 g respectively at the beginning of the experiment.

Rats were housed in standard cages and have free access to water and food. Obesity-induced IR was obtained by the feeding a long-term high-fat diet (HFD) during 24 weeks [26]. The diet included pellets with addition of pork visceral lard as the source of extra fats. The analysis of described HFD [27] demonstrated that total content of fats was at 58%. The fatty acids composition of HFD consisted of 55% saturated fatty acids and 45% unsaturated

fatty acids. The animals from control group received standard rodent chow (Animal Feed Manufacturer "Agrovita", Ukraine) containing 4% of fats with the percentage of SFA and UFA at the level of 38% and 62% respectively. In this study, animals from all the groups were fed *ad libitum*; we did not record the amount of food consumed by the animals.

After a 24-week HFD period, the oral glucose tolerance test was conducted [28]. The rat blood glucose concentration was measured using glucometr Bionime GM300 at the 0 min point after 12-hours of fasting. Then, animals received glucose solution in a dose of 2 g/kg of body weight, and blood glucose concentration was measured after 45, 90, and 150 min. The rats with impaired glucose tolerance (which have blood glucose level higher than 5 mmol/l at the end of the test) were selected and randomly divided into two groups: "HFD" ($n = 11$ and $n = 10$ for 10 m.o. and 24 m.o. groups respectively) and "HFD+NSE" ($n = 14$ and $n = 6$ for 10 m.o. and 24 m.o. groups respectively). Control rats were divided into "Control" ($n = 6$ and $n = 6$ for 10 m.o. and 24 m.o. groups respectively) and "Control+NSE" ($n = 6$ and $n = 8$ for 10 m.o. and 24 m.o. groups respectively) groups.

Rats in "Control+NSE" and "HFD+NSE" groups received the water suspension of NSE *per os* during 2 weeks at a dosage of 50 mg per 1 kg of body weight daily. NSE was synthesized in the Department of Lipid Biochemistry, Palladin Institute of Biochemistry of the NAS of Ukraine according to described previously procedure [71]. During the last two weeks, rats from all groups received a standard diet.

At the end of the experiment, the rats were decapitated under Nembutal anesthesia according to the ethical principles for the conduct with laboratory animals [29]. The abdominal fat pads were removed immediately for further adipocytes isolation.

All experiments involving animals were carried out in accordance with ethical principles with an approval of the Animal Care and Use Committee of the Palladin Institute of Biochemistry of the NAS of Ukraine protocol N2, 05.02.2018.

Lipoproteins content measurement. HDL and LDL cholesterol content in blood plasma was measured using commercial kits (Spinelab, Kharkiv, Ukraine) with a calibrator (Spinreact, Spine).

Adiponectin concentration determination. Plasma adiponectin level was measured using ELISA kit (Invitrogen, USA, catalog number KRP0041).

Adipocytes isolation procedure. Rat abdominal white adipose tissue were digested with solution of Collagenase Type 1 (Sigma-Aldrich, Germany) in HEPES buffer (pH 7.4) according to the modified Rodbell procedure [30, 31]. Then, 2 ml of Krebs-Ringer HEPES Buffer (pH 7.4) was added to 1 g of rat abdominal adipose tissue. Krebs-Ringer HEPES Buffer contains 5 mM D-Glucose, 2% BSA, 135 mM NaCl, 2.2 mM $CaCl_2 \times 2H_2O$, 1.25 mM $MgSO_4 \times 7H_2O$, 0.45 mM KH_2PO_4, 2.17 mM Na_2HPO_4, 10 mM HEPES.

Adipose tissue was thoroughly minced in buffer to 1–2 mm pieces. Buffered tissue fragments were digested with Type 1 Collagenase solution in HEPES buffer (1.25 mg/ml) at 37°C with gentle shaking during 1 h. After incubation the tissue suspension was diluted in 1 ml cold buffer. Isolated adipocytes were separated from undigested tissue by the filtration using the 400-µm nylon mesh and washed by 1 ml of buffer three times. The resulting cell suspension was centrifuged at 1000 rpm for 10 min with further separation of floating adipocytes from the stromal vascular fraction.

Lipids extraction and separation. Total lipids were extracted from adipocytes and purified according to the Blight and Dyer [32] with minor modifications. All the solvents were of chemical grade and purchased from Merck, Germany; methanol was purchased from Honeywell, Germany. Then, 3 ml of chloroform : methanol solvent system (2:1, v/v) was added to 1 ml of adipocyte suspension. The mixture was vortexed during 30 seconds and centrifuged during 15 min at 2500 g. The chloroform layer was transferred to the flask. For total lipid extraction, aditional 2 ml of chloroform was added to residuary methanol fraction, vortexing during 30 second and centrifuged at the same condition. Both of chloroform layers was integrated for further aspiration. Lipid extract was separated on fractions by thin-layer chromatography on Polygram SIL G plates (Macherey-Nagel, Germany) using a solvent system of hexane: diethyl ether: acetic acid with component ratio by volume 85:15:1.

Cholesterol content measurement. After thin-layer chromatography lipid fraction separation, the free and esterified cholesterol were eluted by 3 ml of diethyl ether with further solvent evaporation. The dry residue was assayed on Carlo Erba HRGC 5300 gas chromatograph (Italy) on a glass column (0.5 m) packed with 1.5% OV-1 on 80–100 mesh Chimalite at 250°C using purified cholesterol standard (Sigma-Aldrich, Germany).

Phospholipid composition determination. After the thin-layer chromatography lipid fraction separation, the phospholipid fraction was transferred into solvent mixture of chloroform (2 ml), methanol (1 ml) and distilled water (0.8 ml). After 1 min vortexing and 3 min centrifugation at 1000 g, the lower chloroform layer was transferred to a flask, evaporated and solved in 0.15 ml of benzene for further individual phospholipids (PL) content analysis.

The individual PL content was determined by 2-dimensions (2-D) thin-layer chromatography on Alugram SIL G plates (Macherey-Nagel, Germany). Solvent system for the first dimension contained chloroform: methanol: ammonia: benzene (65:30:6:10, v/v), and the second dimension consisted of chloroform: methanol: acetic acid: water: acetone (5:1:1:0.5:2 v/v) [33, 34]. PL spots were identified using the natural PL standards of phosphatidylcholine, phosphatidylethanolamine, sphingomyelin, phosphatidylinositol, phosphatidylserine, lysophosphatidylcholine (Avanti Polar Lipids, USA). The amount of individual identified PL was estimated by colorimetric measurement of inorganic phosphate [P(i)] using the Vaskovskiy and Kostetskiy method [35] and determined spectrophotometrically as their phosphomolybdenum blue complex in the 815-nm wavelength region using spectrophotometer Spekol 211.

Statistical analysis. The data are presented as mean values ± standard errors of the means (SEM). After analyzing the set of experimental data, we chose a non-parametric analog of the ANOVA test - Kruskal–Wallis test for multiple comparisons to detect the general effects of diet, age, and treatment. For further pairwise comparison between groups, the Mann-Whitney test was used. The statistical of significance was determined at $P < 0.05$ and $P < 0.1$.

Results and Discussion

Animal experimental model. On the 24th week of experiment, the average weight of 10-m.o. and 24-m.o. HFD rats was 500.0 ± 11.5 g and 593.4 ± 24.2 g whereas the weight of control rats from the same age groups was 446.5 ± 20.4 g and 471.80 ± 10.34 g respectively. Fig. 1 demonstrates the dynamics of changes of body weight (*A, B*) and results of oral glucose tolerance test (*C, D*) of two age groups of animals.

In this study we also demonstrated that the rats from different age groups had different response to glucose loading and the results of the oral glucose

tolerance test for 10-m.o. and 24-m.o. animals are presented in the Fig. 1 (*C, D*). Test results demonstrated the absence of a statistically significant difference between the blood glucose concentrations of young and old control rats at the beginning of the test as well as at 150 min. The older animals receiving HFD had worse ability for glucose utilization than younger rats which were dietary fats overloaded and their blood glucose concentrations in 150 min after *per-os* glucose administration were 8.23 ± 0.40 and 6.40 ± 0.19 mmol/l in 24-m.o. and 10-m. o. rats respectively ($P = 0.0006$). The test results showed that at all time points, there was a statistically significant difference between control animals and HFD rats ($P < 0.05$). However, such a difference was absent in 10-m.-o. rats at 45 min of measurement.

Plasma lipoprotein profile. Our study showed that with aging, cholesterol levels in the composition of both low- and high-density lipoproteins did not undergo significant changes. However, the obtained data proved the decisive effect of nutrition on the plasma lipoprotein profile of young rats. The long-term dietary fats overload favors the disturbance of lipoprotein profile of 10-month-old rat plasma: almost 1.5 times increase of the content of plasma LDL-Chol compared to control group ($P = 0.026$) was observed, whereas just a tendency to HDL-Chol decrease was noted ($P = 0.129$). NSE treatment reduced LDL-Chol level ($P = 0.003$) and caused slight growth of HDL-Chol ($P = 0.082$) content in plasma of younger HFD-rats.

Fig. 2 demonstrates the results of plasma LDL-Chol and HDL-Chol measurement.

Long-term high-fat diet had no effect on the concentration of LDL-Chol in the plasma of 24-m.o. rats (Fig. 2). It is known that obesity is accompanied by an increased level of LDL-Chol and a direct correlation between weight gain and LDL-Chol content in the blood plasma is considered as one of the risk factors for cardiovascular diseases. However, research in recent years highlights a more complex relationship between these indicators [14]. In particular, it is suggested that the loss of the correlation between body mass index or waist circumference and the level of plasma LDL-Chol may be associated with metabolic disorders, particularly as a result of aging and be a sign of progressive metabolic disorders accompanying obesity [14].

Thus, the obtained data may indicate that the decrease in LDL-Chol level in young rats with diet-induced obesity when using NSE also occurs due to the intensification of the reverse cholesterol trans-
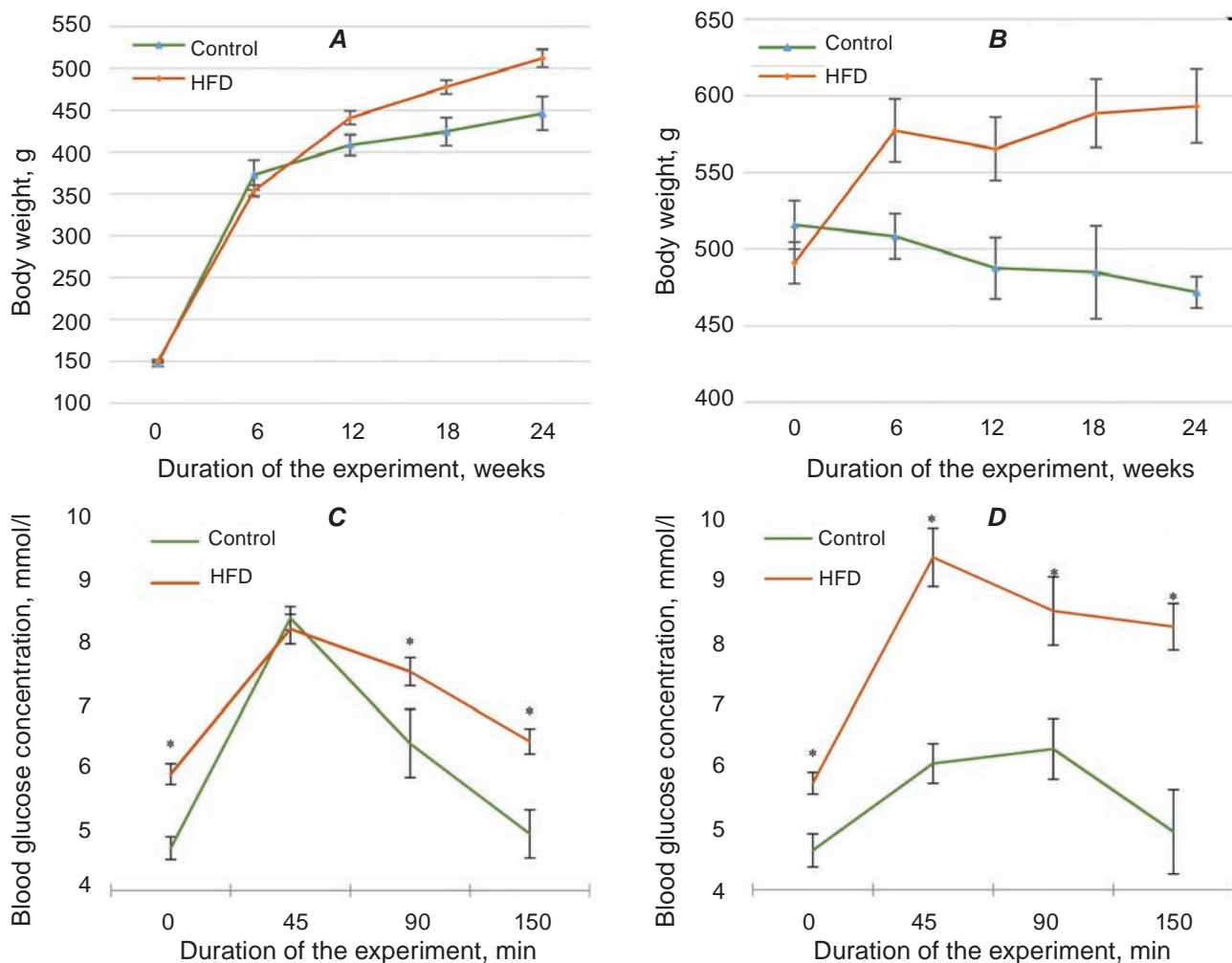
*Fig. 1. The influence of HFD on dynamics of body weight changes of two age groups of rats: **A**, **B** – body weight of 10-month-old and 24-month-old respectively; **C**, **D** –results of oral glucose tolerance test of 10-month-old and 24-month-old rats respectively. Values represented mean ± SEM. *P < 0.05 compared to the "Control" of the same age group*

port, against the background of which an increase in HDL-Chol is observed. One of the main functions of HDL is their ability to promote the reverse transport of cholesterol, absorption of excess cholesterol by peripheral cells and transport to the liver for further elimination. This process is considered the main antiatherogenic effect of HDL [36]. Thus, we hypothesize that the positive influence of NSE on increase in the level of HDL-Chol in the plasma of young HFD rats contributes to the intensification of reverse cholesterol transport and potentially reduces the atherogenic effect of obesity. This effect should be studied better due to the low statistical significance ($P = 0.129$) caused by the result's heterogeneity.

For decades, the hypothesis of a reverse correlation between the content of HDL-Chol in plasma and coronary heart disease was used as the basis for studying the interrelation between the lipoprotein profile and the risks of complications from the cardiovascular system, in particular, the development of coronary heart disease [37, 38]. Although an inverse correlation between plasma HDL-Chol levels and cardiovascular disease risk has been reasonably postulated for many years, recent studies suggest that the antiatherogenic functions of HDL may be independent of their plasma levels [39]. Extraordinarily high and too-low concentrations of HDL-Chol in plasma are associated with increased mortality [40, 41]. Thus, assessing HDL function, characterized as the ability to facilitate the removal of cholesterol from cells, may offer a better prediction of cardiovascular disease than HDL levels alone. Fol-
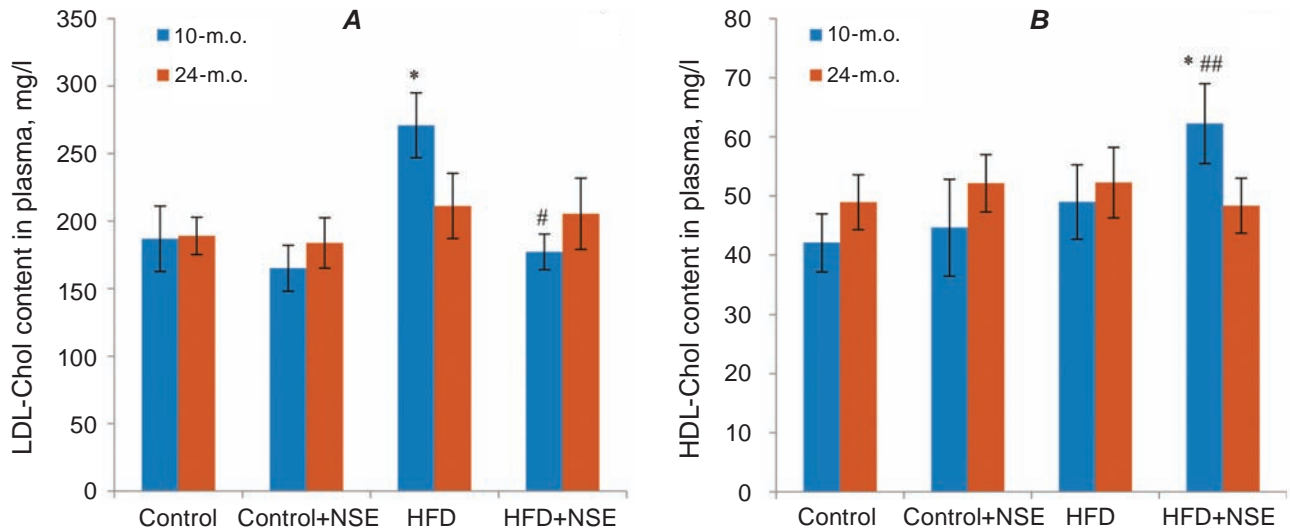
Fig. 2. Plasma lipoprotein profile of 10-month-old and 24-month-old rats: **A** – LDL-Chol content; **B** – HDL-Chol contentment. Values represented mean±SEM. *P < 0.05 compared to the Control of the same age group, #P < 0.05 and ##P < 0.1 compared to the HFD of the same age group

lowing this idea, it was recently demonstrated that the assessment of cholesterol efflux capacity (CEC) predicts the degree of atherosclerosis in humans [39].

Analyzing the obtained data, we also assume that one of the directions of NSE action may be the positive regulation of enzymatic activity, particularly of lipoprotein lipase (LPL). Aging affects lipoprotein recycling, reducing plasma LPL activity by 55–60%. It is similar to the metabolic effect that obesity has on LPL [42]. It is also known that lecithin-cholesterol acyltransferase (LCAT) is one of the key enzymes in HDL metabolism, and the increase in its activity increases HDL-Chol content and reduces the level of proatherogenic apoB-containing lipoproteins. Our previous studies showed the intensification of cholesterol metabolism processes in various tissues [23, 24] under the action of NSE. Also, *in vitro* studies showed the ability of saturated representatives of NAEs to modulate LCAT activity [43]. Therefore, we assume that the positive regulation of LCAT by NSE determines its effect on the lipoprotein profile of blood plasma. In favor of this hypothesis, the data of this study on the effect of NSE on cholesterol content in adipocytes, which are presented below, testify.

Adiponectin level. The changes of adiponectin concentration in 10-m.o. and 24-m.o. rat plasma are shown in Fig. 3.

The findings of our study underscore the significant influence of age on adiponectin levels in rat plasma. In particular, 24-month-old rats displayed
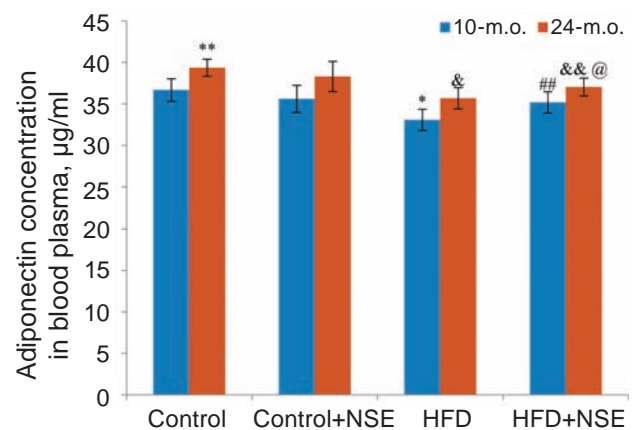

Fig. 3. Adiponectin content in blood plasma 10-month-old and 24-month-old rats. Values represented mean±SEM. *P < 0.05 compared to the 10-m.o. Control group; **P < 0.1 compared to the 10-m.o. Control group; #P < 0.05 compared to the 10-m.o. HFD group; &P < 0.05 compared to the 24-m.o. Control group; &&P < 0.1 compared to the 24-m.o. Control group; @P < 0.05 compared to the 24-m.o. HFD group

an adiponectin level that was more than 7% higher than their 10-month-old counterparts (P = 0.067). Prolonged HFD reduced plasma adiponectin concentration compared to control animals in both the younger (P = 0.034) and elder (P = 0.04) groups. The administration of NSE notably contributed to normalizing adiponectin levels in HFD-rats from

89

younger age group ($P = 0.095$), and did not demonstrated statistically significant changes in 24-month-old HFD-rats ($P = 0.269$).

Adiponectin is an adipokine specifically and abundantly expressed in adipose tissues and improves insulin resistance. According to different studies, plasma levels of adiponectin decrease in obesity, type-2 diabetes, and coronary artery disease [8, 9]. Thus, our results correlate with literature data.

The results of studies in recent years show that the additional administration of adiponectin to animals with HFD-induced insulin resistance contributes to the reduction of ectopic lipid deposits and protein kinase activity in the liver and skeletal muscles and restores their insulin sensitivity [9]. Increased triacylglycerol absorption in epididymal white adipose tissue was shown, which was explained by increased lipoprotein lipase activity. Adiponectin has been suggested to mediate these effects by promoting triacylglycerol accumulation in white adipose tissue, likely by stimulating LPL and stimulation of activation of AMP-activated kinase (AMPK-stimulation) in muscle, leading to increased muscle fat oxidation [9].

Research conducted over the years on rodents has shown that adiponectin deficiency leads to glucose intolerance and hyperlipidemia [10, 11]. Interestingly, induced adiponectin deficiency results in more severe systemic insulin resistance and hyperlipidemia and reduced survival compared to genetically determined deficiency. It suggests that specific compensatory mechanisms may be absent with induced deficiency [11]. Therefore, our study's finding that increasing adiponectin levels in the blood plasma of younger HFD rats is an essential effect of NSE for correcting diet-induced metabolic disorders, holds significant implications for future research and treatments.

It has been clinically established that high adiponectin levels are observed in long-lived people and some of their descendants. Therefore, it is reasonable to assume that adiponectin may be a critically important factor that affects health indicators and life expectancy [12]. Research results [12] convincingly confirm that adiponectin contributes to the normalization of metabolism, maintaining adequate fat distribution, reducing adipose tissue inflammation and decelerating inflammation and fibrosis in the liver. Aged mice with overexpression of adiponectin showed significant improvement in glucose and lipid homeostasis [12]. In our study, there is a trend toward normalization of adiponectin levels in rats of the older age group; however, due to the heterogeneity of the data, a statistically significant difference was not established. Therefore, this indicator requires more careful study.

It is also important to note that the effect of adiponectin on the inflammatory process has been established. The adiponectin level in mammals is inversely proportional to the production of pro-inflammatory cytokines, particularly tumor necrosis factor TNFα [44, 45]. It is noted that high levels of adiponectin correlate with suppression of inflammatory processes of various localization, in particular vascular and adipose. PPARγ (Peroxisome proliferator-activated receptor-γ) is a transcription factor expressed by adipocytes and regulates adipogenesis and lipid deposition [15]. Based on the obtained results and our previous works [46, 47, 70], we assume that the positive effect of NSE on increasing the level of adiponectin in the blood plasma of HFD rats of different ages may be associated with an anti-inflammatory effect, in particular, a decrease in the level of TNFα and an increase transcriptional activity of PPARγ. According to some studies, PPARγ activation results in an increase in the ability of adipocytes to absorb oxidized LDL, reducing their circulating level [15]. In particular, the confirmation of such an effect in our study can be a decrease in the level of plasma LDL-Chol in young HFD rats after NSE treatment.

Adipocyte cholesterol content. The results of total cholesterol content determination in the adipocytes of rats from two age groups are shown in the Fig. 4.

The obtained experimental data showed that the cholesterol content in the rat adipose cells decreased with age. As experimental data showed, control animals of the older group had total cholesterol content in WAT cells reached only about 50% of the level of younger animal corresponding group (Fig. 4). As a result of a long-term HFD, the level of total cholesterol increased in fat cells of rats of both age groups compared to the values of control animals of the same age. As a result of NSE administration, total adipocyte cholesterol content of HFD rats decreased in both age groups, but in 10-m.o. rats this effect manifested more (Fig. 4).

Thus, increasing cholesterol content in adipocytes under long-term exposure to a diet rich in saturated fats is quite natural. This is because saturated fatty acids serve as a substrate for cholesterol synthesis, ensuring a constant supply. The high-fat
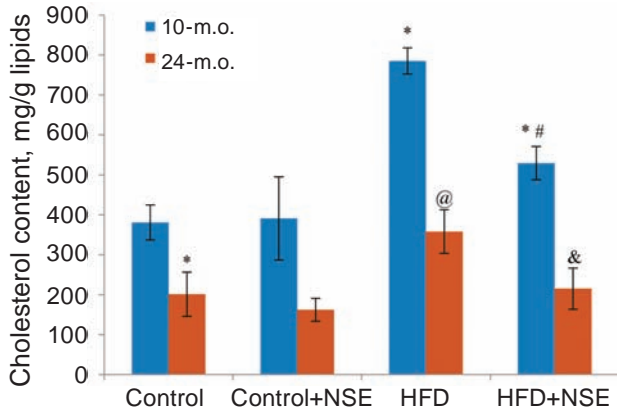
Fig. 4. Total cholesterol content in adipocytes of rats from different age groups. Values represented mean±SEM. *P < 0.05 compared to the 10-m.o. Control group; #P < 0.05 compared to the 10-m.o. HFD group; @P < 0.05 compared to the 24-m.o. Control group; &P < 0.05 compared to the 24-m.o. HFD group

diet in our study contained a small amount of actual cholesterol (0.57 mg/g) [48] but was rich in saturated fatty acids compared to standard chow [48]. We hypothesize that the increase in cholesterol levels in adipocytes of HFD rats of different ages is due to the overproduction of cholesterol from saturated fatty acids. These excess fatty acids are utilized by β-oxidation to produce acetyl-CoA, which is then involved in the cascade of cholesterol synthesis [49, 50].

Compared to young animals, a significant decrease in the level of cholesterol in adipocytes of rats of the older age group may indicate a decrease in the efficiency of cholesterol formation from saturated fatty acids and a decrease in the cholesterol-buffering capacity of adipocytes. It could have implications for the health and functioning of these

cells. Also, considering that cholesterol, along with phospholipids, are the main elements of both the cell membrane of the adipocyte and the membrane of lipid droplets, a decrease in the cholesterol content can lead to the thinning (depletion) of these membranes, and therefore result in the deterioration of their functions.

Since cholesterol is present in adipocytes in free and esterified forms, which have different functions, it is important to investigate the effect of a high-fat diet and aging on the fractional redistribution of cholesterol in adipocytes. Understanding this redistribution can provide insights into the metabolic changes that occur in response to diet and aging, and may have implications for developing interventions to manage cholesterol levels.

Table demonstrates different cholesterol fractions content in adipocytes of rats from two age groups.

These data show that in adipocytes of young and old HFD animals, there was a tendency to increase ($P = 0.135$ and 0.133 respectively) in the percentage of free cholesterol and a decrease in the proportion of esterified, compared with the control. NSE administration significantly decreased free cholesterol content in 10-m.o.-HFD-rat adipocytes and increased the level of esterified cholesterol ($P = 0.048$) but had no effect in 24-m.o.-rat group. Such results support our previous assumptions in previous studies [24] regarding the positive effect of NSE on cholesterol esterification in adipocytes during obesity and the possible mechanism of action. It is known that acylation is one of the main mechanisms of cholesterol removal, in particular, from cell membranes. Lecithin–cholesterol acyltransferase (LCAT) and acyl-CoA: cholesterol acyltransferase (ACAT) are the key enzymes this metabolic pathway catalyzes.

T a b l e. Different fraction cholesterol content in adipocytes of rats from two age groups

| Groups | Different fraction cholesterol content, % of total | | | |
| | 10-m.o. rats | | 24-m.o. rats | |
	Free cholesterol	Esterified cholesterol	Free cholesterol	Esterified cholesterol
Control	81.54 ± 2.99	18.46 ± 2.99	83.46 ± 3.6	16.54 ± 3.6
Control+NSE	81.58 ± 2.00	18.37 ± 2.00	83.65 ± 3.42	16.35 ± 3.42
HFD	86.73 ± 1.86	13.27 ± 1.86	87.38 ± 3.37	12.72 ± 3.34
HFD+NSE	79.99 ± 3.07#	20.01 ± 3.07#	85.38 ± 2.96	14.61 ± 2.97

Note. Values represented mean ± SEM. #P < 0.05 compared to the 10-month-old HFD group

The LCAT bonded the fatty acid from phosphatidylcholine in the sn-2 position to a lipoprotein cleave and transferred it onto the A-ring of cholesterol with further transesterification to the 3-β-hydroxyl group for cholesterol ester formation. Lysophosphatidylcholine is also formed as a by-product of the reaction [51]. In turn, ACAT uses a pool of free fatty acids for cholesterol esterification. However, LCAT is considered to have the primary transport function. We consider the positive regulation of this enzyme as one of the mechanisms of NSE's effect on cholesterol balance, which was already mentioned above.

Adipocyte phospholipid composition. Phospholipids play a critical role in both lipid droplet growth and adipogenesis [52]. Using 2D-thin-layer chromatography, we identified the main phospholipids in rat adipocytes and measured their content. Fig. 5 shows PL classes content in adipocytes of rats from different age groups.

Phosphatidylcholine (PC) is the primary phospholipid in mammalian cells, and in prevalence of tissues, PC is synthesized from choline via the CDP-choline pathway [53]. It is known that PC content typically should grow as a response to increased cholesterol levels in cell membranes [54]. As a result of the analysis, it was found that the amount of PC in adipocytes was about 2.5 times lower in rats aged 24 months compared with 10-m.o. rats ($P = 0.0008$). In the fat cells of younger animals with obesity complicated by IR, the PC content doubled ($P = 0.0625$), while in older rats, there was only 13%-growth of the amount of this phospholipid ($P = 0.095$).

An increase in the group of younger rats, the amount of PC, a quantitatively predominant phospholipid in adipocyte cell membranes, is associated with adipose tissue hypertrophy and proliferative processes against obesity-induced insulin resistance. The data obtained correlates with several other research papers confirming the increase in phosphatidylcholine content in adipose tissue due to obesity and insulin resistance [54]. On the other hand, a significant increase in the PC content in adipocytes on the background of a long-term HFD may lead to an increase in the rigidity of the cell membrane and a decrease in its plasticity and the consequence of such structural changes in the cell membrane receptor functions deterioration and, in particular, impaired insulin sensitivity. A significant reduction of PC in adipocytes of 24-m.o. rats compared to 10-m.o. animals are likely due to depletion of the plasma membrane phospholipid component adequately to the cholesterol proportion. It is known that PC also acts as a surfactant, which is necessary to stabilize the lipid droplet inside the adipocyte and prevent the coalescence of its contents [55]. Thus, the increase in PC in adipose cells of young HFD rats is due to the need to deposit a large amount of triacylglycerols. In old rats, this adipocyte function was impaired due to a decrease in the content of PC. The synthesis of PC to increase lipid droplets is regulated by localized activation of phosphocholine-cytidylyltransferase. PC a of its c cts as a surfactant necessary to stabilize a lipid droplet and prevent the coalescence ontents [55]. Therefore, we assume that the PC reduction in adipocytes of young HFD rats under NSE treatment may be associated with the downregulation of phosphocholine-cytidylyltransferase.

The decrease of PC level in older rat fat cells may also be associated with changes in the activity of phospholipases C and D. According to different authors [56-58], insulin stimulates the PC degradation in cells from insulin-sensitive tissues through the activation of phospholipases C and D activation. Because it is known that hyperinsulinemia is a characteristic marker of aging [59], the activity of phospholipases C and D in experimental animals probably increased also, which could lead to the intensification of PC transformation in adipocytes. Some authors published data on insulin-induced reduction of PC in cells of insulin-dependent tissue, focusing on the simultaneity of high-intensity *de novo* resynthesis of this phospholipid and, at the same time, note that in diabetic adipocytes, phospholipid imbalance is more likely to be due to defects in the synthesis pathways of phosphatidic acid, diacylglycerols and, in fact, phosphatidylcholine [60].

It is also known that membrane-bound phospholipase A2 activity is enhanced with aging, and it correlates well with the reduced fluidity of the membrane [61]. Lysophosphatidylcholine is a product of phospholipase A2-catalyzed PC hydrolysis. Our study also demonstrated that during aging, the amount of LPC in adipocytes increased and its numbers in 24-month-old rats exceeded more than twice the values characteristic of 10-m.o. animals ($P < 0.001$). A similar effect was observed as a result of long-term maintenance of experimental animals of the younger age group on an HFD ($P < 0.001$), while for the older age group, such changes were not found. Thus, one of the mechanisms of the decrease in the PC level and the increase of LPC in rat adipo-
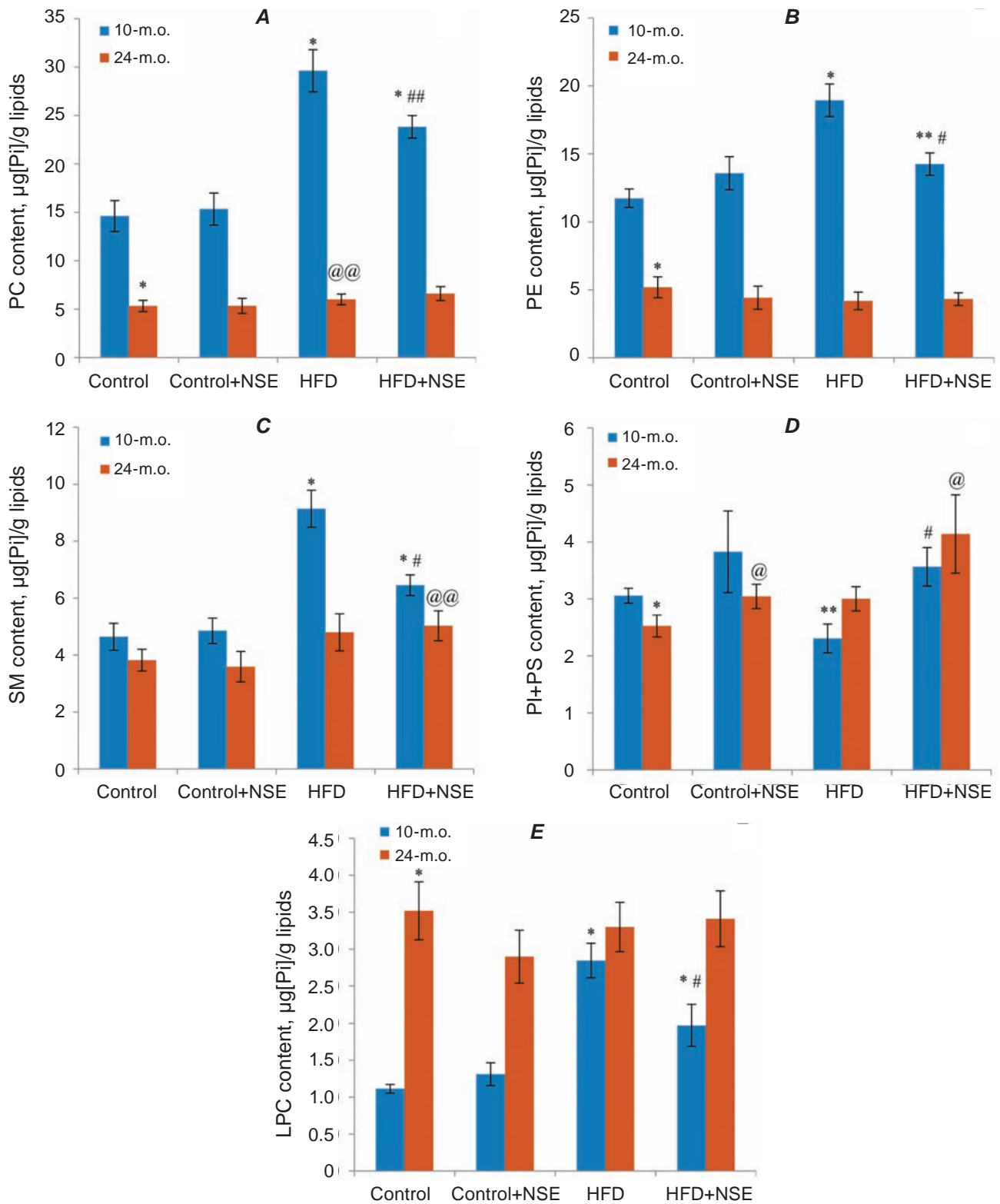
*Fig. 5. Phospholipid classes content in adipocytes of rats from different age groups: **A** – Phosphatidylcholine (PC), **B** – Phosphatidylethanolamine (PE), **C** – Sphingomyelin (SM), **D** – Phosphatidylinositol with Phosphatidylserine (PI+PS), **E** – Lysophosphatidylcholine (LPC). Values represented mean ± SEM. *P < 0.05 and **P < 0.1 compared to the 10-month-old Control group; #P < 0.05 and ##P < 0.1 compared to the 10-month-old HFD group; @P < 0.05 and @@P < 0.1 compared to the 24-month-old Control group*

cytes during aging may be the intensification of the conversion of PC to LPC due to the increased activity of phospholipase A2. The administration of NSE positively affected the normalization of adipocyte LPC content in HFD rats aged 10 months ($P = 0.05$). Also, it contributed to the reduction of this phospholipid in control 24-m.o. animals, but the statistical significance level was insufficient ($P = 0.17$). This effect of NSE can be realized through the downregulation of phospholipase A2. Some publications also described saturated NAEs as regulators of phospholipase A2 activity [62].

The results of determining the content of individual phospholipids in rat adipocytes showed that the reference values of the amount of phosphatidylethanolamine in the older age group were twice less than in the younger age group ($P < 0.001$). In 10-m.o. HFD rats, the PE content was significantly higher than in control animals ($P < 0.001$). At the same time, no such statistically significant changes were observed between the groups of control and HFD rats at 24 months of age ($P = 0.158$). NSE administration for 14 days helped normalize the PE level in the adipose tissue cells of 10-m.o. rats from the HFD group ($P = 0.02$).

The PE content in the insulin-dependent tissue membranes correlates directly with insulin resistance. Some studies demonstrated that PE may affect the activity of hormone-sensitive lipase in adipose tissue; in particular, the growth of PE content can give rise to enzyme activity [63]. It is also known that the ratio of PC to PE plays a significant role in the formation and functioning of lipid droplets and the realization of the energetic activity of mitochondria, and it is inversely correlated with the sensitivity of tissues to insulin. Also, the analysis of recent studies shows that changes in the amount of PE in various tissues are involved in metabolic disorders, particularly insulin resistance, obesity, and atherosclerosis [64]. That is why the normalization of PE content in adipocytes of young obese rats under the NSE treatment may affect the enzyme activity and thus contribute to a decrease in IR development in young rats.

Phospholipid analysis showed that sphingomyelin (SM) content in rat fat cells tends to decrease with aging ($P = 0.12$). The prolonged exposure to dietary fats led to a statistically significant increase in SM content in younge rat adipocytes ($P < 0.001$) and a tendency to increase in older group ($P = 0.17$). In turn, the NSE administration contributed to the normalization of the content of this phospholipid in the younger age group of HFD ($P = 0.01$) animals and did not affect older HFD rats. Some studies indicate the correlation between the PL composition of cell membranes and markers of insulin resistance in insulin-sensitive tissues. In particular, some studies showed that in insulin-sensitive cells, the growth of the membrane SM content inhibits tyrosine kinase, which leads to insulin signaling impairment and contributes to the development of insulin resistance [65, 66]. There are also studies demonstrating that the content of SM in cell membranes correlates directly with its stiffness and influence on insulin sensitivity [66]. Thus, special attention should be paid to studying the mechanisms of direct or indirect effects of NSE on the reduction of SM content in adipocytes of 10-month-old HFD rats.

We showed that in adipocytes of rats aged 24 months, the total content of PI + PS was significantly lower than in 10-m.o. animals ($P = 0.015$). In HFD rats the content of these phospholipids decreased in younger animals (0.067) and tended to increase in older animals (0.11), compared with control rats of the appropriate age groups. The use of NSE was accompanied by an increase in the total content of PI + PS in the adipocytes of control animals from older age group ($P = 0.035$) and younger HFD rats ($P = 0.02$).

It is well known that PI and PS, as anionic phospholipids, are essential in determining the membrane-bound proteins' topology. Thus, protein kinase C (PKC) is one of the most studied transmembrane enzymes, which interacts stoichiometrically with PS in the C2 domain. In vitro studies [67] demonstrated the activation dependence of atypical PKC forms on the PS content. There is also evidence that atypical PKC forms (ζ and ι/λ) involved in activating the glucose-4 transporter translocation, in contrast to other isoforms of its enzyme, are regulated by PS content only [68]. In turn, it is well known that PI is a substrate for forming phosphoinositides, which are the modulators of tyrosine-dependent protein kinase activity [69]. Therefore, the significant decrease in PI and PS content in adipocytes of young HFD and old rats may indicate an adipocyte signaling disorder induced by obesity and aging.

Conclusions. Our study demonstrated that aging and chronic dietary fat overloading lead to changes in adipocyte cholesterol-phospholipid composition, plasma adiponectin level and lipoprotein profile. Prolonged HFD induced glucose intolerance

and led to changes in plasma adiponectin level and adipocyte cholesterol content in different age rats. Significant changes in plasma lipoprotein profile and adipocyte phospholipid composition were observed in younger rats, whereas not detected in elder animals after dietary fats overloading. Based on the study results, we can conclude that HFD causes significantly more pronounced negative changes in animals of the younger age group. At the same time, it does not contribute to such pronounced effects in older animals.

NSE administration positively affected the normalization of plasma adiponectin levels and adipocyte cholesterol content in both age HFD groups. It significantly impacted the normalization of plasma lipoprotein profile and adipocytes phospholipid composition of 10-m.o. HFD rats as well as anionic phospholipids restoring in 24-m.o. rats.

We assume that the obtained results testify mainly in favor of the previously established membrane-stabilizing and anti-inflammatory effects of NSE. The positive effect on investigated parameters makes NSE a prospective agent for treating diet-induced and age-related metabolic disorders threatening cardiovascular diseases.

ЕФЕКТ ЗАСТОСУВАННЯ N-СТЕАРОЇЛЕТАНОЛАМІНУ НА ІНДУКОВАНІ ДІЄТОЮ І СТАРІННЯМ ЗМІНИ У РІВНІ ЛІПОПРОТЕЇНІВ, АДИПОНЕКТИНУ ТА ХОЛЕСТЕРОЛ-ФОСФОЛІПІДНІЙ КОМПОЗИЦІЇ АДИПОЦИТІВ ЩУРІВ

О. С. Ткаченко✉, Г. В. Косякова

Інститут біохімії ім. О. В. Палладіна НАН України, Київ Україна; ✉e-mail: 888oksana.tkachenko@gmail.com

Адипонектин – це адипокін, який секретується жировою тканиною, впливає на метаболізм ліпопротеїнів, зменшує інсулінорезистентність. Відомо, що його рівень знижується при ожирінні та серцево-судинних захворюваннях. Фосфоліпіди та холестерол – це основні компоненти клітинної мембрани, що відіграють ключову роль в акумулюючій та секреторній функціях адипоцитів. N-стеароїлетаноламін (NSE) – це мінорний ліпід, що впливає на ліпідну композицію клітинних мембран. Мета нашого дослідження – дослідити рівень адипонектину та холестеролу в складі ліпопротеїнів низької та високої щільності і холестерол-фосфоліпідну композицію адипоцитів у щурів різного віку (10 та 24 місяці) з індукованим високожировою дієтою ожирінням та ефект NSE на зазначені параметри. Показано, що старіння та хронічне навантаження насиченими жирами приводять до ожиріння, що супроводжується порушенням толерантності до глюкози, проявом дисліпідемії та зміною рівня адипонектину в плазмі крові щурів двох вікових груп. Тривала високожирова дієта призводила до зниження рівня адипонектину в плазмі крові та зростання вмісту холестерину в адипоцитах у щурів різного віку. У молодих щурів спостерігалося значне підвищення рівня холестеролу у складі ліпопротеїнів низької щільності (ЛПНЩ-Хол) і ключових

фосфоліпідів адипоцитів (фосфатидилхоліну, фосфатидилетаноламіну, сфінгомієліну та лізофосфатидилхоліну), у той же час таких змін не виявлено у тварин старшої вікової групи. Зменшення вмісту аніонних фосфоліпідів (фосфатидилінозитол + фосфатидилсерин) також було показано у 10-місячних щурів, які утримувались на високожировій дієті порівняно з контрольними тваринами. Введення NSE позитивно вплинуло на нормалізацію рівня адипонектину в обох вікових групах. Також застосування NSE суттєво вплинуло на зниження рівня ЛПНЩ-Хол та зростання концентрації холестеролу у складі ліпопротеїнів високої щільності (ЛПВЩ-Хол) у плазмі крові 10-місячних щурів, а також на фосфоліпідний склад молодих щурів із ожирінням та відновлення аніонної фосфоліпідної компоненти в адипоцитах щурів віком 24-місяці. Позитивний вплив NSE на досліджувані параметри, дозволяє розглядати його як перспективний засіб для корекції дієт-індукованих та пов'язаних зі старінням метаболічних розладів, які провокують розвиток серцево-судинних захворювань.

К л ю ч о в і   с л о в а: N-стеароїлетаноламін, жирова тканина, дисліпідемія, старіння, холестерол, фосфоліпіди, адипонектин, ліпопротеїни.

References

1. Powell-Wiley TM, Poirier P, Burke LE, Després JP, Gordon-Larsen P, Lavie CJ, Lear SA, Ndumele CE, Neeland IJ, Sanders P, St-Onge MP. Obesity and Cardiovascular Disease: A Scientific Statement From the American Heart Association. *Circulation.* 2021; 143(21): e984-e1010.

2. Stadler JT, Marsche G. Obesity-Related Changes in High-Density Lipoprotein Metabolism and Function. *Int J Mol Sci.* 2020; 21(23): 8985.

3. Stadler JT, Lackner S, Mörkl S, Trakaki A, Scharnagl H, Borenich A, Wonisch W, Mangge H, Zelzer S, Meier-Allard N, Holasek SJ, Marsche G. Obesity Affects HDL Metabolism, Composition and Subclass Distribution. *Biomedicines.* 2021; 9(3): 242.

4. Dziuba OS, Chernyshenko VO, Hudz IeA, Kasatkina LO, Chernyshenko TM, Klymenko PP, Kosiakova HV, Platonova TM, Hula NM, Lugovskoy EV. Blood coagulation and aortic wall integrity in rats with obesity-induced insulin resistance. *Ukr Biochem J.* 2018; 90(2): 14-23.

5. Tchoukalova YD, Sarr MG, Jensen MD. Measuring committed preadipocytes in human adipose tissue from severely obese patients by using adipocyte fatty acid binding protein. *Am J Physiol Regul Integr Comp Physiol.* 2004; 287(5): R1132-R1140.

6. Elgazar-Carmon V, Rudich A, Hadad N, Levy R. Neutrophils transiently infiltrate intra-abdominal fat early in the course of high-fat feeding. *J Lipid Res.* 2008; 49(9): 1894-1903.

7. Kintscher U, Hartge M, Hess K, Foryst-Ludwig A, Clemenz M, Wabitsch M, Fischer-Posovszky P, Barth TF, Dragun D, Skurk T, Hauner H, Blüher M, Unger T, Wolf AM, Knippschild U, Hombach V, Marx N. T-lymphocyte infiltration in visceral adipose tissue: a primary event in adipose tissue inflammation and the development of obesity-mediated insulin resistance. *Arterioscler Thromb Vasc Biol.* 2008; 28(7): 1304-1310.

8. Satoshi Hirako. Adiponectin. Handbook of Hormones (Second Edition). Academic Press, 2021. P. 577-579.

9. Li X, Zhang D, Vatner DF, Goedeke L, Hirabara SM, Zhang Y, Perry RJ, Shulman GI. Mechanisms by which adiponectin reverses high fat diet-induced insulin resistance in mice. *Proc Natl Acad Sci USA.* 2020; 117(51): 32584-32593.

10. Nawrocki AR, Rajala MW, Tomas E, Pajvani UB, Saha AK, Trumbauer ME, Pang Z, Chen AS, Ruderman NB, Chen H, Rossetti L, Scherer PE. Mice lacking adiponectin show decreased hepatic insulin sensitivity and reduced responsiveness to peroxisome proliferator-activated receptor gamma agonists. *J Biol Chem.* 2006; 281(5): 2654-2660.

11. Xia JY, Sun K, Hepler C, Ghaben AL, Gupta RK, An YA, Holland WL, Morley TS, Adams AC, Gordillo R, Kusminski CM, Scherer PE. Acute loss of adipose tissue-derived adiponectin triggers immediate metabolic deterioration in mice. *Diabetologia.* 2018; 61(4): 932-941.

12. Li N, Zhao S, Zhang Z, Zhu Y, Gliniak CM, Vishvanath L, An YA, Wang MY, Deng Y, Zhu Q, Shan B, Sherwood A, Onodera T, Oz OK, Gordillo R, Gupta RK, Liu M, Horvath TL, Dixit VD, Scherer PE. Adiponectin preserves metabolic fitness during aging. *Elife.* 2021; 10: e65108.

13. Manual Kollareth DJ, Chang CL, Zirpoli H, Deckelbaum RJ. Chapter 21 - Molecular mechanisms underlying effects of n−3 and n−6 fatty acids in cardiovascular diseases. Ed. Ntambi JM. Lipid Signaling and Metabolism. Academic Press, 2020. P. 427-453.

14. Laclaustra M, Lopez-Garcia E, Civeira F, Garcia-Esquinas E, Graciani A , Guallar-Castillon P, Banegas JR, Rodriguez-Artalejo F. LDL Cholesterol Rises With BMI Only in Lean Individuals: Cross-sectional U.S. and Spanish Representative Data. *Diabetes Care.* 2018; 41(10): 2195-2201.

15. Chui PC, Guan HP, Lehrke M, Lazar MA. PPARgamma regulates adipocyte cholesterol metabolism via oxidized LDL receptor 1. *J Clin Invest.* 2005; 115(8): 2244-2256.

16. Chang W, Hatch GM, Wang Y, Yu F, Wang M. The relationship between phospholipids and insulin resistance: From clinical to experimental studies. *J Cell Mol Med.* 2019; 23(2): 702-710.

17. Kosiakova GV, Gula NM. The N-stearoylethanolamine effect on the NO-synthase way of nitrogen oxide formation and phospholipid composition of erythrocyte membranes in rats with streptozotocine diabetes. *Ukr Biokhim Zhurn.* 2007; 79(6): 53-59. (In Ukrainian).

18. Goridko TM, Gula NM, Stogniy NA, Meged OF, Klimashevsky VM, Shovkun SA, Kindruk NL, Berdyshev AG. Influence of N-stearoylethanolamine on the lipid peroxidation process and lipid composition of the rat liver under acute morphine intoxication. *Ukr Biokhim Zhurn.* 2007; 79(5): 175-185. (In Ukrainian).

19. Gula NM, Chumak AA, Berdyshev AG, Meged EF, Goridko TM, Kindruk NL, Kosyakova GV, Zhukov OD. Anti-inflammatory effect of N-stearoylethanolamine in experimental burn injury in rats. *Ukr Biokhim Zhurn.* 2009; 81(2): 107-116. (In Ukrainian).

20. Goridko TM, Kosiakova GV, Berdyschev AG, Bazylyanska VR, Margitich VM, Gula NM. The influence of N-stearoylethanolamine on the activity of antioxidant enzymes and on the level of stable NO metabolites in the rat testes and blood plasma at the early stages of streptozotocine-induced diabetes. *Ukr Biokhim Zhurn.* 2012; 84(3): 37-43. (In Ukrainia).

21. Onopchenko OV, Kosiakova GV, Goridko TM, Klimashevsky VM, Hula NM. The effect of N-stearoylethanolamine on liver phospholipid composition of rats with insulin resistance caused by alimentary obesity. *Ukr Biochem J.* 2014; 86(1): 101-110. (In Ukrainian).

22. Onopchenko OV, Kosiakova GV, Oz M, Klimashevsky VM, Gula NM. N-stearoylethanolamine restores pancreas lipid composition in obesity-induced insulin resistant rats. *Lipids.* 2015; 50(1): 13-21.

23. Onopchenko OV, Kosiakova GV, Meged EF, Klimashevsky VM, Hula NM. The effect of N-stearoylethanolamine on cholesterol content, fatty acid composition and protein carbonylation level in rats with alimentary obesity-induced insulin resistance. *Ukr Biochem J.* 2014; 86(6): 119-128.

24. Tkachenko O, Kosiakova H, Klimashevsky V, Berdyshev A, Hula N. The Effect of N-Stearoylethanolamine on the Adipocyte Fatty Acid Composition of Different Age Rats with Obesity-Induced Insulin Resistance. *Eureca: Life Sci.* 2020; (2): 10-23.

25. Tkachenko OS, Hudz IeA, Kosiakova HV, Klymenko PP, Stohnii YM, Didkivskyi VA, Chernyshenko TM, Chernyshenko VO, Platonova TM. Protective action of N-stearoylethanolamine on blood coagulation and arterial changes in spontaneously hypertensive rats fed cholesterol-rich diet. *Ukr Biochem J.* 2020; 92(2): 60-70.

26. Collier GR, Chisholm K, Sykes S, Dryden PA, O'Dea K. More severe impairment of oral than intravenous glucose tolerance in rats after eating a high fat diet. *J Nutr.* 1985; 115(11): 1471-1476.

27. Epps DE, Natarajan V, Schmid PC, Schmid HO. Accumulation of N-acylethanolamine glycero-phospholipids in infarcted myocardium. *Biochim Biophys Acta.* 1980; 618(3): 420-430.

28. Rodbell M. Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. *J Biol Chem.* 1964; 239: 375-380.

29. Mueller WM, Gregoire FM, Stanhope KL, Mobbs CV, Mizuno TM, Warden CH, Stern JS, Havell PJ. Evidence that glucose metabolism regulates leptin secretion from cultured rat adipocytes. *Endocrinology.* 1998; 139(2): 551-558.

30. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol.* 1959; 37(8): 911-917.

31. Vaskovsky VE, Terekhova TA. HPTLC of phospholipid mixtures containing phosphatidyl-

glycerol. *J High Resolut Chromatogr Chromatogr Commun.* 1979; 2: 671-672.

32. Svetashev VI, Vaskovsky VE. A simplified technique for thin-layer microchromatography of lipids. *J Chromatogr.* 1972; 67(2): 376-378.

33. Vaskovsky VE, Kostetsky EY, Vasendin IM. A universal reagent for phospholipid analysis. *J Chromatogr.* 1975; 114(1): 129-141.

34. Cerqueira NM, Oliveira EF, Gesto DS, Santos-Martins D, Moreira C, Moorthy HN, Ramos MJ, Fernandes PA. Cholesterol Biosynthesis: A Mechanistic Overview. *Biochemistry.* 2016; 55(39): 5483-5506.

35. Hussain MM, Strickland DK, Bakillah A. The mammalian low-density lipoprotein receptor family. *Annu Rev Nutr.* 1999; 19: 141-172.

36. Ouimet M, Barrett TJ, Fisher EA. HDL and Reverse Cholesterol Transport. *Circ Res.* 2019; 124(10): 1505-1518.

37. Wilson PW, Abbott RD, Castelli WP. High density lipoprotein cholesterol and mortality. The Framingham Heart Study. *Arteriosclerosis.* 1988; 8(6): 737-741.

38. Rader DJ, Hovingh GK. HDL and cardiovascular disease. *Lancet.* 2014; 384(9943): 618-625.

39. Favari E, Chroni A, Tietge UJF, Zanotti I, Escolà-Gil JC, Bernini F. Cholesterol efflux and reverse cholesterol transport. *Handb Exp Pharmacol.* 2015; 224: 181-206.

40. Madsen CM, Varbo A, Nordestgaard BG. Extreme high high-density lipoprotein cholesterol is paradoxically associated with high mortality in men and women: two prospective cohort studies. *Eur Heart J.* 2017; 38(32): 2478-2486.

41. Rodriguez A. High HDL-Cholesterol Paradox: SCARB1-LAG3-HDL Axis. *Curr Atheroscler Rep.* 2021; 23(1): 5.

42. Mc Auley MT. Effects of obesity on cholesterol metabolism and its implications for healthy ageing. *Nutr Res Rev.* 2020; 33(1): 121-133.

43. Van Meer G, Voelker DR, Feigenson GW. Membrane lipids: where they are and how they behave. *Nat Rev Mol Cell Biol.* 2008; 9(2): 112-124.

44. Wang Y, Wang X, Lau WB, Yuan Y, Booth D, Li JJ, Scalia R, Preston K, Gao E, Koch W, Ma XL. Adiponectin inhibits tumor necrosis factor-α-induced vascular inflammatory response via caveolin-mediated ceramidase recruitment and activation. *Circ Res.* 2014; 114(5): 792-805.

45. Yu H, Gao X, Ge Q, Tai W, Hao X, Shao Q, Fang Z, Chen M, Song Y, Gao W, Liu G, Du X, Li X. Tumor necrosis factor-α reduces adiponectin production by decreasing transcriptional activity of peroxisome proliferator-activated receptor-γ in calf adipocytes. *J Dairy Sci.* 2023; 106(7): 5182-5195.

46. Berdyshev AG, Kosiakova HV, Onopchenko OV, Panchuk RR, Stoika RS, Hula NM. N-Stearoylethanolamine suppresses the pro-inflammatory cytokines production by inhibition of NF-κB translocation. *Prostaglandins Other Lipid Mediat.* 2015; 121(Pt A): 91-96.

47. Onopchenko OV, Kosiakova GV, Klimashevsky VM, Hula NM. The effect of N-stearoylethanolamine on plasma lipid composition in rats with experimental insulin resistance. *Ukr Biochem J.* 2015; 87(1): 46-54.

48. Onopchenko OV, Kosiakova GV, Goridko TM, Berdyshev AG, Mehed OF, Hula NM. The effect of N-stearoylethanolamine on the activity of antioxidant enzymes, content of lipid peroxidation products and nitric oxide in the blood plasma and liver of rats with induced insulin-resistance. *Ukr Biokhim Zhurn.* 2013; 85(5): 88-96. (In Ukrainian).

49. Rousset X, Vaisman B, Amar M, Sethi AA, Remaley AT. Lecithin: cholesterol acyltransferase – from biochemistry to role in cardiovascular disease. *Curr Opin Endocrinol Diabetes Obes.* 2009; 16(2): 163-171.

50. Yeagle PL. Cholesterol and the cell membrane. *Biochim Biophys Acta.* 1985; 822(3-4): 267-287.

51. Yeagle PL. Modulation of membrane function by cholesterol. *Biochimie.* 1991; 73(10): 1303-1310.

52. Esteve Ràfols M. Adipose tissue: cell heterogeneity and functional diversity. *Endocrinol Nutr.* 2014; 61(2): 100-112.

53. Kennedy EP, Weiss SB. The function of cytidine coenzymes in the biosynthesis of phospholipides. *J Biol Chem.* 1956; 222(1): 193-214.

54. Zeghari N, Younsi M, Meyer L, Donner M, Drouin P, Ziegler O. Adipocyte and erythrocyte plasma membrane phospholipid composition and hyperinsulinemia: a study in nondiabetic and diabetic obese women. *Int J Obes Relat Metab Disord.* 2000; 24(12): 1600-1607.

55. Krahmer N, Guo Y, Wilfling F, Hilger M, Lingrell S, Heger K, Newman HW, Schmidt-Supprian M, Vance DE, Mann M, Farese RV Jr, Walther TC. Phosphatidylcholine synthesis

for lipid droplet expansion is mediated by localized activation of CTP:phosphocholine cytidylyltransferase. *Cell Metab.* 2011; 14(4): 504-515.

56. Macaulay SL, Larkins RG. Insulin stimulates turnover of phosphatidylcholine in rat adipocytes. *Mol Cell Biochem.* 1994; 136(1): 23-28.

57. Donchenko V, Zannetti A, Baldini PM. Insulin-stimulated hydrolysis of phosphatidylcholine by phospholipase C and phospholipase D in cultured rat hepatocytes. *Biochim Biophys Acta.* 1994; 1222(3): 492-500.

58. Cazzolli R., Huang P, Teng S, Hughes WE. Measuring phospholipase D activity in insulin-secreting pancreatic beta-cells and insulin-responsive muscle cells and adipocytes. *Methods Mol Biol.* 2009; 462: 241-251.

59. Kurauti MA, Ferreira SM, Soares GM, Vettorazzi JF, Carneiro EM, Boschero AC, Costa-Júnior JM. Hyperinsulinemia is associated with increasing insulin secretion but not with decreasing insulin clearance in an age-related metabolic dysfunction mice model. *J Cell Physiol.* 2019; 234(6): 9802-9809.

60. LeRoith D, Taylor SI, Olefsky JM. Diabetes mellitus: a fundamental and clinical text. Lippincott Williams & Wilkins, 2004. P. 303.

61. Petkova DH, Momchilova AB, Koumanov KS. Age-related changes in rat liver plasma membrane phospholipase A2 activity. *Exp Gerontol.* 1986; 21(3): 187-193.

62. Zolese G, Wozniak M, Mariani P, Saturni L, Bertoli E, Ambrosini A. Different modulation of phospholipase A2 activity by saturated and monounsaturated N-acylethanolamines. *J Lipid Res.* 2003; 44(4): 742-753.

63. Severson DL, Hurley B. Stimulation of the hormone-sensitive triacylglycerol lipase from adipose tissue by phosphatidylethanolamine. *Biochim Biophys Acta.* 1985; 845(2): 283-291.

64. Van der Veen JN, Kennelly JP, Wan S, Vance JE, Vance DE, Jacobs RL. The critical role of phosphatidylcholine and phosphatidylethanolamine metabolism in health and disease. *Biochim Biophys Acta Biomembr.* 2017; 1859(9 Pt B): 1558-1572.

65. Gavrilova NJ, Setchenska MS, Markovska TT, Momchilova-Pankova AB, Koumanov KS. Effect of membrane phospholipid composition and fluidity on rat liver plasma membrane tyrosine kinase activity. *Int J Biochem.* 1993; 25(9): 1309-1312.

66. Torretta E, Barbacini P, Al-Daghri NM, Gelfi C. Sphingolipids in Obesity and Correlated Co-Morbidities: The Contribution of Gender, Age and Environment. *Int J Mol Sci.* 2019; 20(23): 5901.

67. Mosior M, Newton AC. Mechanism of the apparent cooperativity in the interaction of protein kinase C with phosphatidylserine. *Biochemistry.* 1998; 37(49): 17271-17279.

68. Bandyopadhyay G, Sajan MP, Kanoh Y, Standaert ML, Quon MJ, Lea-Currie R, Sen A, Farese RV. PKC-zeta mediates insulin effects on glucose transport in cultured preadipocyte-derived human adipocytes. *J Clin Endocrinol Metab.* 2002; 87(2): 716-723.

69. Sweet LJ, Dudley DT, Pessin JE, Spector AA. Phospholipid activation of the insulin receptor kinase: regulation by phosphatidylinositol. *FASEB J.* 1987; 1(1): 55-59.

70. Kosiakova H, Berdyshev A, Dosenko V, Drevytska T, Herasymenko O, Hula N. The involvement of peroxisome proliferator-activated receptor gamma (PPARγ) in anti-inflammatory activity of N-stearoylethanolamine. *Heliyon.* 2022; 8(11): e11336.

71. Gula NM, Chumak AA, Mehed OF, Goridko TM, Kindruk NL, Berdyshev AH. Immunosuppressive characteristics of N-stearoylethanolamine a stable compound with cannabimimetic activity. *Ukr Biokhim Zhurn.* 2008; 80(1): 57-67. (In Ukrainian).