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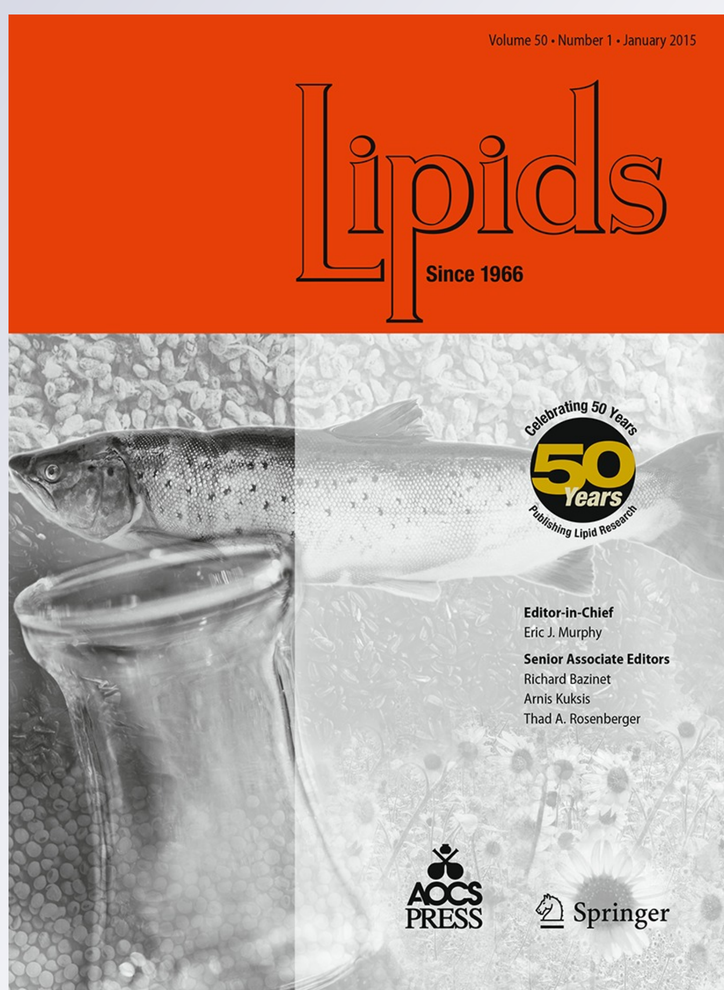

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ORIGINAL ARTICLE

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Abstract This study investigates the protective effect of *N*-stearoylethanolamine (NSE), a bioactive *N*-acylethanolamine , on the lipid profile distribution in the pancreas of obesity-induced insulin resistant (IR) rats fed with prolonged high fat diet (58 % of fat for 6 months). The phospholipid composition was determined using 2D thin-layer chromatography. The level of individual phospholipids was estimated by measuring inorganic phosphorus content. The fatty acid (FA) composition and cholesterol level were investigated by gas–liquid chromatography. Compared to controls, plasma levels of triglycerides and insulin were significantly increased in IR rats. The pancreas lipid composition indicated a significant reduction of the free cholesterol level and some phospholipids such as phosphatidylcholine (PtdCho), phosphatidylethanolamine (PtdEtn), phosphatidylinositol (PtdIns), phosphatidylserine (PtdSer) compared to controls. Moreover, the FA composition of pancreas showed a significant redistribution of the main FA (18:1n-9, 18:2n-6, 18:3n-6 and 20:4n-6) levels between phospholipid, free FA, triglyceride fractions under IR conditions that was accompanied by a change in the estimated activities of Δ9-, Δ6-, Δ5-desaturase. Administration of *N*-stearoylethanolamine (NSE, 50 mg/kg daily per os for 2 weeks) IR rats triggered an increase in the content of free cholesterol, PtdCho and normalization of PtdEtn, PtdSer level. Furthermore, the NSE modulated the activity of desaturases, thus influenced FA composition and restored the FA ratios in the lipid fractions. These NSE-induced changes were associated with a normalization of plasma triglyceride content, considerable decrease of insulin and index HOMA-IR level in rats under IR conditions.

Keywords Experimental insulin resistance · *N*-Stearoylethanolamine · Obesity · Pancreas · Phospholipid · Fatty acid · Triglyceride · Cholesterol

Abbreviations

Cer*P*Cho	Sphingomyelin
DUFA	Diunsaturated fatty acid
FA	Fatty acid
HFD	High fat diet
HOMA-IR	Homeostasis model assessment of insulin resistance index
IR	Insulin resistance
LysoPtdCho	Lysophosphatidylcholine
LysoPtdEtn	Lysophosphatidylethanolamine
MUFA	Monounsaturated fatty acid
NAE	*N*-Acylethanolamine
PL	Phospholipid
PtdCho	Phosphatidylcholine
PtdEtn	Phosphatidylethanolamine
PtdIns	Phosphatidylinositol
PtdSer	Phosphatidylserine
Ptd_2Gro	Diphosphatidylglycerol
PUFA	Polyunsaturated fatty acid
NSE	*N*-Stearoylethanolamine

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SFA	Saturated fatty acid
TAG	Triacylglycerol
USFA	Unsaturated fatty acid

Introduction

Overweight and alimentary obesity are the major problems of modern society with more than half a billion people suffering from these conditions [1]. Alimentary obesity is closely associated with the impairment of carbohydrate and lipid metabolism, causing dyslipidemia, insulin resistance (IR) and type II diabetes. The progression of obesity and glucose intolerance eventually leads to accumulation of saturated fatty acids (FA) and triacylglycerols (TAG) in pancreas tissue, and triggers the development of pancreas pathologies. Furthermore, in clinical studies it was shown that high levels of TAG in pancreatic tissue were converted into toxic intermediates that cause β-cell apoptosis [2]. As a result, increased β-cell failure together with the insulin resistance has been suggested to lead to the development of diabetes [2–4].

Considering the importance of diabetes, it is of a current interest to develop novel treatment modalities that could improve the lipid imbalance of insulin sensitive tissues in obese subjects, and prevent the development of diabetic complications. Recent studies indicate that monounsaturated *N*-acylethanolamine (NAE), *N*-oleylethanolamine (OEA) and saturated *N*-palmitoylethanolamine (PEA) regulate energy homeostasis by interacting with the nuclear (primarily PPARα) and orphan G-protein coupled receptors (GPR55, GPR119) [3, 4]. Recent studies indicate that *N*-stearoylethanolamine (NSE) is able to regulate lipid metabolism via integrating into lipid rafts and activating PPARα [5]. Importantly, in a recent investigation it was suggested that activation of GPR119 results in stimulation of β-cell replication and increase of islets mass, contributing to β-cell regeneration and improvement of diabetes [6]. Furthermore increased expression of PPARγ and PPARα has been shown to induce significant insulin sensitizing, anti-dyslipidemic, antioxidant, anti-inflammatory, and β-cell salvaging activities in type II diabetic rats (high fat diet model with low dose STZ) [7].

In line with earlier findings, the membrane-stabilizing and anti-inflammatory action of NSE was shown on different tissues (liver, heart, skin, testes) in a wide range of pathological conditions in animal models [8, 9]. The main effect of NSE has been suggested to be the improvement of fatty acid imbalance and normalization of phospholipid (PL) levels [10, 11]. In addition, in our previous work we showed that NSE restores liver phospholipid (PL) content and this effect is associated with the reduced plasma insulin level and the improvement of insulin sensitivity in rats with obesity-induced IR [12]. However effects of NSE on pancreatic lipid composition are currently unknown. The aim of this study is to investigate the effect of NSE on the free cholesterol level, the individual PL content and the redistribution of the main FA in pancreas tissue from rats with high fat diet-induced IR.

Materials and Methods

Animal Model

The study was carried out on male Sprague–Dawley rats (200–220 g). All procedures were conducted in accordance with the rules of the Commission on Bioethics of Institute of Biochemistry, National Academy of Sciences and with the "General ethical principles on experiments with animals" of the first National Congress on Bioethics (Kyiv, 2001). Rats were housed in standard cages with free access to food and water.

Obesity-induced IR was attained by feeding a prolonged high-fat diet (HFD) (58 % fat:23 % proteins:10 % carbohydrates for 6 months) as described earlier [13]. The amount of lipids in the diet was increased by addition of lard to the pellet diet, which contained a high level of palmitic (24 % of total FA) and stearic (28 % of total acids) acids. Cholesterol content of lard was not at a high level (0.57 mg/g of lard). The HFD–FA composition was at a ratio of 55 % saturated (SFA) to 45 % unsaturated FA (USFA). Control rats during the experiment were on normal pellet diet (4 % fat:23 % proteins:65 % carbohydrates) with SFA/USFA ratio 38 %/62 %, respectively. Throughout the experiments, the rats were gaining weight gradually. On the 24th week, the average weight of HFD rats was 410–430 g (due to visceral fat) in comparison with control rats of 330–350 g.

Six months after HFD period, we conducted the oral glucose tolerance test [14]. The rats with impaired glucose tolerance (the level of blood glucose within 150 min after the oral glucose administration was higher than 5 mmol/l) were selected and divided randomly into two groups: IR ($n = 9$) and IR + NSE ($n = 10$). Control rats were further subdivided into control ($n = 10$) and NSE ($n = 7$) groups. Animals in NSE and IR + NSE groups were orally received the water suspension of NSE for 2 weeks at the dose of 50 mg body weight kg^{-1}.

This particular dose of NSE has been chosen as an optimal reacting dose for the biological effect investigations. Schmid HH et al. [15] earlier reported the exact concentration of NSE during the experiment of dog coronary artery occlusion. Previously in the experiment with

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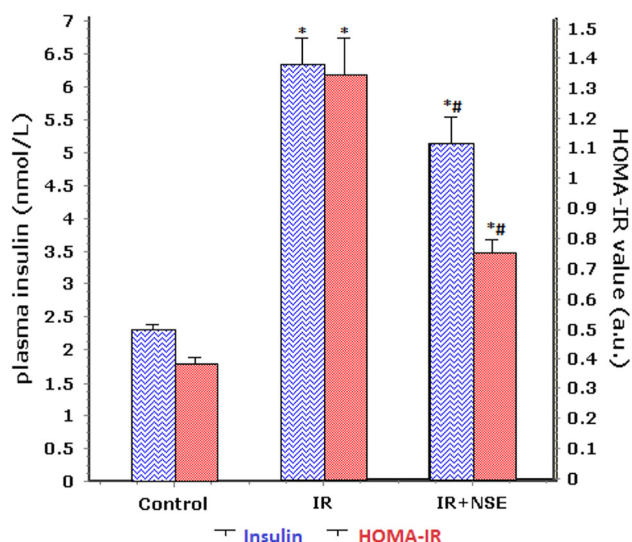

Fig. 1 Plasma fasting insulin level (nmol/L) and index of insulin resistance HOMA-IR (a.u.) in control and IR rats. *Values* represented mean ± SEM. *$p < 0.05$, compared to control rats; #$p < 0.05$, compared to the IR group

radiolabeled PEA, structural homologue of NSE, we found that 0.95 % of the per os administrated *N*-[(9,10-3*H*)-palmitoyl]-ethanolamine was accumulated in brain, suggesting its penetration through gastrointestinal tract and blood–brain barrier [16]. In vivo experiments showed that pharmacological treatments with saturated *N*-acylethanolamines such as NSE able to increase local concentrations of these compounds [17].

The establishment of IR was confirmed based on the results of fasting plasma insulin levels (measured by ELISA kit, DRG, Germany) and HOMA-IR value [calculated by fasting insulin (nmol/L) × fasting glucose (mmol/L)/22.5]. The results of these experiments were previously published [14] and presented in the Fig. 1. At the end of the experiments, the rats were decapitated under Nembutal anesthesia (50 mg/kg bodyweight). The pancreas was immediately removed and frozen at −80 °C until further analysis.

Plasma Triglyceride Content Determination

The total plasma TAG level was determined using a commercially available multi-enzyme kit and standard calibration procedures (Felicit diagnostic, Ukraine).

Phospholipid and Fatty Acid Profile Determination

The pancreas was homogenized in physiological saline solution. Total lipids were extracted from 10 % homogenate and purified by the methods of Bligh and Dyer [18]. The content of total pancreatic PL expressed by the level of inorganic phosphate [P(i)] in the lipid extracts were assayed using the molybdenum blue staining method [19] and determined spectrophotometrically as their phosphomolybdenum blue complex in the 815-nm wavelength region.

The individual PL content was determined by lipid extract separation with 2D thin-layer chromatography. Solvent system for the first dimension were chloroform (65):methanol (30):ammonia (6), benzene (10) (v/v), and the second dimension were with chloroform (5):methanol (1):acetic acid (1):water (0.5):acetone (2) (v/v) [20, 21]. The level of individual PL was estimated by colorimetric measurement of P(i) in each separated PL spot, using the Vaskovskiy and Kostetskiy method [1919].

The FA composition of pancreas was investigated from three different lipid fractions (PL, free FA and TAG) that were separated from lipid extract by thin-layer chromatography using a solvent system of hexane/diethyl ether/acetic acid (85:15:1, v/v). After the separation, samples of all fractions were methylated [22]. Fatty acids methyl esters (FAME) were analyzed by gas–liquid chromatography. A Carlo Erba gas chromatograph (model HRGC 5300 Italy with flame ionization detector) equipped with a glass packed column (length: 3.5 m, internal diameter: 3 mm), completed with 10 % SP-2300 phase (Silar 5CP) on "Chromosorb W/HP" was used to separate FAME. The temperature was programmed from 140 to 250 °C at 2 °C/min. The FAME were identified by comparing each peak's retention time with FA methyl ester standards (Sigma Serva, Germany). The relative amount was quantified by integrating the area under the specific peak and dividing the results by the total area for all FA.

Cholesterol Content Determination

After separation of lipid extract by thin-layer chromatography (hexane/diethyl ether/acetic acid (85:15:1, v/v), the cholesterol fraction was taken and eluted by 3 ml of diethyl ether. Following evaporation of the solvent, the dry residue was assayed by gas–liquid chromatography on a glass column (0.5 m) packed with 1.5 % OV-1 on 80–100 mesh Chimalite at 250 °C. The concentration of cholesterol was calculated in each sample by the difference between peak areas of the sample and the peak of the purified cholesterol standard.

Estimation of Desaturase Indices

It is known that the FA desaturase activity may be estimated, using relative product-to-precursor indices. In this study, the following ratios were used: oleinic/stearic acids for Δ9-desaturase, linolenic/linoleic acids for Δ6-desaturase, arachidonic/linolenic acids for Δ5-desaturase [23].

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Recent studies presented percentages of FA for estimation of desaturases [24]. Moreover, McLauren Dorrance et al. [23] reported that the weight FA quantification was not significantly different from the percentage quantification.

Statistical Analysis

The data, presented as mean values ± standard errors of the means (SEM) from different studied groups were compared by one-way analysis of variance (ANOVA) followed by the Tukey post test. The statistical of significance was determined at the level of $p < 0.05$.

Results

Plasma Triglyceride Levels

The results indicate that the TAG levels in rats with IR increased significantly to 68 % higher than control values (Fig. 2). On the other hand, the plasma level of TAG from IR rats that received water suspension of NSE was 63 % lower than those of IR-group of rats (Fig. 2).

Pancreas Phospholipid Composition

Investigations of PL content indicated that the total PL level of IR-group was lower (334.21 ± 33.14; $p < 0.001$) than that of controls (456.52 ± 24.99). Moreover, the individual PL composition was significantly affected by the development of obesity-induced IR (Fig. 3). In particular, IR-group showed a significant reduction in contents of

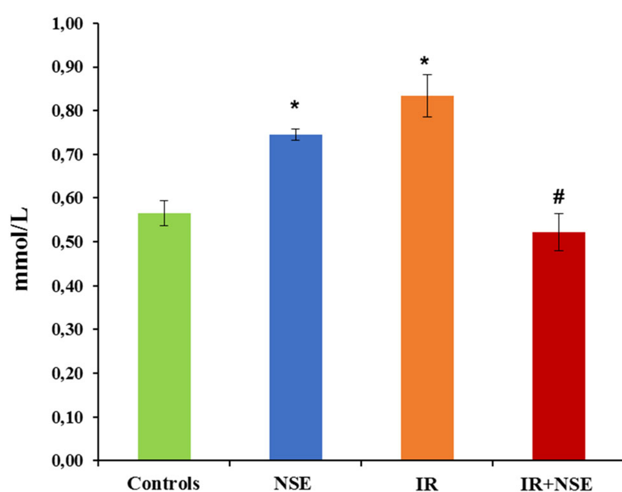

Fig. 2 Plasma triglyceride level (mmol/L) in control and IR rats. Data shown mean ± SEM. Values in control ($n = 10$), NSE ($n = 7$), IR ($n = 9$), IR + NSE ($n = 10$) were compared. *$p < 0.001$, compared to the control rats; #$p < 0.001$, compared to the IR group

phosphatidylcholine (PtdCho) 34 %, phosphatidylethanolamine (PtdEtn) nearly 39 %, phosphatidylinositol (PtdIns) 72 %, phosphatidylserine (PtdSer) 54 % compared to control animals (Fig. 3a, c). The investigation of the pancreatic sphingomyelin (Cer*P*Cho), diphosphatidylglycerol (Ptd_2Gro), lysophosphatidylcholine (LysoPtdCho) and lysophosphatidylethanolamine (LysoPtdEtn) level showed no significant changes in IR group of rats compared to controls (Fig. 3b, d).

The treatment of NSE had no considerable effect on total PL level (353.32 ± 13.82), however, its administration caused a statistically significant increase in the content of the main PL (PtdCho, PtdEtn, PtdSer) in pancreas of rats exposed to HFD (Fig. 3a, c).

Pancreas Cholesterol Content

The determination of the free cholesterol content in the rat pancreas showed a considerable decrease in the IR group, which was significantly lower than those of controls (Fig. 4). The administration of NSE did not cause a statistically significant change in the cholesterol content in NSE-control group. However, NSE caused a significant increase in free pancreatic cholesterol level in the obesity-induced IR group (Fig. 4).

The Fatty Acid Profile of Pancreas

The results of experiments indicated that the main pancreatic FA composition (Table 1) showed a considerable redistribution of the FA level between lipid fractions in the IR group of rats. The PL fraction contained less saturated fatty acids (SFA), primarily 16:0 and 18:0, polyunsaturated acid (PUFA) 20:4n-6 and more monounsaturated acid (MUFA) 18:1n-9 in the IR group compared to controls. The free FA (FFA) fraction and the TAG fraction in the IR group contained more 18:0, 18:3n-6 and less 16:0, 16:1n-9, and diunsaturated fatty acids (DUFA) mainly 18:2n-6, whereas no considerable changes were detected in the 18:1n-9 TAG level. The decreased level of 20:4n-6 TAG and a tendency of 20:4n-6 FFA to decrease was found in the pancreas of the IR rats compared to the controls. The NSE treatment of rats with obesity-induced IR resulted in increased content of 16:0 FFA, 16:0 TAG, 20:4n-6 PL, decreased levels of 18:3n-6 FFA, 18:3n-6 TAG and a tendency of 18:0 to reduce compared to IR group. The level of 18:1n-9 was even higher in all lipid fractions of IR + NSE group compared to IR group.

Indices of Fatty Acid Desaturation

Using the ratios defined earlier, we estimated the main desaturase activity indices in different lipid fractions

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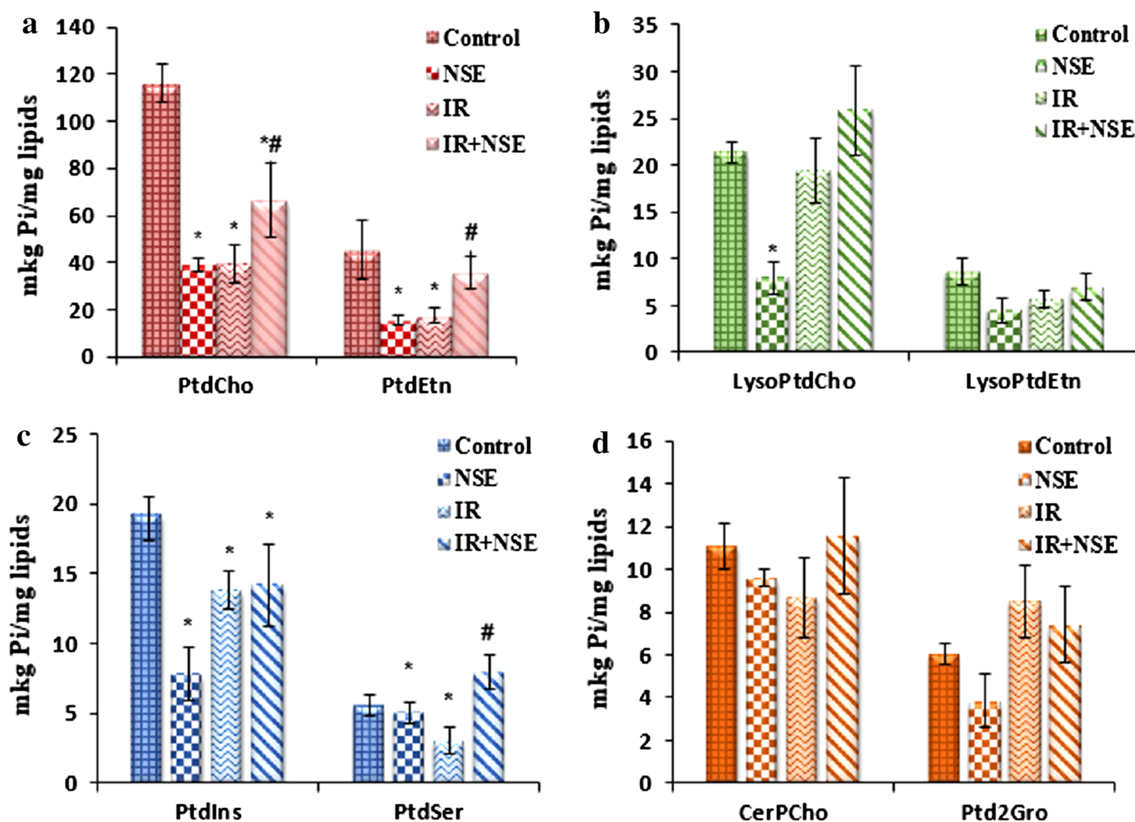

Fig. 3 Pancreatic phospholipid composition (μg Pi/mg lipids) in control and HFD rats. **a** Phosphatidylcholine (PtdCho), phosphatidylethanolamine (PtdEtn) level. **b** Lysophosphatidylcholine (LysoPtdCho), lysophosphatidylethanolamine (LysoPtdEtn) level. **c** Phosphatidylinositol (PtdIns), phosphatidylserine (PtdSer) level. **d** Diphosphotidilglycerol (Ptd2Gro), sphingomyelin (CerPCho) level. *Columns* represented mean ± SEM. Values in control ($n = 10$), NSE ($n = 7$), IR ($n = 9$), and IR + NSE ($n = 10$) were compared. *$p < 0.05$, compared to the control rats; #$p < 0.05$, compared to the IR group

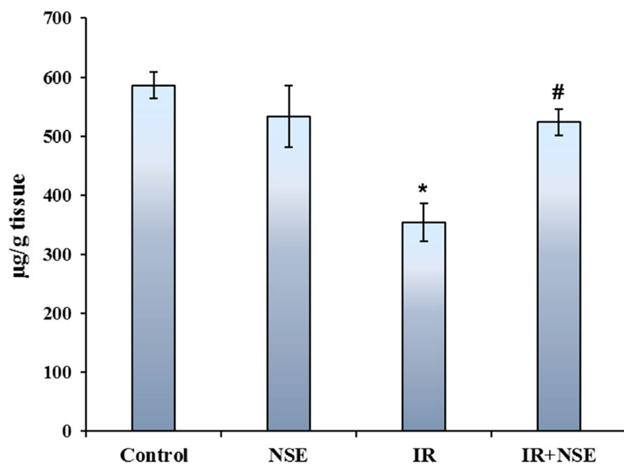

Fig. 4 Pancreatic free cholesterol content (μg/g tissue) in control and HFD rats. *Values* represented mean ± SEM. Values in control ($n = 10$), NSE ($n = 7$), IR ($n = 9$), and IR+NSE group ($n = 10$) were compared. *$p < 0.05$, compared to the control rats; #$p < 0.05$, compared to the IR group

(Table 2). The estimated index of Δ5-desaturase in the pancreas lipid fraction from IR rats was significantly lower than those in controls (Table 2), whereas Δ6-desaturase activity index was increased in the FFA and TAG lipid fractions and had a tendency to increase in PL. In the FFA and TAG fractions of IR group, the Δ9-desaturase index (Stearoyl-CoA-desaturase-1) was approximately half of the controls. However, in the PL fraction, Δ9-desaturase index showed a three-fold increase in the IR group compared to controls.

The NSE administration to rats in IR group caused a decrease in the Δ6-desaturase index of the TAG fraction and led to normalization in the FFA fraction compared to the IR group. The PL, TAG Δ5-desaturase index remained unaltered by NSE treatment, meanwhile in the FFA fraction, the Δ5-desaturation index was significantly increased after the NSE administration. In pancreases of NSE administered rats, the Δ9-desaturase index showed a tendency to increase in all investigated fractions in comparison to the IR group.

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Table 1 The distribution of main fatty acids (% of total fatty acid level) between lipid fractions in rat pancreas

Lipid fraction	Fatty acid	Experimental group			
		Control	NSE	IR	IR + NSE
16:0	PL	45.4 ± 1.1	45.0 ± 1.6	35.4 ± 1.2*	35.4 ± 1.3*
	FFA	46.7 ± 1.4	41.1 ± 2.3*	33.6 ± 1.4*	38.6 ± 1.3*#
	TG	37.5 ± 1.1	35.4 ± 1.4	28.8 ± 1.0*	33.3 ± 1.8#
16:1n-9	PL	2.8 ± 0.2	2.3 ± 0.2*	1.1 ± 0.0*	1.3 ± 0.2*
	FFA	6.5 ± 0.4	6.7 ± 0.6	3.3 ± 0.4*	2.8 ± 0.2*
	TG	7.7 ± 0.6	7.3 ± 0.8	4.3 ± 0.5*	4.8 ± 0.8*
18:0	PL	23.2 ± 0.8	23.4 ± 0.9	20.0 ± 1.1*	19.0 ± 1.1*
	FFA	12.4 ± 1.1	14.4 ± 1.1	27.1 ± 0.8*	25.3 ± 1.7*
	TG	9.4 ± 0.7	10.1 ± 1.4	25.9 ± 0.8*	21.5 ± 2.5*
18:1n-9	PL	11.5 ± 0.7	11.4 ± 0.5	26.8 ± 1.9*	30.9 ± 2.5*
	FFA	20.4 ± 0.3	22.8 ± 1.0*	26.1 ± 0.8*	27.2 ± 1.0*
	TG	27.1 ± 0.9	27.6 ± 0.9	26.6 ± 1.1	27.5 ± 1.4
18:2n-6	PL	5.2 ± 0.5	4.9 ± 0.8	6.8 ± 0.3*	6.5 ± 0.2*
	FFA	7.2 ± 0.5	7.5 ± 0.6	4.3 ± 0.4*	3.3 ± 0.2*
	TG	10.5 ± 0.9	11.4 ± 1.4	7.7 ± 0.6*	7.4 ± 1.0*
18:3n-6	PL	0.01 ± 0.004	0.02 ± 0.006	0.04 ± 0.01	0.03 ± 0.01
	FFA	0.04 ± 0.008	0.05 ± 0.02	0.08 ± 0.01*	0.03 ± 0.004#
	TG	0.07 ± 0.02	0.06 ± 0.02	0.13 ± 0.02*	0.05 ± 0.01#
20:3n-6	PL	0 ± 0	0 ± 0	0 ± 0	0.65 ± 0.01
	FFA	0.35 ± 0.003	0 ± 0	0 ± 0	0 ± 0
	TG	0 ± 0	0 ± 0	0 ± 0	0.2 ± 0.002
20:4n-6	PL	0.32 ± 0.06	0.25 ± 0.09	0.03 ± 0.02*	0.2 ± 0.06#
	FFA	0.24 ± 0.02	0.34 ± 0.03*	0.22 ± 0.02	0.28 ± 0.05
	TG	0.54 ± 0.09	0.57 ± 0.14	0.29 ± 0.05*	0.21 ± 0.05*
Total SAFA	PL	78.7 ± 1.1	79.3 ± 1.5	63.7 ± 2.2*	59.8 ± 2.7*
	FFA	63.4 ± 0.8	59.6 ± 2.1	67.4 ± 3.1	61.6 ± 2.4
	TG	51.3 ± 0.9	50.0 ± 1.2	59.4 ± 0.4*	55.7 ± 1.3*#
Total USFA	PL	21.3 ± 1.1	20.6 ± 1.5	36.3 ± 2.1*	40.3 ± 2.7*
	FFA	35.9 ± 0.6	40.4 ± 2.1*	35.7 ± 0.4	36.0 ± 0.7
	TG	48.6 ± 0.9	50.0 ± 1.2	40.9 ± 0.5*	43.5 ± 1.4*
MUFA	PL	15.7 ± 0.8	15.5 ± 0.7	29.4 ± 2.0*	33.5 ± 2.7*
	FFA	29.1 ± 0.5	31.7 ± 1.4	31.1 ± 0.4*	31.7 ± 1.0*
	TG	37.4 ± 0.6	37.9 ± 0.5	32.8 ± 0.6*	35.8 ± 1.5
DUFA	PL	5.2 ± 0.5	4.8 ± 0.8	6.7 ± 0.3*	6.4 ± 0.2*
	FFA	7.2 ± 0.5	8.3 ± 0.9	4.3 ± 0.5*	3.3 ± 0.2*
	TG	10.5 ± 0.9	11.4 ± 1.4	7.6 ± 0.6*	7.4 ± 1.0*
PUFA	PL	0.33 ± 0.06	0.38 ± 0.09	0.08 ± 0.03*	0.22 ± 0.05#
	FFA	0.26 ± 0.03	0.4 ± 0.04*	0.3 ± 0.03	0.3 ± 0.1
	TG	0.61 ± 0.09	0.65 ± 0.13	0.45 ± 0.05	0.34 ± 0.07*

PL phospholipid, *FFA* free fatty acid, *TG* triacylglycerol

* $p < 0.05$, compared to the control rats

# $p < 0.05$, compared to the IR group

Discussion

The present study shows that HFD overload induces a decrease in the total PL and free cholesterol content in rat pancreas that correlated with an increased pool of TAG. As it was reported earlier, these factors lead to an accumulation of TAG and causes development of pancreas steatosis [2].

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Table 2 Pancreatic estimated Δ9-, Δ6-, Δ5- desaturase activity indexes for different lipid fractions of control and HFD-fed rats

Desaturase	Control	NSE	IR	IR + NSE
Phospholipids				
Δ5-Desaturase (20:4/18:3 ratio)	20.7 ± 3.7	13.1 ± 9.9	0.2 ± 0.08*	31.4 ± 31.2
Δ6-Desaturase (18:3/18:2 ratio)	0.003 ± 0.001	0.005 ± 0.001	0.006 ± 0.001	0.005 ± 0.001
Δ9-Desaturase (18:1/18:0 ratio)	0.5 ± 0.04	0.5 ± 0.04	1.4 ± 0.1*	1.8 ± 0.3*
Free fatty acids				
Δ5-Desaturase (20:4/18:3 ratio)	6.6 ± 0.9	8.3 ± 3.8	2.9 ± 0.3*	11.3 ± 1.4*#
Δ6-Desaturase (18:3/18:2 ratio)	0.006 ± 0.001	0.006 ± 0.002	0.019 ± 0.002*	0.008 ± 0.001#
Δ9-Desaturase (18:1/18:0 ratio)	1.7 ± 0.1	1.6 ± 0.1	0.9 ± 0.08*	1.2 ± 0.1*
Triacylglycerols				
Δ5-Desaturase (20:4/18:3 ratio)	14.0 ± 3.8	15.321 ± 6.1	4.1 ± 1.5*	3.4 ± 1.4*
Δ6-Desaturase (18:3/18:2 ratio)	0.006 ± 0.001	0.006 ± 0.002	0.015 ± 0.002*	0.013 ± 0.002*
Δ9-Desaturase (18:1/18:0 ratio)	3.0 ± 0.2	3.0 ± 0.3	1.0 ± 0.09*	1.9 ± 0.4*#

* $p < 0.05$, compared to the control rats

# $p < 0.05$, compared to the IR group

The results of PL measurements suggest that the reduction of PtdCho, PtdEtn (important in organization of the lipid bilayer) and PtdSer, PtdIns (important in signal transduction), may be an underlying cause for membrane damage and cell dysfunction observed in diabetic conditions. Decreased PtdCho and PtdEtn content did not correlate with the change in their lysoforms level, suggesting that these changes were not associated with the phospholipase A_2 alteration. Thus, reduction in PL levels may be mainly associated with the impairment of de novo PL synthesis in obesity-induced IR. In our earlier studies, it was found that, in agreement with present findings, decrease in hepatic anionic PL (PtdSer, PtdIns) correlates highly with the increased level of plasma insulin and HOMA-IR value in IR conditions [12]. It is well known that cellular PL are primarily involved in membrane lipid bilayer organization, creating certain membrane properties such as fluidity, curvature and permeability. Therefore, lipid bilayer composition is an important target for regulation of signaling by membrane proteins such as insulin receptor [25, 26]. The major components of surrounding lipid microenvironment of transmembrane proteins are cholesterol, Cer*P*Cho and SFA (primarily 16:0) which are able to pack closely and constitute highly ordered area with a lesser degree of fluidity compare to the surrounding membrane [27]. In our experiment, we found a decrease in cholesterol, 16:0 levels and individual PL composition in pancreas of rats with IR suggesting that the membrane lipid bilayer structure was impaired in this group which may affect insulin signaling.

It is important to note that plasma insulin can regulate its own secretion via insulin receptors in β-cells of the pancreas. The results of studies reporting both a positive and negative effect of insulin on its own secretion are currently unclear [28]. However, recent studies on normal mouse and human β-cells suggested that insulin via phosphatidylinositol-4,5-bisphosphate 3-kinase induced opening of K_{ATP} channels causes hyperpolarization and a subsequent decrease in insulin secretion [29].

The NSE administration normalizes the plasma TAG pool and restores the lipid bilayer structure by increasing the main PL content, cholesterol and 16:0 levels in the pancreas of rats with HFD-induced IR. This effect of NSE is accompanied by an improvement in insulin signaling (reduced HOMA-IR value) and a reduced plasma insulin content.

Meanwhile, in rats without IR that were treated by NSE we found an opposite effect on the TAG level and PL composition. The mechanisms responsible for these changes are still poorly understood; however, most investigations of NSE's activity did not show significant changes in biochemical parameters from intact animals. In agreement with previous studies NSE compensate metabolic disturbances mainly under stress-related pathological conditions, including diabetes, thus showing its adaptogenic property [30]. Therefore, we specifically investigated the biological effect of NSE under pathological lipid profile impairment and its possible use in the pharmacotherapy of diabetes complications connected to dyslipidemia.

Another important factor that affects physical properties of membrane PL and TAG is the degree of FA unsaturation. Membrane-bound acyl-CoA desaturases are the key enzymes that take part in de novo synthesis of USFA. Three desaturases, Δ5, Δ6 and Δ9, are present in humans [31]. In our study, the analysis of FA content in IR rat pancreas showed a significant redistribution of the main FA between lipid fractions and changes in the estimated activity of acyl-CoA desaturases. Particularly, the decreased level of 16:0—the precursor for 18:0 synthesis was found. The level of 18:0 was also decreased compared to control rats suggesting its consumption for 18:1n-9 formation under IR condition. This was confirmed by increased activity of Δ9-desaturase in PL fraction as a compensatory mechanism for β-cell death. Earlier it was shown that pancreas cells are highly sensitive to SFA

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(16:0; 18:0) induced lipotoxicity [32]. In addition, enhanced 18:1n-9/18:0 ratio namely in PL compartment suggest the distribution of Δ9-desaturase product (18:1n-9) in membrane PL composition, thus increasing the total level of unsaturated PL. High level of USFA in PL fraction together with decreased cholesterol and total PL content is likely to trigger an increase in plasma membrane fluidity and curvature that may alter signal transduction mechanisms [33, 34]. In the pancreas of IR rats treated with NSE, the level of 18:1n-9 did not change significantly which may be connected to the 18:0 lipotoxicity and the short time of NSE administration. However, NSE increased PtdCho and cholesterol content that may correct membrane fluidity and restore the function of the insulin receptor which is confirmed by the reduced hyperinsulinemia.

Furthermore, under obesity-induced IR the PUFA n-6 content was significantly affected, in particular the index of the first desaturase—Δ6 that converts 18:2n-6 to 18:3n-6, the substrate for 20:4n-6 was increased in all lipid fractions compared to control subjects. Meanwhile the level of 20:4n-6 PL was considerably decreased, suggesting its involvement in eicosanoid formation. The 20:4n-6 in membrane PL is a precursor in the biosynthesis of prostaglandins, hydroxyeicosatetraenoic acids and leukotrienes, which play a role in the initiation and regulation of inflammation [35]. Additionally, increased conversion of 20:4n-6 PL to 12-hydroxyeicosatetraenoic acid by 12-lypoxigenase in the pancreas of rats impair β-cell function, cell viability and influence insulin secretion, cytotoxicity and kinase activation [36]. It is known that 20:4n-6 PL can act as a target for reactive oxygen species, forming lipid peroxides and thus damaging PL bilayers. This finding is accordingly in support of our earlier study, where the level of lipid peroxidation products was significantly high and the activity of antioxidant enzymes was reduced in the liver of IR rats [14]. Another mechanism of 20:4n-6 reduction is an increased level of 18:1n-9 that acts as an inhibitor of Δ5- and Δ6-desaturase and elongase-5. This inverse relationship between percentages of 18:1n-9 and 20:4n-6 in human serum PL was recently presented [37]. However, in our study we found a higher level of 18:1n-9 alongside with increased 20:4n-6 content in the PL fraction of IR rats treated by NSE. This finding may confirm the hypothesis concerning the increased peroxidation of 20:4n-6 PL and its transformation into eicosanoids under IR conditions.

The administration of NSE to rats with an IR, influenced the n-6 PUFA synthesis pathway by normalizing the 20:4n-6 in PL and increasing the Δ5-desaturase index in the PL and FFA fractions. This effect of NSE indicates the reduction of pro-oxidative processes that may be associated with its antioxidant properties. In a rat model of hypoxic hypoxia, the inhibitory effect of NSE on non-enzymatic lipid peroxidation was detected, thus showing its membrane protective action [38]. In agreement with these findings, earlier studies supported the idea of a reduction in lipid peroxidation processes under NSE administration in different rat models [11, 14].

Conclusion

Our results confirm that a high intake of saturated fats for long time periods induces the development of dyslipidemia and steatosis in rat pancreas and this process may be an underlying factor in the pathogenesis of type II diabetes. It appears that the enhanced level of circulated TAG and their accumulation in pancreas tissue influence de novo synthesis of PL and FA in diet-induced IR. The reduction of the main PL and free cholesterol can lead to impairment of insulin sensitivity and enhanced secretion of insulin. Importantly, the NSE administration decreased the plasma pool of TAG, increased some pancreas PL and free cholesterol content that could contribute to the restoration of the lipid environment surrounding insulin receptors. Furthermore, NSE improved the altered FA composition of pancreas lipid fractions and modulated the main Acyl-Co-*A* desaturases in rats with IR. Finally, the decrease in plasma insulin content by NSE was associated with the restoration of lipid composition and increased insulin sensitivity in pancreas of rats with HFD-induced IR.

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Conflict of interest The authors declare that there is no conflict of interest.

References

1. World health organization (2014) Obesity and overweight. Fact sheet No 311, Accessed Apr 2014 http://www.who.int/mediacentre/factsheets/fs311/en/
2. Szczepaniak LS, Victor RG, Mathur R, Nelson MD, Szczepaniak EW, Tyer N, Chen I, Unger RH, Bergman RN, Lingvay I (2012) Pancreatic steatosis and its relationship to β-cell dysfunction in humans: racial and ethnic variations. Diabetes Care 35:2377–2383
3. Matias I, Gonthier MP, Petrosino S, Docimo L, Capasso R, Hoareau L, Monteleone P, Roche R, Izzo AA, Di Marzo V (2007) Role and regulation of acylethanolamides in energy balance: focus on adipocytes and beta-cells. Br J Pharmacol 152:676–690
4. Lambert DM, Muccioli GG (2007) Endocannabinoids and related *N*-acylethanolamines in the control of appetite and energy metabolism: emergence of new molecular players. Curr Opin Clin Nutr Metab Care 10:735–744
5. Artmann A, Petersen G, Hellgren LI, Boberg J, Skonberg C, Nellemann C, Hansen SH, Hansen HS (2008) Influence of dietary fatty acids on endocannabinoid and *N*-acylethanolamine levels in rat brain, liver and small intestine. Biochim Biophys Acta 1781:200–212

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6. Ansarullah LuY, Holstein M, DeRuyter B, Rabinovitch A, Guo Z (2013) Stimulating β-Cell regeneration by combining a GPR119 agonist with a DPP-IV inhibitor. PLoS ONE 8:e53345
7. Sharma AK, Bharti S, Kumar R, Krishnamurthy B, Bhatia J, Kumari S, Arya DS (2012) Ameliorates insulin resistance and β-cell dysfunction via modulation of PPARγ, dyslipidemia, oxidative stress, and TNF-α in Type 2 diabetic rats. J Pharmacol Sci 119:205–213
8. Hula NM, Chumak AA, Berdyshev AH, Mehed' OF, Horid'ko TM, Kindruk NL, Kosiakova HV, Zhukov OD (2009) Anti-inflammatory effect of *N*-stearoylethanolamine in experimental burn injury in rats. Ukr Biokhim Zh 81:107–116
9. Gorid'ko TM, Kosiakova HV, Berdyshev AH, Bazylians'ka VR, Margitych VM, Gula NM (2012) 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 Zh 84:37–43
10. Kosiakova HV, Hula NM (2007) 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 Zh 79:53–59
11. Horid'ko TM, Hula NM, Stohniĭ NA, Mehed' OF, Klimashevs'kyĭ VM, Shovkun SA, Kindruk NL, Berdyshev AH (2007) Effect of *N*-stearoylethanolamine on the lipid peroxidation process and lipid composition of the rat liver in acute morphine intoxication. Ukr Biokhim Zh 79:175–185
12. Onopchenko OV, Kosiakova GV, Horid'ko TM, Klimashevsky VM, Hula NM (2014) The effect of *N*-stearoylethanolamine on liver phospholipid composition of rats with high-fat diet-induced insulin resistance. Ukr Biokhim Zh 86:101–110
13. Svegliati-Baroni G, Candelaresi C, Saccomanno S, Ferretti G, Bachetti T, Marzioni M, De Minicis S, Nobili L, Salzano R, Omenetti A, Pacetti D, Sigmund S, Benedetti A, Casini A (2006) A model of insulin resistance and nonalcoholic steatohepatitis in rats: role of peroxisome proliferator-activated receptor-alpha and n-3 polyunsaturated fatty acid treatment on liver injury. Am J Pathol 169:846–860
14. Onopchenko OV, Kosiakova HV, Horid'ko TM, Berdyshev AH, Mehed' OF, Hula NM (2013) 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 Zh 85:88–96
15. Epps DE, Natarajan V, Schmid PC, Schmid HO (1980) Accumulation of *N*-acylethanolamine glycerophospholipids in infarcted myocardium. Biochim Biophys Acta 618:420–430
16. Artamonov M, Zhukov O, Shuba I, Storozhuk L, Khmel T, Klimashevsky V, Mikosha A, Gula N (2005) Incorporation of labelled *N*-acylethanolamine (NAE) into rat brain regions in vivo and adaptive properties of saturated NAE under x-ray irradiation. Ukr Biokhim Zh 77:51–62
17. Dalle Carbonare M, Del Giudice E, Stecca A, Colavito D, Fabris M, D'Arrigo A, Bernardini D, Dam M, Leon A (2008) A saturated *N*-acylethanolamine other than *N*-palmitoyl ethanolamine with anti-inflammatory properties: a neglected story. J Neuroendocrinol Suppl 1:26–34. doi:10.1111/j.1365-2826.2008.01689.x
18. Bligh EG, Dyer WI (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917
19. Vaskovsky VE, Kostetsky EY, Vasendin IM (1975) A universal reagent for phospholipid analysis. J Chromatogr 114:129–141
20. Vaskovsky VE, Terekhova TA (1979) HPTLC of phospholipid mixtures containing phosphatidylglycerol. J High Resol Chromatogr 2:671–672
21. Svetashev VI, Vaskovsky VE (1972) A simplified technique for thin-layer microchromatography of lipids. J Chromatogr 67:376–378
22. Ichihara K, Fukubayashi Y (2010) Preparation of fatty acid methyl esters for gas-liquid chromatography. J Lipid Res 51:635–640
23. McLauren Dorrance A, Graham D, Dominiczak A, Fraser R (2000) Inhibition of nitric oxide synthesis increases erythrocyte membrane fluidity and unsaturated fatty acid content. Am J Hypertens 13:1194–1202
24. Cedernaes J, Alsiö J, Västermark A, Risérus U, Schiöth HB (2013) Adipose tissue stearoyl-CoA desaturase 1 index is increased and linoleic acid is decreased in obesity-prone rats fed a high-fat diet. Lipids Health Dis 12:2. doi:10.1186/1476-511X-12-2
25. Anderson N, Borlak J (2008) Molecular mechanisms and therapeutic targets in steatosis and steatohepatitis. Pharmacol Rev 60:311–357
26. Parpal S, Karlsson M, Thorn H, Strålfors P (2001) Cholesterol depletion disrupts caveolae and insulin receptor signaling for metabolic control via insulin receptor substrate-1, but not for mitogen-activated protein kinase control. J Biol Chem 276:9670–9678
27. Calder PC, Yaqoob P (2007) Lipid rafts–composition, characterization, and controversies. J Nutr 137:545–547
28. Leibiger IB, Leibiger B, Berggren PO (2008) Insulin signaling in the pancreatic β-cell. Annu Rev Nutr 28:233–251
29. Persaud SJ, Asare-Anane H, Jones PM (2002) Insulin receptor activation inhibits insulin secretion from human islets of Langerhans. FEBS Lett 510:225–228
30. Hula NM, Kosiakova HV, Kindruk NL, Khmel' TO (2005) Effect of *N*-stearoylethanolamine on the level of stable NO metabolites in different pathological conditions which are accompanied by oxidative stress. Ukr Biokhim Zh 77:113–119
31. Nakamura MT, Nara TY (2004) Structure, function, and dietary regulation of Δ6, Δ5, and Δ9 desaturases. Annu Rev Nutr 24:345–376
32. Brown JM, Rudel LL (2010) Stearoyl-coenzyme A desaturase 1 inhibition and the metabolic syndrome: considerations for future drug discovery. Curr Opin Lipidol 21:192–197
33. Ollila S, Hyvönen MT, Vattulainen I (2007) Polyunsaturation in lipid membranes: dynamic properties and lateral pressure profiles. J Phys Chem B 111:3139–3150
34. Elmendorf JS (2004) Fluidity of insulin action. Mol Biotechnol 27:127–138
35. Hardwick JP, Eckman K, Lee YK, Abdelmegeed MA, Esterle A, Chilian WM, Chiang JY, Song BJ (2013) Eicosanoids in metabolic syndrome. Adv Pharmacol 66:157–266
36. Chen M, Yang ZD, Smith KM, Carter JD, Nadler JL (2005) Activation of 12-lipoxygenase in proinflammatory cytokine-mediated beta cell toxicity. Diabetologia 48:486–495
37. Høstmark AT, Haug A (2013) Percentages of oleic acid and arachidonic acid are inversely related in phospholipids of human sera. Lipids Health Dis 12:106
38. Gulaya NM, Kuzmenko AI, Margitich VM, Govseeva NM, Melnichuk SD, Goridko TM, Zhukov AD (1998) Long-chain *N*-acylethanolamines inhibit lipid peroxidation in rat liver mitochondria under acute hypoxic hypoxia. Chem Phys Lipids 97:49–54