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# THE EFFECT OF N-STEAROYLETHANOLAMINE ON THE ADIPOCYTE FATTY ACID COMPOSITION OF DIFFERENT AGE RATS WITH OBESITY-INDUCED INSULIN RESISTANCE

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**Abstract**

Chronic hypernutrition and high fat diet (HFD), rich in saturated fatty acids leads to molecular changes in insulin sensitive tissues and is followed by dyslipidemia. That is why the aim of our study was to investigate the fatty acid (FA) composition of phospholipids (PL), free fatty acids (FFA), triacylglycerol (TAG) and cholesterol esters (CE) of adipocytes in different age rats with HFD-induced insulin resistance (IR) and its changes under N-stearoylethanolamine (NSE) administration.

The experimental model was induced on rats in age 10-month-old and 24-month-old by 6-month HFD and confirmed by the oral glucose tolerance test. NSE was administrated as water suspension per os in a dosage 50 mg/kg daily during 2 weeks. Adipocytes were isolated from abdominal fat using Type 1 Collagenase solution. Adipocytes lipid extract was separated on the fractions by thin-layer chromatography. The fatty acid composition of lipid fractions was analyzed by gas-liquid chromatography. Experimental data were processed statistically using Student's t-test.

It was demonstrated, that prolonged HFD induces IR and leads to changes in FA profile of adipocytes PL, TAG, CE and composition of FFA in rats from two age groups. The results we obtained shoved that the aging process affects the fatty acid composition of adipocytes. Particularly, there was a significant decrease in the amount of fatty acids in the fractions of phospholipids, triacylglycerols and cholesterol esters together with a decrease in the percentage of unsaturated fatty acids. It was also demonstrated, that HFD significantly alters the fatty acid composition of all investigated adipocytes lipid fractions of younger age group rats, while similar changes were much less manifested in older age group of animals. NSE administration had a positive effect on the normalization of the fatty acid composition of the studied lipid fractions of both age group rat adipocytes.

This study demonstrated that prolonged HFD induces obesity, increases the risk of type 2 diabetes development and leads to changes in adipocytes FA profile in rats from two age groups. As far as NSE administration had a positive effect on normalization of FA composition of adipocytes, we can consider NSE as a prospective agent for the treatment of obesity-induced complications and correction of age-related dyslipidemia.

**Keywords**: fatty acid composition, obesity, adipose tissue, dyslipidemia, N-stearoylethanolamine.

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## 1. Introduction

According to the World Health Organization, the percentage of older people is steadily increasing and is projected to double by 2050 compared to 2015 and will exceed one fifth of all the human population [1]. At the same time, along with the economic development, food traditions and habits have been changed and now the so-called "Western type of diet", characterized by an excess of simple carbohydrates and saturated fats, is extremely widespread. As a result, the rapidly increasing prevalence of chronic obesity causes diseases, including metabolic disorders such as dyslipidemia and insulin resistance, leads to cardiovascular disease, type 2 diabetes and the metabolic syndrome [2, 3]. The situation is further complicated by the fact that aging is accompanied with

a redistribution of the ratio of subcutaneous and abdominal adipose tissue towards the increase of the latter, and excess deposition of visceral fat is recognized as a source of chronic inflammatory process and the above mentioned metabolic disorders [4].

It is well known, that fatty acids (FA) are one of the major components of lipids, which biochemical properties depend mainly on the fatty acid composition. Currently, there are a number of papers [5–8], devoted to the fatty acid analysis of adipose tissue as a whole and its individual lipid fractions. At the same time, the question remains how the prolonged dietary fat overload affects the fatty acid composition of adipose tissue and whether there are differences in FA composition of different age groups of animals. Given the important role of adipose tissue in the regulation of eating behavior, lipid metabolism, energy expenditure, etc., it is important to maintain the structural and functional state of adipocyte membranes, which in turn depends largely on the composition of FAs.

Nowadays, the important role of the endocannabinoid system in most vital processes regulation in the organism has been proved [9, 10]. It is known, that the endocannabinoid system includes representatives of a class of minor lipids of N-acylethanolamines (NAEs), which exhibit cannabimimetic properties and a wide range of biological activity [9, 11].

In our previous studies [12–14], the positive effect of N-stearoylethanolamine (NSE) on the normalization of the lipid profile of various tissues and organs in different pathological conditions, including obesity and insulin resistance, was shown.

That is why, the aim of this study was to investigate the effect of NSE on the fatty acid content of various lipid fractions (phospholipids, free fatty acids, triacylglycerols and cholesterol esters) of adipocytes of two age group rats with alimentary obesity-induced insulin resistance (IR).

## 2. Materials and methods

### 2. 1. Animal model

The study was carried out on male outbred rats in age 10-month-old (10-m.o.) and 24-month-old (24-m.o.) with average weight 150.5±1.76 g and 491±13.52 g respectively at the beginning of the experiment. 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.

Rats were housed in standard cages with free access to food and water. Obesity-induced IR was obtained by the feeding a long-term high-fat diet (HFD) during 24 weeks [15]. The diet included pellets with addition of visceral lard as the source of extra fats. The analysis of described HFD [16] demonstrated that total the 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 the control group received standard pellet diet, containing 4 % of fats with the percentage of SFA and UFA at the level of 38 % and 62 % respectively.

After 6-months HFD period, the oral glucose tolerance test was conducted [17]. 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” ($n$=11 and $n$=10 for 10 m.o. and 24 m.o. groups respectively) and “IR+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 “IR+NSE” groups received the water suspension of NSE per os during 14 days in a dose of 50 mg per 1 kg of body weight. At the end of the experiment, the rats were decapitated under Nembutal anesthesia according to the ethical principles for the conduct with laboratory animals [18].

The abdominal fat pads were removed immediately for further adipocytes isolation.

### 2. 2. Adipocytes isolation

Abdominal fat pads were digested with Type 1 Collagenase solution in HEPES buffer (pH 7.4) according to the modified Rodbell procedure [19, 20]. 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 $CaCl_2x_2H_2O$, 1.25 mM $MgSO_4X7H_2O$, 0.45 mM $KH_2PO_4$, 2.17 mM $Na_2HPO_4$, 10 mM HEPES.

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

### 2. 3. Lipids extraction from adipocytes

Total lipids from the adipocytes were extracted and purified according to the Blight and Dyer methods [21] with minor modifications. Then, 3 ml of solvent system of chloroform/methanol (2:1, v/v) was added to 1 ml of adipocyte suspension and after 30 seconds vortexing, the mixture was centrifuged during 15 minutes at 2500 G. The lower chloroform layer was transferred to the flask. After that, 2 ml of chloroform was added to residuary methanol fraction, vortexing during 30 seconds and centrifuged again at the same condition for total lipids extraction. The removed chloroform layers were integrated for further aspiration.

Lipid fractions separation was carried by thin-layer chromatography using a solvent system of hexane: diethyl ether: acetic acid with component ratio by volume 85:15:1.

### 2. 4. Fatty acid composition determination

After separation of lipid extract by thin-layer chromatography, fractions of phospholipids (PL), free fatty acids (FFA), triacylglycerols (TAG) and cholesterol esters (CE) were removed from the plates and methylated using 3M HCl in methanol [22]. The quantitative analysis of FA methyl esters was performed using gas-liquid chromatography on the chromatograph GC7890 Agilent on a Supelco SP-2560 capillary column (100 m×0.25 mm×0.2 µm) using an internal standard the caprylic acid (C8:0) (Sigma). The identification of individual FAs was performed using chromatograph software according to the calibration table of 37 fatty acid standards (Sigma).

### 2. 5. Statistical analysis

The data, presented as mean values±standard errors of the means (SEM) from different studied groups, were compared by the Student's unpaired *t*-test. The statistical difference of significance was determined at $P<0.05$ and $P<0.1$.

## 3. Results and discussion

### 3. 1. Experimental model of obesity-induced insulin resistance

On the 24th week, the average weight of HFD rats was 500±11.5 g and 593.4±24.2 g in comparison with 446.4±19.23 g and 471.8±10.34 g of control rats in 10-m.o. and 24-m.o. groups respectively. The results of the oral glucose tolerance test for two age groups of animals are presented in **Fig. 1**.

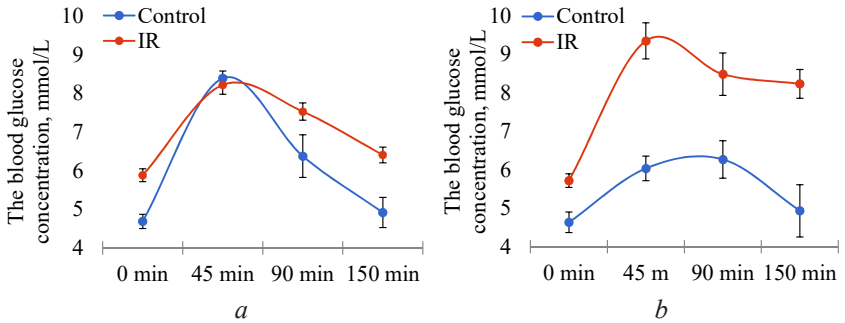


**Fig. 1.** Results of oral glucose tolerance test: *a* – 10-month-old rats; *b* – 24-month-old rats

### 3. 2. Phospholipid fatty acid composition

Many cellular functions, such as enzymatic activity, hormonal response, and membrane permeability, are known to depend on the physical and chemical properties of the plasma membrane lipid bilayer, which consists mainly of phospholipids. Therefore, the imbalance in the phospholipid composition of cells at the condition of obesity and type 2 diabetes influences membrane fluidity, permeability and, as a consequence, the activity of enzymes, in particular, protein kinases, involved in insulin signaling. It is known, that the properties of phospholipids are mainly determined by their FA composition.

The results of our fatty acid composition study showed that in the phospholipid fraction of adipocytes of rats of two age groups are represented in different amounts of saturated fatty acids (C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C21:0, C22:0, C23:0, C24:0) and unsaturated, among which monoenic (C16:1, C18:1, C20:1, C22:1n9, C24:1n9), dienoic (C18:2n6, C20:2, C22:2n6) and polyenoic (C18:3n3, C20:3n6, C20:3n3, C20:4n6, C20:5n3, C22:6n3). The highest proportion of all fatty acids, identified in the phospholipid fraction, included saturated C16:0 and C18:0 and unsaturated C18:1n9c and C18:2n6c, which accounted for more than 75 % of the total FAs.

The results of our study showed that saturated fatty acids (SFA) and unsaturated (UFA), in particular monounsaturated (MUFA), bi-unsaturated (Bi-UFA) and polyunsaturated (PUFA) were determined in PL fraction of rat adipocytes and **Table 1** summarizes the rat adipocyte phospholipid fatty acids content.

**Table 1**

Fatty acid classes represented in phospholipids of rat adipocyte

| | Age, month | Fatty acids content, mg/g lipids | | | |
|---|---|---|---|---|---|
| | | Control | Control+NSE | IR | IR+NSE |
| Total FA | 10 | 1.022±0.256 | 0.894±0.032 | 3.016±0.724$^{*}$ | 2.757±1.025$^{\#\#}$ |
| | 24 | 0.347±0.100$^{*}$ | 0.870±0.201$^{@}$ | 0.590±0.150$^{@@}$ | 1.248±0.417$^{@\&\&}$ |
| SFA | 10 | 0.527±0.157 | 0.420±0.076 | 1.515±0.304$^{*}$ | 1.353±0.496$^{*}$ |
| | 24 | 0.136±0.031$^{*}$ | 0.361±0.084$^{@}$ | 0.212±0.057 | 0.444±0.172$^{@\&\&}$ |
| UFA | 10 | 0.496±0.099 | 0.474±0.106 | 1.501±0.448$^{*}$ | 1.405±0.532$^{*}$ |
| | 24 | 0.211±0.069$^{*}$ | 0.508±0.118$^{@}$ | 0.378±0.094$^{@@}$ | 0.805±0.252$^{@\#\#}$ |
| MUFA | 10 | 0.358±0.093 | 0.339±0.075 | 1.044±0.291$^{*}$ | 1.089±0.429$^{*}$ |
| | 24 | 0.136±0.038$^{*}$ | 0.317±0.081$^{@}$ | 0.244±0.062$^{@@}$ | 0.551±0.197$^{@\&\&}$ |
| Bi-UFA | 10 | 0.119±0.009 | 0.094±0.022 | 0.387±0.149$^{*}$ | 0.279±0.097$^{**}$ |
| | 24 | 0.057±0.026$^{*}$ | 0.164±0.035$^{@}$ | 0.105±0.030 | 0.215±0.063$^{@\&\&}$ |
| PUFA | 10 | 0.019±0.002 | 0.041±0.014$^{**}$ | 0.070±0.022$^{*}$ | 0.037±0.011$^{*\#\#}$ |
| | 24 | 0.018±0.006 | 0.028±0.004 | 0.029±0.008 | 0.039±0.010$^{\&\&}$ |

*Note: Values represented mean±SEM. * and ** – p<0.05 and p<0.1 compared to the 10-m.o. "Control" group respectively; # and ## – p<0.05 and p<0.1 compared to the 10-m.o. "IR" group respectively; @ and @@ – p<0.05 and p<0.1 compared to the 24-m.o. "Control" group respectively; & and && – p<0.05 and p<0.1 compared to the 24-m.o. "IR" group respectively*

As data in the **Table 1** show, in adipocytes of the younger age group rats the total amount of FAs, incorporated to the PL fraction, was significantly higher than in animals of the older age group. The prolong HFD increased the total phospholipid FAs of 10-m.o. and 24-m.o. rats, which can be explained by adipocyte hypertrophy, invariably accompanying obesity and, as a consequence, by an increase in the quantity of PLs, as major components of cell membranes [5].

The NSE did not have a statistically significant effect on the level of total FAs in PL fraction of fat cells of younger rats (both the "Control" and "IR" groups). At the same time, a pronounced

effect of NSE was observed in animals of the older age group: as a result of NSE administration the total FA content of PL more than doubled in both control and IR-rats. The percentage ratio of saturated and unsaturated FAs in rat adipocyte phospholipids is shown on **Fig. 2**.

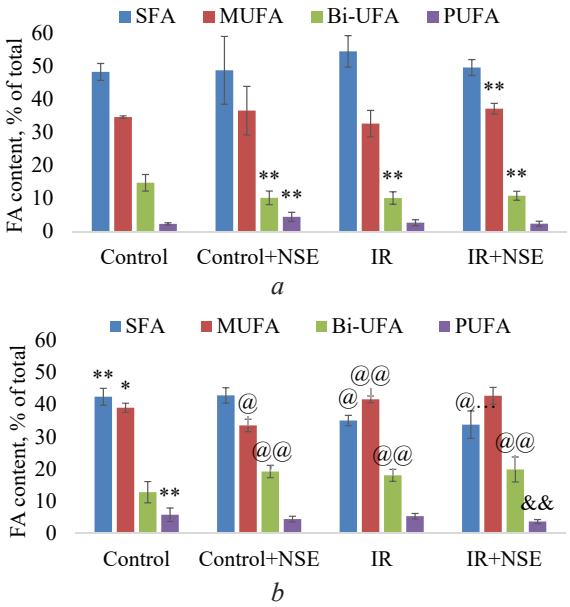


**Fig. 2.** Percentage of fatty acid classes, represented in phospholipids of rat adipocyte: *a* – 10-m.o. rats, *b* – 24-m.o. rats. Values represented mean±SEM. * and ** – $p<0.05$ and $p<0.1$ compared to the 10-m.o. "Control" group respectively; # and ## – $p<0.05$ and $p<0.1$ compared to the 10-m.o. "IR" group respectively; @ and @@ – $p<0.05$ and $p<0.1$ compared to the 24-m.o. "Control" group respectively; & and && – $p<0.05$ and $p<0.1$ compared to the 24-m.o. "IR" group respectively

The analysis of the obtained data showed that the ratio of SFA and UFA in phospholipids of adipocytes of control rats slightly shifted towards UFA (48.29±2.56 % to 51.71±2.56 % in the younger group and 42.40±2.62 % to 57.6±2.62 % in older age group). Of particular interest are the results, regarding the effect of long-term dietary fat overloading. The younger age group tended to increase the percentage of SFA and to reduce UFA, accordingly. At the same time, in older rats that were fed by HFD, the degree of unsaturation of the adipocyte phospholipid FA composition significantly increased (the ratio of SFA to UFA was 35.02±1.61 % to 64.98±1.61 %).

The unsaturated fatty acids of PL fraction were represented by monoenic, dienoic and polyenic FAs. In older group the percentage of MUFA was significantly higher than that of the younger age group (38.98±1.43 % and 34.64±0.36 % of total FAs of PL fraction respectively). After 14 days of NSE administration to 24-m.o. control rats, the proportion of MUFA of fatt cell PL decreased to 10-m.o. animals level and was 33.51±1.94 %, whereas the absolute quantitative content increased significantly (**Table 1**).

Analazying the experimental data, there were found a tendency for increase of the Bi-UFA content twice in IR-rats compared to controls from relevant age groups, however, their percentage in younger age group decreased (from 14.76±2.51 % in «Control» to 10.15±1.88 % of total fatty acids in «IR» group), whereas in the older group there was an increase in Bi-UFA (from 12.8±3.29 % in control to 18.03±1.86 % in IR-rats, respectively). NSE administration led to a quantitative and percent decrease in the level of dienoic FAs in control animals of the younger age group (up to 0.094±0.022 mg/g lipids and 10.20±2.06 %) and their growth in older rats of the respective group (to 0.164±0.035 mg/g lipids and 19.21±1.92 %) and caused no changes in obese rats of both age groups.

Data on the content of polyunsaturated fatty acids in phospholipids showed that their content was at the same level in animals of both age groups, but the percentage of polyenoic FAs in

older rat adipocytes was twice higher than the values of younger animals (5.82±2.1 % compared to 2.31±0.35 %). Long-term high fat loading leads to a significant increase in the content of PUFA of adipocyte PL in 10-m.o. rats (**Table 1**), while the percentage of this FAs remains unchanged. Long-term dietary fat overloading also led to an increase in the amount of PUFA in older rats, but to a much lesser extent compared to younger rats.

Due to the administration of NSE for 2 weeks, the amount of PUFAs in PL fraction of adipocytes of younger control rats doubled (**Table 1**), and the percentage of this class of FAs also increased significantly (from 2.31±0.35 % in the control group to 4.45±1.38 % in the group "Control+NSE", respectively). The effect of NSE was observed to a much lesser extent on the older animal model. At the same time, obese and insulin-resistant rats at 24 months of age had a tendency to increase the amount of polyenic FAs while reducing their percentage (from 0.029±0.008 mg/g lipids and 5.36±0.85 % in the "IR" group to 0.0399±0.0019 mg/g lipids and 3.7±0.62 % in the «IR+NSE» group, respectively), whereas in younger animals PUFA amount decreased, but their percentage content had no changes from 0.07±0.022 mg/g lipids and 2.68±0.91 % in the «IR» group to 0.037±0.011 mg/g lipids and 2.37±0.76 % in the "IP+NSE" group respectively).

### 3. 3. Free fatty acid composition

In the pool of free fatty acids in adipocytes there were identified SFA (C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0,C21:0, C22:0, C23:0, C24:0), MUFA (C16:1, C17:1, C18:1, C20:1, C22:1n9, C24:1n9), Bi-UFA (C18:2n6, C20:2, C22:2n6) and PUFA (C18:3n6, C18:3n3, C20:3n6, C20:3n3, C20:4n6, C20:5n3, C22:6n3). The largest part of FFA was represented mainly by C16:0, C18:0, C18:1n9c and C18:2n6c.

The data of the main free fatty acid classes are shown in **Table 2**.

**Table 2**
Free fatty acid classes, represented in rat adipocyte

| | Age, month | Fatty acids content, mg/g lipids | | | |
|---|---|---|---|---|---|
| | | Control | Control+NSE | IR | IR+NSE |
| Total FA | 10 | 2.161±0.250 | 2.714±0.645 | 16.058±4.325$^{*}$ | 8.867±2.534$^{*\#\#}$ |
| | 24 | 4.682±1.032$^{*}$ | 2.940±0.679$^{@@}$ | 4.437±1.027 | 4.567±1.029 |
| SFA | 10 | 1.097±0.163 | 1.234±0.281 | 4.794±0.927$^{*}$ | 3.863±0.983$^{*}$ |
| | 24 | 1.875±0.431$^{*}$ | 1.108±0.278$^{@@}$ | 1.754±0.385 | 1.821±0.406 |
| UFA | 10 | 1.065±0.088 | 1.758±0.545 | 7.337±1.953$^{*}$ | 5.004±1.565$^{**}$ |
| | 24 | 2.807±0.608$^{*}$ | 1.833±0.404$^{@@}$ | 2.683±0.650 | 2.746±0.629 |
| MUFA | 10 | 0.787±0.060 | 1.115±0.267 | 4.663±1.084$^{*}$ | 3.718±1.138$^{*}$ |
| | 24 | 2.224±0.502$^{*}$ | 1.343±0.332$^{@@}$ | 1.839±0.411 | 2.108±0.497 |
| Bi-UFA | 10 | 0.229±0.017 | 0.344±0.105 | 2.607±0.872$^{*}$ | 1.256±0.432$^{*\#}$ |
| | 24 | 0.530±0.123$^{*}$ | 0.471±0.082 | 0.747±0.255 | 0.563±0.150 |
| PUFA | 10 | 0.048±0.014 | 0.020±0.000$^{*}$ | 0.068±0.012 | 0.031±0.013$^{\#}$ |
| | 24 | 0.053±0.009 | 0.019±0.006$^{@}$ | 0.097±0.020$^{@}$ | 0.075±0.020 |

*Note: Values represented mean±SEM. * and ** – p<0.05 and p<0.1 compared to the 10-m.o. "Control" group respectively; # and ## – p<0.05 and p<0.1 compared to the 10-m.o. "IR" group respectively; @ and @@ – p<0.05 and p<0.1 compared to the 24-m.o. "Control" group respectively; & and && – p<0.05 and p<0.1 compared to the 24-m.o. "IR" group respectively*

In our study, it was shown, that the total pool of FFAs in adipocytes was higher twice in control rats of the older age group as compared to that of the younger group. In younger animals, HFD caused a rapid increase in the total amount of FFA more than 5-fold compared to controls. However, in rats aged 24 months, no changes were found in the amount of FFA of adipocytes after prolonged loading by dietary fats (**Table 2**).

It is known from the literature, that obesity is accompanied by the chronic low-grade inflammation, which is caused by an increased level of circulating FFA [23]. The obtained data can be explained by the fact that in the case of a large amount of dietary fats, adipocytes intensively extract excess FFA from the bloodstream for further conversion by β-oxidation. Obviously, during the aging, this function of fat cell lipids attenuates and they, on the one hand, are not able to absorb fatty acids under the condition of HFD, and on the other – not so effectively metabolize them [5, 24]. After the administration of NSE for 2 weeks in 10-m.o. animals with obesity-induced IR, the level of total FFA of adipocytes decreased by almost 1.5 times, similarly as in control 24-m.o. (**Table 2**). At the same time, NSE administration did not cause significant effects on the FFA content in both the control younger rats and the older IR-rats.

The percentage distribution of main fatty acids in FFA pool are shown in **Fig. 3**.

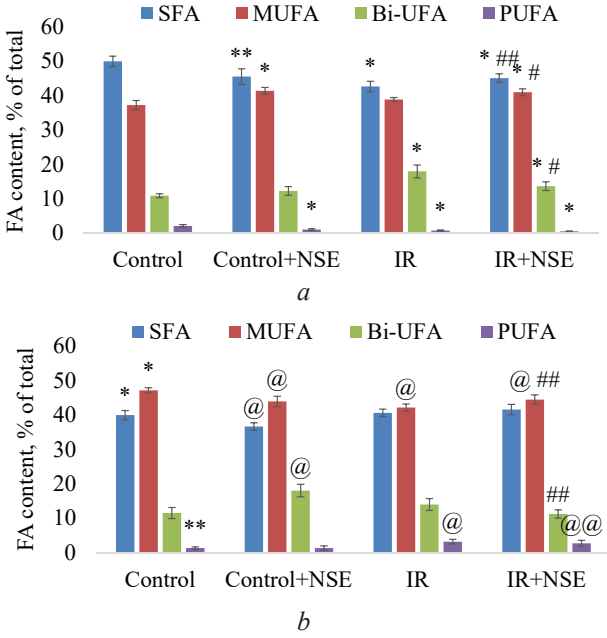


**Fig. 3.** Percentage of free fatty acid classes, represented in rat adipocyte: *a* – 10-m.o. rats, *b* – 24-m.o. rats. Values represented mean±SEM. * and ** – $p<0.05$ and $p<0.1$ compared to the 10-m.o. "Control" group respectively; # and ## – $p<0.05$ and $p<0.1$ compared to the 10-m.o. "IR" group respectively; @ and @@ – $p<0.05$ and $p<0.1$ compared to the 24-m.o. "Control" group respectively; & and && – $p<0.05$ and $p<0.1$ compared to the 24-m.o. "IR" group respectively

However, in the control rats of the younger age group, the ratio of saturated and unsaturated FFA was equivalent (49.88±1.52 % to 50.12±1.52 %, respectively) and varied toward increasing the percentage of unsaturated FA in the process of aging (SFA – 39.96±1.36 %; UFA – 60.04±1.36 %) and as a result of long-term consumption of excess dietary fats (SFA – 42.58±1.53 %; UFA – 57.42±1.53 %). In turn, no statistically significant difference in the proportion of SFA: UFA was observed in obese 24-m.o. rats compared to control animals of the same age.

As a result of 14 days NSE administration, in control animals of both age groups, an increase in the saturation of adipocyte FFA pool was recorded. However, in 10-m.o. obese rats, the use of NSE caused a decrease in the proportion of UFA to 54.98±1.27 %; and, at the same time, in group of 24-m.-o. IR-rats, NSE had no significant effect on the UFA content.

Among the UFAs, the representatives of the monoenoic subgroup proved to be the most numerous. In healthy animals of older age, the MUFA level significantly exceeded the rates of younger rats (2.224±0.502 mg/g lipids and 47.15±0.79 %, respectively, compared to 0.787±0.06 mg/g lipids and 37.19±1.34 %). The long-term HFD caused a significant increase in the amount of adipocyte monounsaturated FFA in 10-m.o. rats, but their percentage did not change statistically significant.

Contrary to these data, the group of 24-m.o. rats, consuming additional dietary fats, showed a slight decrease in the quantity and a statistically significant decrease in the percentage content of monoenic FAs among total FFAs up to 42.18±1.04 %. Probably, the increase of the MUFA content in young rats with obesity-induced IR occured due to an increase in Δ9-desaturase activity under dietary fat overloading [25]. In particular, increases in the oleic acid content are usually associated with an increase in the activity of stearoyl-CoA-desaturase-1 (SCD1), which in turn contributes to the development of IR in HFD feeding conditions [26]. In our studies, the administration of NSE contributed to the normalization of the content of monounsaturated FFA in adipocytes of IR animals. Such an NSE effect may be due to inhibition of SCD1 expression [27].

Significant fluctuations were observed in the levels of dienoic FFA.Thus, under the influence of long-term consumption of excess fats, in adipocytes of 10-m.o. rats the content of unsaturated FFA increased by almost 10 times (**Table 2**). The administration of NSE led to the twice decrease in these parameters in IR-animals, although no statistically significant changes were observed in the control animals of the younger age group. In rats aged 24 months, the content of Bi-UFA was twice higher than the reference values of younger animals, but their percentage did not undergo significant changes in the aging process ("Control" values at the age of 24 months were 0.530±0.123 mg/g lipids and 11.52±1.61 %). The prolong HFD tended to increase the quantitative and percentage content of UFA (up to 0.747±0.255 mg/g lipids and 14±1.71 %), and the use of NSE contributed to the normalization of these parameters (for the "IR+NSE" group at 24 months of age – 0.563±0.15 mg/g lipids and 11.24±1.21 %). Thus, the growth of the total pool of FFA in rat adipocytes under conditions of prolonged fat loading may be the result of an increase in desaturase activity, and the positive effect of NSE on the normalization of the dienoic FFAs content is clearly related to the suppression of SCD1, a key enzyme of the synthesisof monoenic FAs, which are the substrate for the production FAs with more double bonds [27].

The smallest subgroup, represented in the total pool of FFA, were polyenoic fatty acids. No statistically significant differences were observed in the figures of the content of these FFAs between two age groups (**Table 2**). Rats of both age groups with obesity-induced IR had an increase in the content of PUFA, but statistically significant differences were only in older rats The NSE effect was in a significant reduction in the number of PUFA as in control animals of both age groups, as in 10-m.o. IR-rats (and a similar trend in 24-m.o. animals with IR (**Table 2**).

### 3. 5. Triacylglycerol fatty acid composition

The results of fatty acid analysis of rat adipocytes demonstrated that in the triacylglycerol fraction there were present SFA (C12:0, C14:0, C15:0, C16:0, C17:0, C18:0,C20:0, C22:0, C23:0, C24:0), MUFA (C16:1, C17:1, C18:1, C20:1, C22:1n9, C24:1n9), Bi-UFA (C18:2n6 i C20:2) and PUFA (C18:3n6, C18:3n3, C20:3n6). However, C16:0, C18:0, C18:1n9c and C18:2n6c had a significant quantitative prevalence over other fatty acids.

**Table 3** summarizes the data of the triacylglycerol fatty acids content.

As it was expected, triacylglycerols were the most fatty acid-rich adipocyte lipid fraction. The complexity of interpreting obtained results was due to a significant variation in the individual results for each animal. There were no statistically significant differences between the reference values for rats of different age groups, but some tendency was observed for the decrease in the FA content in the triacylglycerol fraction as a result of aging (**Table 3**).

It was shown, that in group of 10-m.o. rats the content of FAs, isolated from the fraction of triacylglycerols, significantly increased after 6-month HFD, whereas for 24-m.o. rats the content of triacylglycerols FAs did not undergo statistically significant changes. Thus, our study demonstrated that during the aging, the function of adipocytes as energy depots alters and manifests itself in the inability to adequately respond to the intake of excess dietary fats [5, 24]. After the oral administration of NSE for 2 weeks the content of FAs in TAG of adipocytes of younger IR-rats significantly decreased. In the case of control animals of both age groups, no statistically significant effects on the quantity of total TAG FAs were found, however, there was a tendency for slight increase compared to the control animals of the respective age groups (**Table 3**).

**Table 3**

Fatty acid classes, represented in triacylglycerols of rat adipocyte

| | Age, month | Fatty acids content, mg/g lipids | | | |
|---|---|---|---|---|---|
| | | Control | Control+NSE | IR | IR+NSE |
| Total FA | 10 | 56.503±22.074 | 83.297±13.218 | 119.145±16.382* | 79.651±16.148# |
| | 24 | 46.958±6.604 | 49.049±15.016 | 56.999±8.323 | 47.797±6.390 |
| SFA | 10 | 12.846±4.347 | 21.047±3.361** | 44.641±7.676* | 26.568±4.563*# |
| | 24 | 14.605±1.844 | 17.079±3.897 | 20.353±3.087@@ | 17.344±2.185 |
| UFA | 10 | 45.134±18.684 | 62.250±9.911 | 74.504±9.081** | 53.082±11.889## |
| | 24 | 32.353±5.100 | 31.970±11.124 | 36.924±5.619 | 30.453±4.237 |
| MUFA | 10 | 21.594±8.142 | 30.002±5.382 | 55.957±7.736* | 34.790±6.347# |
| | 24 | 20.051±3.374 | 22.118±5.501 | 27.204±3.991@@ | 24.319±3.188 |
| Bi-UFA | 10 | 21.827±9.493 | 31.914±4.639 | 18.370±2.190 | 18.057±6.001 |
| | 24 | 12.149±2.634 | 9.735±5.566 | 9.192±2.366 | 6.075±1.185@@ |
| PUFA | 10 | 0.236±0.100 | 0.334±0.037 | 0.177±0.035 | 0.235±0.093 |
| | 24 | 0.153±0.034 | 0.116±0.071 | 0.249±0.151 | 0.059±0.017@@ |

*Note: Values represented mean±SEM. * and ** – p<0.05 and p<0.1 compared to the 10-m.o. "Control" group respectively; # and ## – p<0.05 and p<0.1 compared to the 10-m.o. "IR" group respectively; @ and @@ – p<0.05 and p<0.1 compared to the 24-m.o. "Control" group respectively; & and && – p<0.05 and p<0.1 compared to the 24-m.o. "IR" group respectively*

The results of the TAG fatty acid analysis in percentage values are presented in **Fig. 4.**

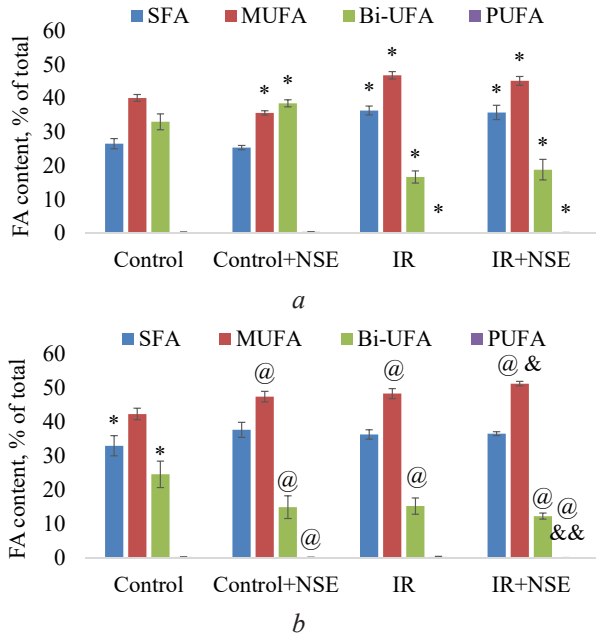


**Fig. 4.** Percentage of triacylglycerol fatty acid classes, represented in rat adipocyte: *a* – 10-m.o. rats, *b* – 24-m.o. rats. Values represented mean±SEM. * and ** – $p<0.05$ and $p<0.1$ compared to the 10-m.o. "Control" group respectively; # and ## – $p<0.05$ and $p<0.1$ compared to the 10-m.o. "IR" group respectively; @ and @@ – $p<0.05$ and $p<0.1$ compared to the 24-m.o. "Control" group respectively; & and && – $p<0.05$ and $p<0.1$ compared to the 24-m.o. "IR" group respectively

The experimental data demonstrated that most of the adipocyte TAG FAs of both age groups of rats were represented by unsaturated FAs and in the aging process their proportion significantly reduced (from 73.47±1.5 % in healthy rats aged 10 months to 67.09±2.95 % in animals 24 months

of age). The HFD-rats were characterized by a significant increase in the amount of SFA and their percentage (**Table 3** and **Fig. 4**). NSE administration significantly reduced the amount of both SFA and UFA in animals aged 10 months, but did not affect their percentage. In the 24-m.o. rats, both the quantitative and the percentages of SFA and UFA did not undergo statistically significant changes under the influence of NSE.

The largest group among the unsaturated fatty acids of rat adipocyte triacylglycerols was monoenic FAs and their quantitative and percentage content increased in IR-rats of both age groups compared to the reference values (up to 55.957±7.736 mg/g lipids and 46.8±1.12 % in the «IR» group compared to 21.594±8.142 mg/g lipids and 40.07±1.01 % in the «Control» group in 24 m.o. rats and up to 27.204±3.99 mg/g of lipids and 46.80±1.12 % in the «IR» group compared to 21.594±8.142 mg/g lipids and 40.07±1.01 % in the «Control» group in 10-m.o. rats.

The NSE significantly decreased the MUFA content in TAG of younger rat adipocytes, but their percentage remained constant. A slightly different situation was observed in the older age group: there was a tendency for a decrease in the content of FAs with one double bond in IR-rats, administered NSE, but their percentage increased significantly (to 51.15±0.65 %).

The results of the FA analysis showed that the process of physiological aging in rats is characterized by a significant decrease in the proportion of Bi-UFA in adipocyte triacylglycerols (from 33.02±2.36 % to 24.53±3.88 %), which was also observed as a result of the prolonged saturated fats-reach diet (in 10-month and 24-month IR rats the values were 16.66±1.80 % and 15.23±2.38 %, respectively). It was interesting to note, that the effect of the use of NSE on healthy rats of different age groups was opposite: a tendency to increase in quantitative indicators and a significant increase in the percentage to 38.52±1.05 % in 10-m.o. rats and a significant decrease to 14.89±3.33 % in control 24-m.o. animals and a similar trend in older IR -rats.

The least represented in TAG fraction of adipocytes was polyunsaturated fatty acids, whose proportion was less than 1 % of total FAs. There was a tendency to decrease the content of PUFAs in the composition of rat adipocyte TAG of the older age group compared to younger animals (**Table 3**).

### 3. 6. Fatty acids of cholesterol esters

The experimental data showed that in rat adipocytes in the process of cholesterol esterification there were involved saturated fatty acids (C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, C23:0, C24:0), monounsaturated (C14:1, C16:1, C18:1, C18:1n9t, C20:1, C22:1n9, C24:1n9), biunsaturated (C18:2n6, C20:2) and polyunsaturated (C18:3n3, C20:3n6, C20:3n3). It is noteworthy that more than 80 % of all FAs identified in the cholesterol ester fraction were C16:0, C18:0 , C18:1n9c and C18:2n6c.

The results of the fatty acid analysis of cholesterol esters are presented in **Table 4**.

The results of the study showed that in the cholesterol esters fraction, the amount of fatty acids was three times lower in adipocytes of 24-m.o. rats, compared with 10-m.o. animals (**Table 4**). Long-term dietary fat overloading significantly increased the content of FAs, involved in cholesterol esterification in adipocytes of both age group animals, almost twice (**Table 4**). Such data may indicate an intensification of esterification processes of free cholesterol in adipocytes, the increase of which is a characteristic of the condition of obesity-induced insulin resistance, which was shown in our previous works [13]. As a result of NSE application, the FA content in the CE fraction in control animals of the younger age group was significantly decreased, whereas for IR-rats this effect was not observed to a statistical significance.

The percentage ratio of saturated and unsaturated FAs in rat adipocyte cholesterol esters is shown on **Fig. 5**.

As a result of the analysis, it was found, that the process of physiological aging was accompanied by a decrease in the amount of both saturated and unsaturated fatty acids of the cholesterol esters fraction (**Table 4**), and the ratio of SFA to UFA varied in the direction of decrease of saturated fractions (SFA: UFA in younger age group was 37.28±0.63 % to 62.72±0.63 %, and in older – 33.92±1.72 % to 66.08±1.72 %).

**Table 4**

Fatty acid classes, represented in cholesterol esters of rat adipocyte

| | Age, month | Fatty acids content, mg/g lipids | | | |
|---|---|---|---|---|---|
| | | Control | Control+NSE | IR | IR+NSE |
| Total FA | 10 | 7.832±0.588 | 5.779±0.414* | 15.129±4.692* | 12.040±1.437* |
| | 24 | 2.015±0.388* | 2.323±0.446 | 4.300±0.874@ | 3.585±0.787@ |
| SFA | 10 | 2.909±0.200 | 1.932±0.114* | 5.484±1.643* | 4.723±0.580* |
| | 24 | 0.661±0.109* | 0.731±0.120 | 1.623±0.320@ | 1.356±0.310@ |
| UFA | 10 | 4.923±0.393 | 3.847±0.301* | 9.645±3.115** | 7.317±0.873* |
| | 24 | 1.355±0.282* | 1.593±0.326 | 2.677±0.565@ | 2.229±0.487@@ |
| MUFA | 10 | 3.874±0.285 | 2.449±0.155* | 7.156±2.111** | 6.066±0.699* |
| | 24 | 0.870±0.151* | 1.050±0.189 | 2.033±0.392@ | 1.714±0.371@ |
| Bi-UFA | 10 | 1.029±0.110 | 1.332±0.160** | 2.477±1.211 | 1.230±0.191 |
| | 24 | 0.479±0.139* | 0.522±0.176 | 0.632±0.228 | 0.505±0.169 |
| PUFA | 10 | 0.019±0.010 | 0.065±0.030 | 0.012±0.007 | 0.020±0.013 |
| | 24 | 0.006±0.003 | 0.021±0.011 | 0.012±0.009 | 0.010±0.005 |

*Note: Values represented mean±SEM. * and ** – p<0.05 and p<0.1 compared to the 10-m.o. "Control" group respectively; # and ## – p<0.05 and p<0.1 compared to the 10-m.o. "IR" group respectively; @ and @@ – p<0.05 and p<0.1 compared to the 24-m.o. "Control" group respectively; & and && – p<0.05 and p<0.1 compared to the 24-m.o. "IR" group respectively*

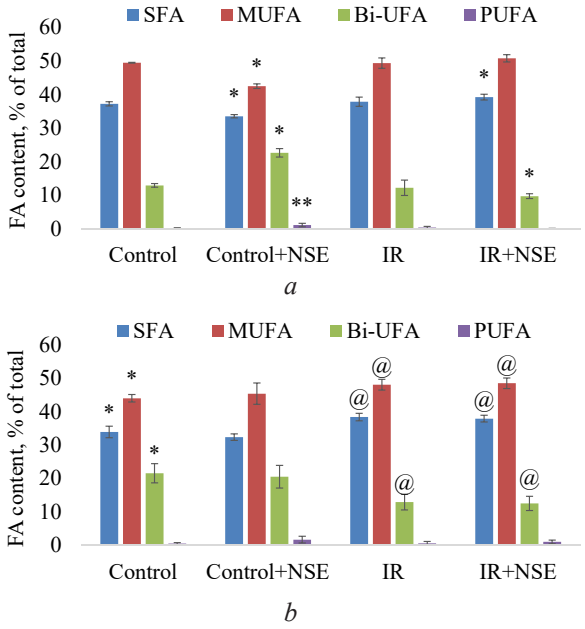


**Fig. 5.** Percentage of cholesterol esters fatty acid classes, represented in rat adipocyte: *a* – 10-m.o. rats, *b* – 24-m.o. rats. Values represented mean±SEM. * and ** – $p<0.05$ and $p<0.1$ compared to the 10-m.o. "Control" group respectively; # and ## – $p<0.05$ and $p<0.1$ compared to the 10-m.o. "IR" group respectively; @ and @@ – $p<0.05$ and $p<0.1$ compared to the 24-m.o. "Control" group respectively; & and && – $p<0.05$ and $p<0.1$ compared to the 24-m.o. "IR" group respectively

In 10-m.o. rats with obesity-induced IR the content of both SFA and UFA in the cholesterol esters composition doubled (**Table 4**), but their percentage remained constant. At the same time, in 24-m.o. IR rats the increase in SFA and UFA was accompanied by changes in their ratio in the direction of increasing their saturation (the amount of SFA increased to 38.40±1.14 %, while UFAs were presented at the level of 61.60±1.14 %).

The NSE did not make statistically significant changes in these parameters in IR-rats of both age groups, whereas a significant decrease in the content of saturated and unsaturated FAs, involved in cholesterol esterification in 10-m.o. control rats, treated with NSE, was found and their ratio changed towards reducing SFA to 33.57±0.5 %.

It is interesting to note, that the distribution of MUFA and Bi-UFA in the composition of rat adipocyte CE was completely opposite in different age groups. It was found, that the content of both monoenic and dienoic FAs significantly decreased during aging (**Table 4**). In this case, the proportion of MUFA decreased in older rats (up to 44.01±1.13 % in comparison with 49.49±0.11 % in young animals), and the percentage of Bi-UFA increased significantly (up to 21.57±2.87 % compared to 12.98±0.54 % in young animals).

It was also found, that at the condition of experimental IR in 10-m.o. rats, followed by a twofold increase in the amount of MUFA and Bi-UFA in the composition of CE (**Table 4**), their percentages remained unchanged, whereas 24-m.o. rats were characterized by an increase in both quantity and percentages of MUFA up to 48.10±1.61 % and a significant decrease in the percentage of Bi-UFA up to 12.92±2.36 %. The effect of NSE was manifested only in the group of healthy rats of younger age in some increase of the dienoic FA content and their percentage to 22.7±1.25 %.

PUFAs were the least represented class in the composition of adipocyte cholesterol esters. Due to their minimal amounts and significant variation of data, a tendency was observed to decrease their content in 24-m.o. rats compared to 10-m.o.. It should also be noted, that the administration of NSE promoted an increase of the content of cholesterol esters PUFAs in control rats of both age groups (**Table 4**).

**4. Conclusions**

In this work, we obtain data that described the N-stearoylethanolamine effect on the adipocyte fatty acid composition of two different age groups of rats during aging and in the conditions of prolonged high-fat diet induced insulin resistance.

Therefore, the age characteristics of the adipocyte lipid fractions fatty acid composition of rats at the age of 10 months and 24 months were established and the study results demonstrated, that phospholipids, triacylglycerols and cholesterol esters were depleted of FAs in older rats, whereas their pool of free fatty acids increased significantly compared to the younger animals.

Thus, it was shown, that in the process of aging, there is likely to be a redistribution of fatty acids between the phospholipid and FFA fractions. This situation was also reflected in some classes of FAs: the decrease in the number of SFA, MUFA and Bi-UFA of phospholipids of older rats was accompanied by their quantitative increase in FFA pool. The decrease of the FAs content in the fractions of triacylglycerols and cholesterol esters in the older age group is consistent with the literature regarding the reduction of the depositing role of fat cells with age.

The experimental data obtained demonstrated that a prolonged HFD, provoking obesity and insulin sensitivity disorders, alters the adipocyte fatty acid composition. In particular, the FAs content of all tested lipid fractions was significantly increased in IR animals from the younger age group, which is a characteristic of adipocytes hypertrophy, whereas no statistically significant changes were observed in older rats. The experimental data obtained showed that the content of FFA in 10-month-old IR rats significantly decreased after NSE administration, which was also observed in the 24-month-old control rats. These data are associated with the anti-inflammatory effect of NSE, described in previous works, since, according to the literature, the excess FFA provokes the development of chronic low-grade inflammation, which is a characteristic of the aging and obesity.

As the study showed the positive effect of N-stearoylethanolamine on normalization of the adipocytes fatty acid profile of rats from two age groups at thenormal phisiological condition and pathology we can consider NSE as a prospective agent for the treatment of obesity-induced insulin resistance and correction of age-related dyslipidemia.

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