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AGE-DEPENDENT METABOLIC AND MORPHOLOGICAL RESPONSE OF RAT ADIPOSE TISSUE TO A HIGH-FAT DIET

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Obesity and related metabolic disorders remain one of the most pressing problems of modern biomedicine. Both obesity and aging cause functional and structural changes in the adipose organ, but the age-specificity of adipose tissue's response to high-fat diet (HFD) remains poorly understood. The aim of the study was to analyze correlation between glucose, lipoproteins and adiponectin plasma levels, to determine Δ9-desaturase activity in adipocyte and to explore the morphological state of adipose tissue in rats of different ages. The experiment was carried out on male Wistar rats for 24 weeks, when young animals reached the age of 10 months, and older – 24 months. Animals of both age groups were divided into kept on a standard rodent diet or a diet with addition of pork visceral lard. The fatty acid composition was identified by gas-liquid chromatography with mass-detection, HDL and LDL cholesterol content – by commercial kits, adiponectin levels – using ELISA kit. The activity index of Δ9-desaturase was calculated as the ratio of oleic to stearic acid. It was shown that the saturated/ unsaturated fatty acids ratio in a standard pelleted feed was 1:4, whereas in the lard it was close to 1:1. No correlation between increased body weight and glucose level, and a positive correlation between adiponectin and HDL cholesterol levels were found in younger rats. In the older rats a positive correlation between body weight and glucose level, and a significant negative correlation between adiponectin and LDL cholesterol levels was observed. Activity of Δ9-desaturase in adipocytes was found to be increased with aging. When animals were kept on a HFD diet, Δ9-desaturase activity in younger group was increased to a large extent, while in older group it decreased significantly as compared with age-matched controls. Morphological analysis showed age-related differences in the morphology, cellular adaptation, and inflammation development in brown and white adipose tissue in response to a dietary fat overload. The results of our study facilitate the identification of potential biomarkers for the prevention and treatment of obesity and associated diseases across different age groups.

K e y w o r d s: obesity, aging, adipose tissue, high-fat diet, adiponectin, lipoproteins, Δ9-desaturase activity, morphological analysis.

Obesity and related metabolic disorders constitute a serious burden on the public health system and a challenge for modern biomedicine. In the context of a rapidly aging world population, understanding the interaction between age-related physiological changes and lifestyle factors, particularly dietary habits, is critical for elucidating the key determinants of metabolic health and developing effective preventive strategies.

A review of current studies suggests that long-term excessive dietary intake of saturated fatty acids is associated with adverse metabolic outcomes, including impaired glucose tolerance, dyslipidemia, insulin resistance (IR), and increased cardiometabolic risk [1, 2]. However, the nature and severity of these changes, induced by high-fat diet (HFD) largely depend on age [3, 4]. Despite significant progress in understanding the metabolic consequences

of HFD, the age-specificity of adipose tissue's response remains poorly understood.

It is known that adipose tissue in mammals comprises white and brown adipose tissue. Both types of adipose tissue perform distinct functions. Thus, white adipose tissue stores excess energy as triacylglycerols and secretes adipokines, while brown adipose tissue mediates non-contractile thermogenesis. Accordingly, the structural features of both types of adipose tissue are determined by their functions, and obesity and aging are two important factors that cause structural and functional changes in the adipose organ [5, 6].

Among the relatively wide range of adipokines synthesized by white adipose tissue, adiponectin holds a special place as an anti-inflammatory and insulin-sensitizing adipokine, which, according to modern research, can be considered an integral marker of metabolic health. According to the literature, a decrease in adiponectin levels is associated with obesity, insulin resistance, worse glucose tolerance, dyslipidemia, and increased cardiometabolic risk [6, 7]. Recent reviews have described a positive role for adiponectin in improving carbohydrate and lipid metabolism, while patients with metabolic syndrome and type 2 diabetes usually have reduced adiponectin levels, which also correlate with high-density lipoproteins (HDL) cholesterol levels in the blood [7]. Measuring plasma glucose, lipoproteins, and adiponectin is essential for assessing the age-dependent metabolic response of adipose tissue to a high-fat diet, as these markers integratively reflect disturbances in carbohydrate and lipid metabolism, adipose tissue endocrine function, and their interrelated contribution to insulin sensitivity and cardiometabolic risk.

An important aspect of determining metabolic status is also the analysis of correlations between systemic indicators – body weight, glucose level, lipoprotein profile and the concentration of adiponectin, a hormone of adipose tissue that plays a leading role in maintaining metabolic homeostasis and protective mechanisms against insulin resistance [8-10]. Studies show that a comprehensive analysis of these relationships helps us better understand the mechanisms underlying metabolic disorders, especially with respect to age.

Adipose tissue serves as a central regulator of energy homeostasis and is sensitive to nutritional challenges. Consequently, morphological and functional alterations, including hypertrophy, hyperplasia, extracellular matrix remodeling, modifications in the lipid pool, and activation of inflammatory pathways, occur in adipocytes during HFD exposure. Notably, stearoyl-CoA desaturase-1 (SCD1 or Δ9-desaturase) plays a pivotal role in these processes as a key enzyme that regulates the balance between saturated and monounsaturated fatty acids. SCD1 activity influences lipogenesis and lipolysis, modulates free fatty acid concentrations in the bloodstream, and impacts systemic insulin sensitivity [11-13]. However, current literature lacks studies that elucidate changes in SCD1 enzyme activity in adipocytes during aging in humans or rodents. Investigating SCD1 activity expands understanding of age-dependent metabolic responses of adipose tissue to a high-fat diet, as this enzyme integrates dietary lipid signals with adipocyte lipid remodeling, inflammatory status, and insulin sensitivity. These processes are differentially regulated during aging and are critical determinants of systemic metabolic adaptation.

Given the above, our work aimed to study the age-related features of obesity development induced by a long-term high-fat diet excess of saturated fatty acids with particular emphasis on correlations among key metabolic and biochemical parameters and on remodeling of white and brown adipose tissue in a rat model across two age groups.

We devoted this work to an extended analysis of the experimental model, described in detail in our previous works [14, 15]. A feature of our experimental model was the study of the effect of a long-term diet with an excess of saturated fats on rats of two age groups fed ad libitum. We have analyzed the fatty acid composition of the diet in detail, conducted correlation analyses of key metabolic parameters, and assessed the age-specificity of adipose tissue's response to dietary stress. The obtained results allow us to understand better the fundamental mechanisms underlying age-related differences in the development of obesity and associated metabolic dysfunctions induced by a long-term high-fat diet excess of saturated fatty acids.

Materials and Methods

High-fat diet model in rats of two age groups. The model of obesity and insulin resistance induced by a long-term HFD has been described in detail in our previous work [14]. The animal model was conducted on male Wistar rats of two age groups during 24 weeks and in the end of experiment younger ani-

mals were 10-month-old (10-m.o.) and older were 24-month-old (24-m.o.). Rats were kept in vivarium conditions in standard cages with free access to water and food in accordance with the General Ethical Principles of Experiments on Animals (Ukraine, 2001), which are consistent with the provisions of the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" (Strasbourg, 1986). The study was approved by the Animal Care and Use Committee of the Palladin Institute of Biochemistry of the NAS of Ukraine.

The rats were randomly divided into "HFD" groups (n = 11 and n = 10 for 10-m.o. and 24-m.o. respectively) and "Control" groups (n = 6 and n = 6 for 10-m.o. and 24-m.o. respectively). Rats from the experimental group were feeding a long-term high-fat diet during 24 weeks. The diet included pellets with addition of pork visceral lard as the source of extra fats. The animals from control group received standard rodent chow. In this study, animals from all the groups were fed ad libitum. At the end of the experiment, the rats were decapitated under Nembutal anesthesia according to the ethical principles for the conduct with laboratory animals. The blood samples were collected after anesthesia by heart puncture, and 3.8% sodium citrate was added immediately after collection. Plasma was then prepared by centrifugation at 1000 rpm for 30 min. Interscapular brown adipose tissue (BAT) and visceral white adipose tissue (WAT) samples were removed immediately for further analysis.

Fatty acid analysis of rat diets. Analysis of pelleted diet and lard was performed by gas-liquid chromatography on an Agilent GC7890 chromatograph with an Agilent 5977B GC/MSD mass detector using a HP-5MS capillary column (30 m × 250 μm × 0.25 μm) with helium as the carrier gas (flow rate: 1 ml/min) and a programmed temperature of 60°C for 1 min, then raised to 280°C at 5°C/min, the final temperature of 280°C was maintained for 15 min. Total time 60 min, injector split 20:1, sample volume injected 1 μl. Preliminary extraction of lipids from the samples was performed by the method of Blight and Dyer [16] with further methylation with 3 M HCl in methanol for chromatographic analysis.

Measurement of blood glucose levels. The concentration of glucose in the blood of rats was measured using a Bionime GM300 glucometer after a 12-hour fast. Blood was collected from the tail vein of rats.

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

Measurement of adiponectin concentration. Adiponectin levels in blood plasma were measured using an ELISA kit (Invitrogen, USA, catalog number KRP0041).

Determination of the calculated activity of Δ9-desaturase. The activity index of Δ9-desaturase was calculated as the ratio of the amount of oleic acid to stearic acid in the free fatty acid fraction of white adipocytes. The quantitative determination of fatty acid (FA) content in adipocytes was performed as described in detail in our previous work [15].

Histological analysis was performed on white and brown adipose tissue samples from rats. After decapitation, adipose tissue samples were isolated and fixed in 10% neutral formalin for 24 h. Then it was dehydrated in ethanol of increasing concentrations (70, 80, 90 and 96%) and embedded in paraffin. Tissue sections with a thickness of 5 μm were made on a microtome HM 325 (Microm, Germany). The samples were stained with hematoxylin and eosin according to Ehrlich and examined using an Olympus BX51 microscope (Olympus, Japan).

Morphometric analysis of brown adipose tissue was performed using ImageJ (National Institutes of Health, USA).

Statistical analysis. Statistical data processing was performed using Microsoft Office Excel software. For statistical analysis, ANOVA (analysis of variance) was used, with subsequent pairwise comparisons using Studen's t-test. The normality of the data distribution was assessed using the Shapiro-Wilk Test. We used two-way ANOVA with independent samples to assume diet and age interactions, with further pairwise comparisons between groups using Student's t-test. Correlation analysis was performed using the special CORELL function to calculate pairwise Pearson correlation coefficients r. Probability was assessed at the $P < 0.05$ level.

Results and Discussion

Fatty acid composition of rat diets. After extracting total lipids from samples of standard pelleted rodent diet and pork visceral fat (lard), we determined that the total fat content in the studied samples was 4% and 91.5%, respectively. As a result of the analysis by gas-liquid chromatography with mass detection, we determined the composition

Table 1. Fatty acid composition of standard pelleted rodent diet and lard

No	Formula	Name	Proportion of total fatty acids, %	
			Pellets	Lard
1	C14:0	Myristic	0.96 ± 0.07	1.04 ± 0.19
2	C15:0	Pentadecanoic	–	0.13 ± 0.05
3	C16:1n-7	Palmitoleic	0.71 ± 0.08	1.48 ± 0.33
4	C16:0	Palmitic	15.85 ± 0.33	23.76 ± 0.88
5	C17:1n-7	Heptadecenoic	–	0.16 ± 0.05
6	C17:0	Margaric	–	0.24 ± 0.05
7	C18:2n-6	Linoleic	40.22 ± 0.23	6.05 ± 0.67
8	C18:1n-9	Oleic	30.51 ± 0.62	42.52 ± 1.16
9	C18:1n-9 trans	Elaidic	–	2.80 ± 0.09
10	C18:0	Stearic	4.42 ± 0.07	20.28 ± 1.18
11	C20:4n-6	Arachidonic	–	0.17 ± 0.02
12	C20:2n-6	Eicosadienoic	–	0.16 ± 0.03
13	C20:5n-3	Eicosapentaenoic	0.33 ± 0.02	–
14	C20:1n-9	Gondeic	2.42 ± 0.10	0.73 ± 0.10
15	C20:0	Arachidic	0.27 ± 0.02	0.19 ± 0.04
16	C22:6n-3	Docosahexaenoic	0.38 ± 0.04	–
17	C22:2n-6	Cis-13,16-docosahexaenoic	1.78 ± 0.18	–
18	C22:0	Behenic	0.17 ± 0.02	–

Note. Values are presented as mean ± SEM. Analyses were conducted with $n = 5$ for each diet type

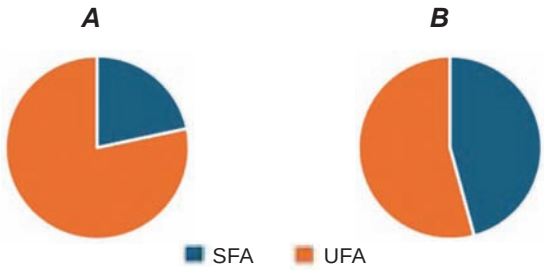

*Fig. 1. Percentage ratio of saturated and unsaturated fatty acids in rat diets: **A** – standard pelleted diet, **B** – lard; SFA – saturated fatty acids, UFA – unsaturated fatty acids*

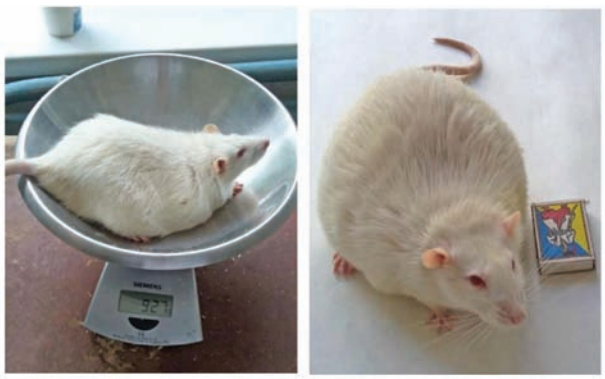

Fig. 2. Representative images of a rat after 24 weeks of a high-fat diet

of the standard pelleted diet, which was received by control rats of both age groups, as well as pork lard, which was used as the primary source of fat in the diet of HFD-rats. The results are presented in Table 1.

Fig. 1 shows the ratio of saturated and unsaturated fatty acids in pelleted diet and lard samples

The graph indicates that in pelleted feed, the ratio of saturated to unsaturated fatty acids is 1:4, whereas in lard it is close to 1:1. These findings are consistent with the literature [17, 18].

Development of the experimental model. Long-term maintenance of rats from two age groups on HFD resulted in a significant increase in body weight compared to age-matched controls [14]. A representative image of a rat after 24 weeks of HFD is presented in Fig. 2.

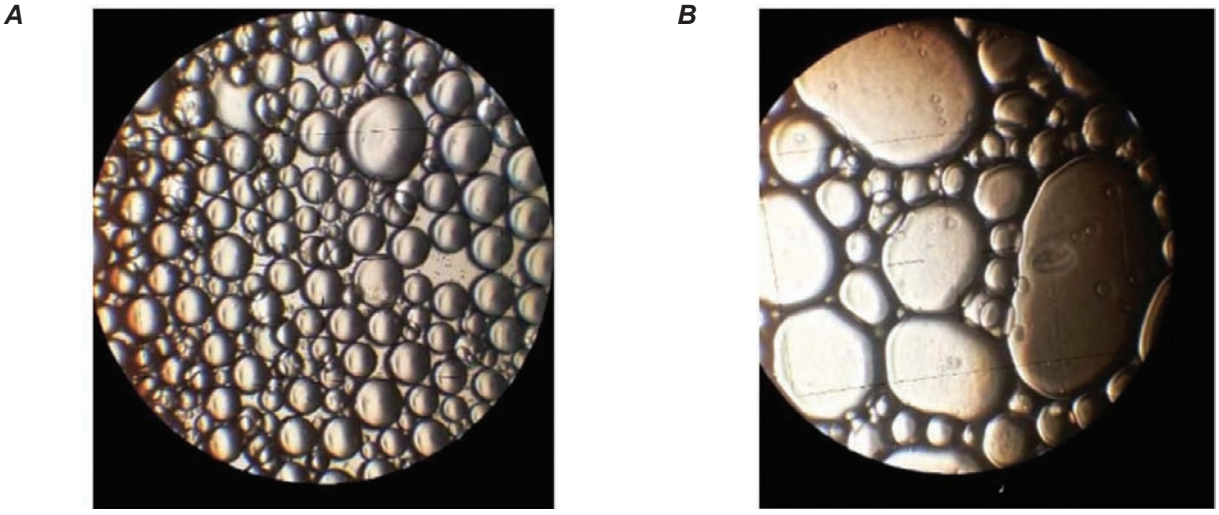

Fig. 3. Representative images of adipocytes suspension from white adipose tissue of 10-month-old rats: ***A*** *– control group,* ***B*** *– HFD group. The image was obtained during microscopic control of adipocyte isolation from tissue samples using collagenase (microscope Lumam I3, ×20 objective; camera of a Samsung SM-G530H smartphone)*

To analyze the lipid composition of adipocytes from white adipose tissue of rats in two age groups, adipocytes were isolated using type 1 collagenase [14]. Control was carried out using a light microscope. Representative images of isolated adipocytes are presented in Fig. 3. Since, from the very beginning, we did not aim to capture images of isolated fat cells, but only to evaluate the effectiveness of the protocol, the images were taken with a non-professional camera. However, in the context of our work and given the clarity of the images obtained, we consider it appropriate to present them to demonstrate the impact of a long-term HFD on white adipose tissue cells of rats. Fig. 3,*B* shows an increase in the number of hypertrophied adipocytes in the visceral white adipose tissue of HFD-rats compared to age-matched controls (Fig. 3, *A*).

It is well known that fat cell hypertrophy is associated with pathophysiological conditions, particularly metabolic disorders and systemic insulin resistance [19]. It is also well established that adipocyte size is highly variable and, for example, in hu-

Table 2. Correlation analysis of metabolic model parameters in rats from two age groups

Parameters being analyzed	Pearson's *r* correlation coefficient	
	10-month-old	24-month-old
Body weight: Glucose	0.132	0.744 ($P < 0.001$)
LDL-C: Body weight	0.500 ($P < 0.05$)	0.440 ($P < 0.1$)
LDL-C: Glucose	-0.197	0.188
HDL-C: Body weight	0.117	-0.160
HDL-C: Glucose	0.100	-0.123
LDL-C:HDL-C	0.386	0.358
Adiponectin: Body weight	0.191	-0.274
Adiponectin: Glucose	-0.010	-0.095
Adiponectin: HDL-C	0.595 ($P < 0.05$)	-0.319
Adiponectin: LDL-C	0.209	-0.541 ($P < 0.05$)

Note. Calculations and analyses of the statistical significance of correlation coefficients were performed for groups of rats aged 10 months ($n = 17$) and 24 months ($n = 16$) [14]

mans, the diameter of mature white fat cells from the same fat depot can vary by more than 10-fold [20].

Correlation analysis of the parameters of the HFD model in rats of different ages. According to modern scientific literature [6, 7], metabolic disorders induced by lifestyle and nutrition, as well as by aging, should be considered comprehensively, accounting for many interrelated factors. That is why, to fully assess the impact of a long-term HFD on rats of two age groups, we conducted a correlation analysis to identify relationships among indicators key to the metabolic model's characteristics. In particular, correlations between animal weight, blood glucose levels, plasma low- and high-density lipoprotein cholesterol levels, and plasma adiponectin levels were analyzed. The results are presented in Table 2.

Numerous studies [3, 4] and our previous studies [14, 21, 22] indicate that a long-term high-fat diet causes not only obesity but also impaired glucose tolerance and insulin sensitivity.

In this study we analyzed the relationship between body weight and blood glucose levels in rats of two age groups. As a result of the correlation analysis, we found that in 10-month-old rats, there was no statistically significant correlation between weight and glucose levels, whereas in the 24-month-old group, there were a positive correlation. Such unexpected results, at first glance, can be explained by the inclusion of compensatory anti-hyperglycemic mechanisms that are activated in younger animals and that, in turn, fade with aging.

In our work [14], it was shown that, with age, the cholesterol levels in both low- and high-density lipoproteins did not change significantly. However, it was demonstrated that a long-term overload of dietary fats on the change of the lipoprotein profile of the plasma of young rats towards an increase in the content of LDL-C compared to age-matched controls was decisive. At the same time, the diet had no significant effect on similar indicators of rats of the older age group [14].

The literature data indicate a direct correlation between body mass index and low-density lipoprotein levels. Usually, an increase in LDL-C in blood plasma with increasing body weight is considered one of the risk factors for the development of cardiovascular disease in obesity. However, recent studies highlight a more complex relationship between these indicators [23]. In particular, it is suggested that the loss of the positive correlation between body mass index and LDL-C levels in blood plasma may be associated with metabolic disorders, particularly with aging, and may be a sign of progressive metabolic disorders that accompany obesity [23].

The results of our study demonstrated that in younger rats, there is a direct correlation between LDL-C plasma levels and body weight (Table 2). At the same time, the results of the correlation analysis showed that in older rats, the direct correlation between body weight and LDL-C levels is weakened (Table 2). At the same time, our study found no correlation between LDL-C levels and blood glucose in rats of both age groups.

One of the main functions of HDL is promoting reverse cholesterol transport: the uptake of excess cholesterol by peripheral cells and its transport to the liver for further excretion. This process is considered to be the main antiatherogenic effect of HDL [24]. According to our study, no statistically significant correlations were found between plasma HDL-C levels and glucose concentration or body weight in rats across both age groups. It is also important to note that, when analyzing the lipoprotein profile of rat plasma, we observed a tendency toward a positive correlation among lipoprotein fractions. However, we did not find a statistically significant correlation between LDL-C and HDL-C in either age group.

For decades, the hypothesis of an inverse correlation between plasma HDL-C levels and coronary heart disease has been the basis for studying the relationship between the lipoprotein profile and the risks of cardiovascular complications [25, 26]. Although the leading hypothesis for a long time was an inverse correlation between plasma HDL-C levels and the risk of cardiovascular diseases, in particular coronary heart disease, more recent studies suggest that the antiatherogenic functions of HDL may be independent of its plasma levels [25-27]. Moreover, extremely high and too low plasma HDL-C concentrations are associated with increased mortality [28, 29]. It follows that the assessment of the functional activity of HDL in removing cholesterol from cells is a more relevant prognostic marker of cardiovascular diseases than the level of HDL itself. Following this idea, it has recently been demonstrated that cholesterol efflux capacity (CEC) assessment predicts the degree of atherosclerosis in humans [27].

Adiponectin is an adipokine that is specifically and highly expressed in adipose tissue and improves insulin resistance. According to various studies, plasma adiponectin levels are reduced in obesity, type 2 diabetes mellitus, and coronary heart disease [8, 9]. Previously published data from our studies demonstrate a significant effect of age on plasma

adiponectin levels in rats. In particular, adiponectin levels in 24-month-old rats were higher than in 10-month-old rats, and prolonged HFD decreased plasma adiponectin concentrations in both younger and older groups [14].

In this study, we found a positive correlation between adiponectin and HDL-C levels in the plasma of 10-month-old rats (Table 2), but no such correlation was observed in 24-month-old rats. In contrast, a significant negative correlation between adiponectin and LDL-C levels was observed in the older age group (Table 2), but not in the younger age group. However, there was no statistically significant correlation between plasma adiponectin levels and body weight or blood glucose levels in rats of both age groups.

A number of studies [30] show that adiponectin contributes to the normalization of metabolism, the maintenance of adequate fat distribution, the reduction of inflammation in insulin-sensitive tissues, and the reduction of circulating pro-inflammatory cytokines [31, 32], and increased adiponectin expression in aging mouse studies has been shown to improve glucose and lipid homeostasis [30] significantly.

There is currently a large body of clinical work investigating the relationship between adiponectin and lipid profiles in patients. Some studies [33] describes that the negative correlation between adiponectin and large floating very low density lipoproteins (VLDL) in healthy individuals is consistent with adiponectin-mediated induction of LPL and VLDL receptors, important determinants of VLDL catabolism [34-37]. The likely mechanism underlying the reduction in VLDL production by the liver under the influence of adiponectin is to reduce the supply of free fatty acids to the liver by decreasing their release from adipocytes and increasing their uptake by skeletal muscle, as well as to suppress hepatic lipase expression [35, 38, 39]. The negative associations between adiponectin and large floating VLDL and small dense LDL observed in healthy volunteers but not in patients with metabolic syndrome are consistent with previous studies examining the association between adiponectin and lipoprotein subclasses in healthy adults [40, 41], as well as in obese and lean adolescents [42] and patients with type 2 diabetes [43].

The results of our work in an animal model help better understand the possible mechanisms of mutual influence and changes in key indicators of metabolic status induced by a long-term high-fat diet across different age groups.

Estimated activity of Δ9-desaturase. Adipose tissue has the ability to absorb and release free fatty acids (FFA) simultaneously [44]. In our previous work [15], we observed changes in the FA composition of rat adipocytes at different ages. In particular, a significant increase in the FFA pool has been demonstrated both during aging and long-term dietary fat loading [15]. We hypothesized that the redistribution of FA composition towards increased monounsaturated fatty acids (MUFA) content in young HFD-rats occurred due to increased Δ9-desaturase activity during dietary fat overload [45].

Stearoyl-CoA desaturase-1 (SCD1) is an enzyme involved in the conversion of saturated palmitate and stearate into monounsaturated palmitoleate and oleate. According to the literature, it is more appropriate to use the 18:1/18:0 index to assess SCD1 activity, since the key reaction involving SCD1 remains the desaturation of stearate to oleate [46, 47].

The calculated Δ9-desaturase activity was calculated as the product/precursor ratio, i.e., the amount of oleic acid to stearic acid:

Δ9-desaturase activity = C18:1/C18:0.

The Δ9-desaturase activity was calculated in the FFA fraction of adipocytes [15]. The results are presented in Fig. 4.

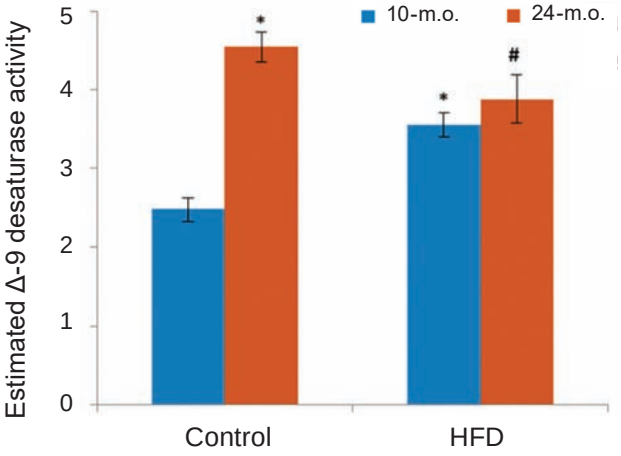

*Fig. 4. Estimated Δ9-desaturase activity in the free fatty acid fraction of adipocytes of rats of two age groups. Values are presented as mean ± SEM. *P < 0.0001 compared to the control group of 10 months of age; #P < 0.05 compared to the control group of 24 months of age [15]*

Fig. 4 shows that, with aging, Δ9-desaturase activity increased physiologically, with values in the 24-m.o. group almost twice those in the 10-m.o. group. We also showed that in younger rats, Δ9-desaturase activity increased significantly with prolonged HFD, whereas in old HFD rats it decreased significantly compared to age-matched controls.

We previously showed [15] that in healthy older animals, the level of MUFA significantly exceeded that of younger rats both in absolute quantity and in percentage of the total HFD pool. Long-term HFD caused a significant increase in the content of MUFA in adipocytes of 10-m.o. rats, but their percentage did not change significantly. That is, the balance was maintained. Contrary to these data, in the 24-month-old group of rats fed additional dietary fat, there was a slight decrease in the amount and a statistically significant decrease in the percentage of monounsaturated fatty acids among total fatty acids [15]. These data correlate with the estimated Δ9-desaturase activity, which was reduced in older HFD-rats compared with age-matched controls (Fig. 4).

The literature reports that increased SCD1 activity, which directly leads to increased MUFA formation and, consequently, triacylglycerol formation, may enhance lipid mobilization from adipose tissue (lipolysis). Changes in the fatty acid pool affect the delivery of substrates to the liver for the resynthesis of VLDL, which in turn are precursors of LDL. Studies show that SCD1 in white adipose tissue may promote the mobilization of triacylglycerols, thereby modulating systemic lipid mobility [48].

Increased monounsaturated oleic acid content is typically associated with elevated SCD1 activity, which contributes to the development of IR under HFD conditions [49]. MUFAs function as lipid signaling mediators that upregulate the expression of adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL), thereby promoting triacylglycerol (TAG) hydrolysis in white adipose tissue adipocytes. This mechanism is supported by *in vivo* studies in mice, in which experimental overexpression of SCD1 or oleate supplementation increased ATGL and HSL expression and enhanced lipolysis, whereas SCD1 knockout mice exhibited reduced responses [48]. ATGL is a key enzyme that catalyzes the initial step in the hydrolysis of triacylglycerols to diacylglycerols and fatty acids. This process is essential for mobilizing lipids from adipose tissue depots. Therefore, the Δ9-desaturase activity index in the free fatty acid fraction reflects the functional state of adipocytes and can serve as an indicator of adipose tissue metabolic activity during aging and in response to high-fat diet.

Study of changes in adipose tissue structure. Aging and long-term exposure to a high-fat diet share several pathophysiological mechanisms: both lead to tissue remodeling, accumulation of senescent cells, chronic low-grade inflammation, and disruption of metabolic homeostasis, particularly in adipose tissue. HFD often accelerates or intensifies age-related changes [50]. In our study we conducted histological analysis of selected samples of white and brown adipose tissue from rats of two age groups is presented in Fig. 5.

Histological examination of white and brown adipose tissue from rats of two age groups showed that, in the control groups, both 10- and 24-month-old rats, the cells of both fat types generally retain characteristic morphological features. In WAT of control rats from both age groups, adipocytes have a large transparent cytoplasm with peripherally located basophilic nuclei (Fig. 5, *A*, 1, 6,), and vessels demonstrate normal histoarchitectonics (Fig. 5, *A*, 2, 7). In the BAT of control rats aged 10 months, the typical polygonal cell shape, granular eosinophilic cytoplasm, and centrally located nuclei are preserved (Fig. 5, *B*, 1); blood vessels are of the normal structure (Fig. 5, *B*, 2). However, in 24-month-old rats, an age-related transformation of BAT is observed: there is a cytoplasm condensation (Fig. 5, *B*, 5) the cytoplasm becomes clearer, and the nuclei shift to the periphery, bringing the tissue morphology closer to that of white adipose tissue, reducing the difference between fat cell types (Fig. 5, *B*, 7). The blood vessels that pass through BAT have a normal structure; their endothelium is not changed, and the extracellular matrix is of moderate optical density with single fibroblasts (Fig. 5, *B*, 6).

In HFD-rats at 10 months of age, the WAT structure is largely preserved (Fig. 5, *A*, 3, 5). However, immune cells (leukocytes, lymphocytes) are observed in the vascular lumen (Fig. 5, *A*, 4), indicating an early vascular and tissue response to dietary fat loading. In BAT of HFD-rats of this age group, changes are also minimal. However, erythrocytes and protein accumulations are observed in the vascular lumen, suggesting an increased load on the microcirculatory bed.

In 24-month-old rats on a HFD, WAT retains the general adipocyte structure (Fig. 5, *A*, 9), but edema is occasionally observed in the perivascular

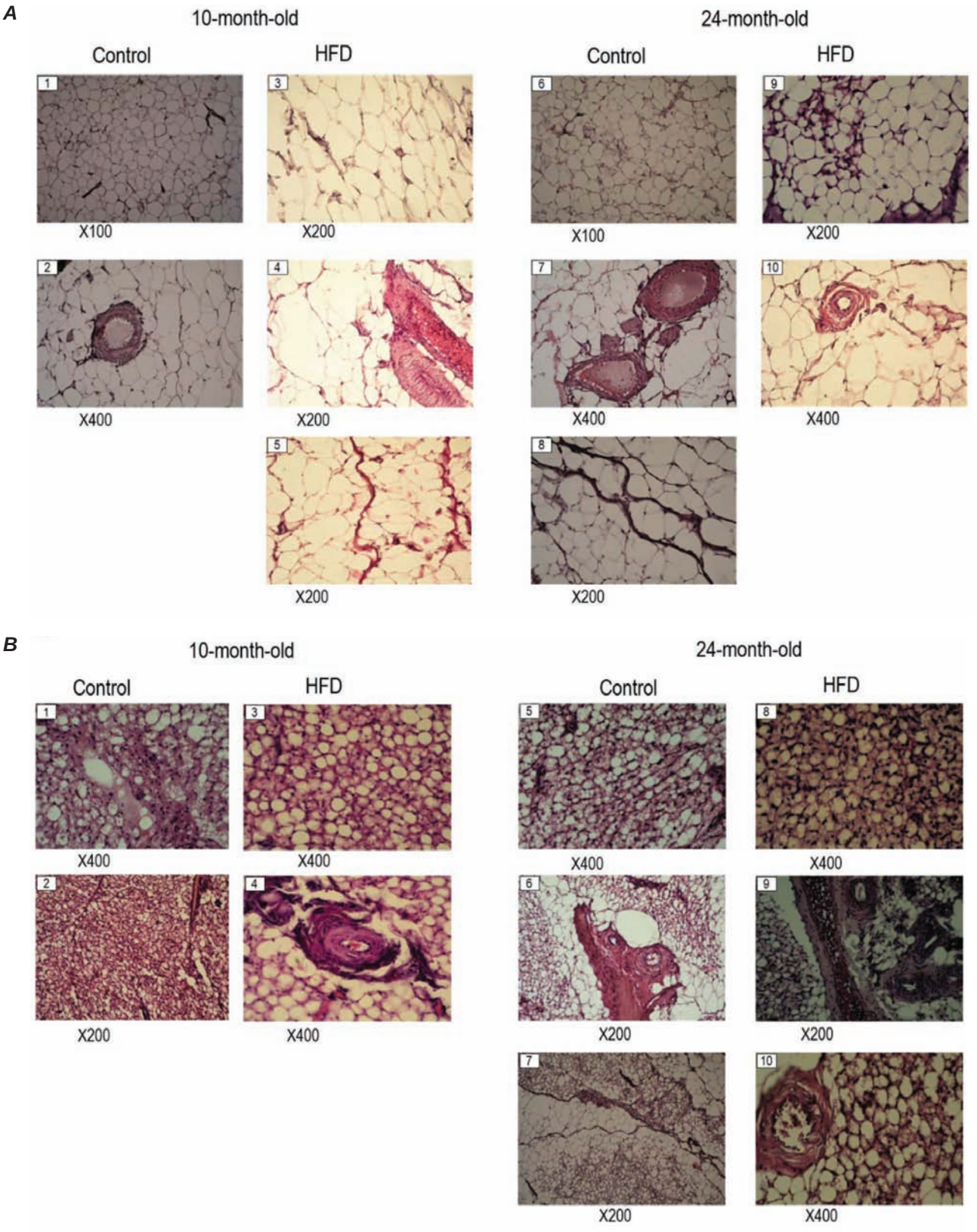

*Fig. 5. Representative images of white adipose tissue (**A**) and brown adipose tissue (**B**) of 10-month-old and 24-month-old rats from the "Control" and "HFD" groups*

space, which may reflect the development of a local inflammatory process (Fig. 5, *A*, 10). The most pronounced changes are observed in the BAT of this group: significant accumulation of lipid droplets in cells (Fig. 5, *B*, 8), the presence of lymphocytes and leukocytes in the arterial lumen (Fig. 5, *B*, 9), and signs of edema in some smooth muscle cells and components of the vascular wall connective tissue (Fig. 5, *B*, 10). This indicates an age-related increase in the sensitivity of brown adipose tissue to metabolic stress and an increase in inflammatory phenomena under the influence of HFD.

Among the identified morphological changes in WAT of younger rats, hypertrophy and signs of adipocyte hyperplasia were more pronounced than in older rats. It confirms the activation of lipogenesis in young rats following prolonged exposure to excess saturated fat in the diet. In older rats, this mechanism is probably not fully implemented. At the same time, histological analysis of brown adipose tissue in rats indicates a probable decrease in its functional activity during aging. Obtained experimental data allow to assume that such a negative manifestation is intensifying under prolonged HFD, which directly leads to a decrease in energy expenditure and further progression of obesity.

Currently, several studies have shown that BAT in rodents undergoes degeneration during aging. Such involution of brown fat is characterized by increased lipid deposition, larger lipid droplets, reduced mitochondrial content, decreased expression of thermogenic genes, and, consequently, decreased thermogenic activity [51-53]. Some studies have shown that a similar degenerative process can be modeled in rodents by maintaining them on a high-fat diet [54, 55]. Thus, the relationship between decreased BAT thermogenic activity and impaired metabolic health suggests that preventing BAT involution or reactivating it has significant potential in the prevention and treatment of metabolic disorders [56]. However, the mechanisms responsible for maladaptive BAT processes, especially during aging, remain poorly understood [56].

According to current scientific knowledge, brown adipose tissue undergoes negative changes at the structural and functional levels under the influence of obesity, insulin resistance and aging. Common pathological features include lipid "bleaching" of brown adipocytes [57, 58], mitochondrial [58, 59], decreased expression of thermogenic genes (in particular uncouple protein-1, UCP1), impaired sympathetic and vascular support, as well as

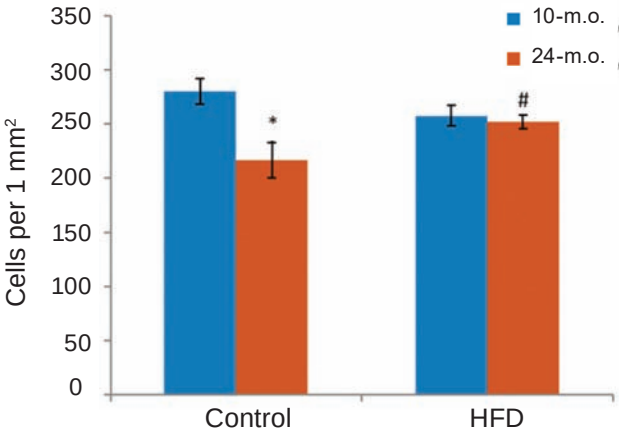

*Fig. 6. Brown adipose tissue cellularity in rats of two age groups. Values are presented as mean ± SEM. *P < 0.01, compared with the "Control" group aged 10 months; #P < 0.05 compared with the "Control" group aged 24 months*

local inflammation and cellular senescence. These changes reduce the absorption of glucose and fatty acids by BAT, reduce systemic energy expenditure and contribute to metabolic dysfunction of the whole organism [60, 61].

In line with the aim of our study, we also performed morphometric analysis of brown adipose tissue samples from healthy and HFD rats across two age groups. The results of the morphometric analysis are shown in Fig. 6.

The results of our study showed that the cellularity of brown adipose tissue significantly decreases during aging ($P = 0.005$). In 10-month-old rats maintained on a HFD, there was a tendency toward decreased cellularity in brown adipose tissue compared with controls ($P = 0.083$). In 24-month-old rats, prolonged dietary fat loading, in contrast, led to a statistically significant increase in cellularity in brown fat compared with the corresponding age-matched control ($P = 0.041$).

Thus, our study demonstrates a statistically significant decrease in brown fat cellularity in aging rats. We believe that this may indicate an age-related involution of brown adipose tissue. From another perspective, we suggest that the results obtained may also indicate a different mechanism of action of prolonged HFD on brown adipose tissue in rats of different ages. Thus, it is likely that in young rats, prolonged dietary fat loading results in hypertrophy of brown adipocytes due to their involvement in the absorption of excess triacylglycerols, whereas in old

rats, the response is realized through hyperplasia. A similar response from brown fat was described in another study more than 40 years ago [62]. In any case, both the hypertrophy and hyperplasia pathways should be considered in terms of ensuring the functional activity of brown fat, in particular, thermogenesis, which is ensured by a sufficient level of tissue lipoprotein lipase activity in conditions of excessive intake of triacylglycerols and the need for their effective oxidation with the release of energy in the form of heat.

Conclusions. We investigated age-dependent metabolic and morphological responses of adipose tissue to a long-term high-fat diet excess of saturated fatty acids, with particular emphasis on correlations among key metabolic and biochemical parameters and on remodeling of white and brown adipose tissue in rats.

We demonstrated that long-term high-fat diet induces obesity and metabolic homeostasis disruption in rats in an age-dependent manner, characterized by altered glycemic regulation, age-specific shifts in adiponectin–lipoprotein relationships, changes in Δ9-desaturase activity reflecting impaired adipocyte function. The results of our study also show age-related differences in the morphology, cellular adaptation, and development of inflammation in brown and white adipose tissue in response to excess dietary fat.

A comprehensive approach to studying the influence of age and dietary habits on the biochemical and morphological parameters of adipose tissue enables us to understand the mechanisms of metabolic disorders better and identify potential biomarkers for the prevention and treatment of obesity and associated diseases, particularly type 2 diabetes and cardiovascular disorders.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ВІКОВО-ЗАЛЕЖНА МЕТАБОЛІЧНА ТА МОРФОЛОГІЧНА ВІДПОВІДЬ ЖИРОВОЇ ТКАНИНИ ЩУРІВ НА ДІЄТУ З ВИСОКИМ ВМІСТОМ ЖИРІВ

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Ожиріння та пов'язані з ним метаболічні порушення залишаються однією з найактуальніших проблем сучасної біомедицини. Як ожиріння, так і старіння спричиняють функціональні та структурні зміни жировій тканині, проте вікова специфічність реакції жирової тканини на дієту з високим вмістом жирів (ВЖД) залишається недостатньо вивченою. Метою дослідження було проаналізувати кореляцію між рівнями глюкози, ліпопротеїнів та адипонектину в плазмі крові, визначити активність Δ9-десатурази в адипоцитах та дослідити морфологічний стан жирової тканини у щурів різного віку. Експеримент проводили на самцях щурів лінії Вістар протягом 24 тижнів коли молоді тварини досягли віку 10 місяців, а старші – 24 місяці. Тварини обох вікових груп були розділені на тих, хто утримувався на стандартному раціоні та на раціоні з додаванням свинячого вісцерального жиру. Жирнокислотний склад визначали методом газорідинної хроматографії з мас-детекцією; вміст холестеролу ЛПВЩ і ЛПНЩ оцінювали за допомогою комерційних наборів; рівень адипонектину визначали із застосуванням набору ELISA. Індекс активності Δ9-десатурази розраховували як співвідношення олеїнової до стеаринової кислоти. Показано, що співвідношення насичених/ненасичених жирних кислот у стандартному гранульованому кормі становило 1:4, тоді як у салі

воно наближалося до 1:1. У молодших щурів не було виявлено кореляції між збільшенням маси тіла та рівнем глюкози, водночас спостерігалася позитивна кореляція між рівнями адипонектину та холестеролу ЛПВЩ. У старших щурів спостерігалася позитивна кореляція між масою тіла та рівнем глюкози, а також негативна кореляція між рівнями адипонектину та холестеролу ЛПНЩ. Було виявлено, що активність Δ9-десатурази в адипоцитах зростає з віком. За умов ВЖД у молодшій групі активність Δ9-десатурази достовірно підвищувалася, тоді як у старшій – знижувалася порівняно з віковим контролем. Морфологічний аналіз показав вікові відмінності в морфології, клітинній адаптації та розвитку запалення в бурій та білій жировій тканині у відповідь на тривале перевантаження дієтарними жирами. Отримані результати підкреслюють суттєву роль вікового фактора у формуванні метаболічних і морфологічних змін жирової тканини та сприяють ідентифікації потенційних біомаркерів для профілактики й лікування ожиріння та пов'язаних із ним захворювань.

Ключові слова: ожиріння, старіння, жирова тканина, високожирова дієта, адипонектин, ліпопротеїни, активність Δ9-десатурази, морфологічний аналіз.

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