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Research paper

Pathophysiological justification of age- and gender-dependent morphological changes in the adipose tissue in rat models of metabolic syndrome

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Abstract

Introduction: The mechanisms of metabolic syndrome (MS) is one of the urgent issues in medicine. Regional distribution of the adipose tissue should be diagnosed at clinical examination, as the morphometric parameters of the cells of the active adipose tissue components may indicate the metabolic state.

Aim: The aim of the study was to evaluate the differences in morphological and histological parameters of the adipose tissue associated with the development of MS in animals of different ages and gender.

Material and methods: An experimental study was carried out on 144 WAG/G Sto white rats, divided into three study groups. Group 1 included young immature rats, 3 months old; group 2 consisted of 48 sexually mature rats, aged 5–6 months; group 3 consisted of 48 old rats, 18 months old. Each group was divided into 2 subgroups, control and experimental, and was additionally divided according to gender.

Results and discussion: The body mass indices and specific weights of mesenteric, epididymal, retroperitoneal and subcutaneous adipose tissue were determined in rats, as well as morphological characteristics of adipocytes of the adipose tissue. It was shown that histological and morphological changes in the adipose tissue of the animals were age- and gender-dependent, and that obesity is associated with chronic inflammation of the adipose tissue.

Conclusions: The results of the study can be used for further determination of possible age and gender differences in the adipose tissue involvement in the development of chronic inflammation, as well as monitoring and correction of adipose tissue dysfunction in MS.

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1. INTRODUCTION

Metabolic syndrome (MS) is a combination of pathological components associated with carbohydrate and lipid metabolism disorders,[1] which ultimately result in the development of type 2 diabetes mellitus, a number of cardiovascular diseases (CVDs),[2] liver and kidney diseases, etc. MS belongs to the so-called multimorbidity states, which are characterized by a combination of pathogenetic mechanisms, where impaired primary regulation can potentiate the development of dysregulation of interacting integrative systems, cause complications, and ultimately result in the development of 'mutual burden syndrome.'[3] The issue of studying the mechanisms of MS is one of the urgent issues of medicine. The most common factor in the initiation of MS is considered an exogenous-constitutional obesity, where adipocyte hypertrophy stimulates the synthesis of the adipose tissue (AT) active components, which leads to numerous metabolic and hemodynamic disorders, which sooner or later result in the development of obesity-associated diseases. Depending on the topographic location, the AT has morphological, physiological and pathophysiological features. In rodents, AT is classified according to its regional location and subcutaneous and visceral types are distinguished. The subcutaneous (white) AT (SAT) is located in the subcutaneous fat tissue and is represented by small adipocytes. It has low glucose and lipid metabolism.[4] The visceral AT (VAT) is associated with internal organs and is distributed throughout the body.[5] The VAT is represented by several subtypes: mesenteric AT (MAT) located along the intestine; retroperitoneal AT (RPAT) – located behind the kidneys, and epididymal AT (EDAT) – located in the small pelvis around the testes or ovaries.[6] The mesenteric VAT has the highest metabolic activity among the above VAT subtypes. It consists predominantly of small adipocytes and is characterized by high lipid mobility, vascular density and intense blood flow. The EDAT is characterized by less intensive glucose and lipid metabolism, it is dominated by large adipocytes. The RPAT is represented by medium and large adipocytes.[7]

These morphological and functional differences in the AT types cause their unequal increase and distribution between different VAT deposits during positive energy load, and the distribution indices are predictors of diseases associated with obesity. For example, many studies show a positive correlation between upper body obesity and cardiac and metabolic disease,[8] with more visceral than subcutaneous AT activity.[9] On the other hand, fat accumulation in the lower body, especially in the subcutaneous gluteofemoral cavity, is known to protect against diseases associated with obesity.[10] Therefore, today the diagnosis of the regional distribution of the AT is one of the important parameters to be determined during clinical examination. However, quantitative determination of the AT only characterizes its total content in the body and does not reflect gender or age characteristics. This encourages the search for new, more specific methods for AT evaluation taking into account age and gender. Determination of morphological age- and gender-dependent characteristics, from a practical point of view, can be important and necessary for understanding the initial mechanisms of obesity and can become a diagnostic predictor of the main risk factors for obesity – type 2 diabetes mellitus and associated clinical and metabolic complications.[11;12]

Many authors admit that the morphometric parameters of the AT cells may indicate metabolic state. Thus, the methods for radiocarbon dating of the lipids and mathematical modeling have shown that impaired ability of the VAT to accumulate lipids results in lipotoxicity of the liver,[13,14] kidneys, heart and pancreas, which leads to cell apoptosis and further metabolic changes in the body – dyslipidemia, metabolic cardiomyopathy, type 2 diabetes mellitus, liver steatohepatitis, etc.[15,16] A set of data gives a clear idea of the AT histological features in obesity and MS. Other studies emphasize that not all obese people have the same risk of MS. For example, people with the same level of obesity may have different insulin sensitivity.[17] Therefore, the study of pathophysiological mechanisms based on the AT morphometric and biochemical parameters, taking into account age and gender aspects, remains relevant.

2. AIM

The aim of the study was to evaluate the differences in morphological and histological parameters of the AT associated with the development of MS in animals of different age and gender.

3. MATERIAL AND METHODS

Included patients were AL cases with available demographic To achieve this purpose, an experimental study was carried out on 144 WAG/G Sto white rats. Each age group consisted of a control and study subgroups of animals, where the animals were divided by gender (Table 1).

Group 1 included young immature rats (36 animals), 3 months old, with an initial mean body weight of 170.0 ± 7.8 g, which were divided into 2 subgroups, control (1K) and experimental (1A). Subgroup 1K was a control group and

Table 1. Distribution of animals into study groups in accordance with the objectives of the experiment.

Experiment series	Number of animals					
	Group 1 Young (sexually immature)		Group 2 Adult (sexually mature)		Group 3 Old (post-reproductive)	
	female	male	female	male	female	male
Control group (K)	6	6	6	6	6	6
Experimental model of MS (A)	18	18	18	18	18	18

consisted of 12 intact healthy rats (6 of each sex). These animals remained on the standard diet for the entire duration of the experiment. Subgroup 1A of young rats was divided into females and males, 18 animals in each. Group 2 consisted of 48 sexually mature rats, aged 5–6 months, with initial mean body weight of 240.0 ± 14.7 g. The rats were divided into 2 subgroups, control (2K) and experimental (2A). Each subgroup was additionally divided according to gender (Table 1). Group 3 consisted of 48 old rats, 18 months old, with an initial mean body weight of 360 ± 21.6 g. Subgroup 3K consisted of 6 old females and 6 old males, and the subgroup 3A – 18 females and 18 males (Table 1).

The experiment was carried out on the animals which had the closest resemblance to the histomorphological parameters of humans, which makes it possible to extrapolate the results of the study to humans with sufficient confidence.

MS was induced in rats by a combination of high-fat and high-carbohydrate diets with pharmacological correction of the inhibition of physiological satiety in animals. This MS model was developed by the authors; a patent was obtained.[20] The animals were removed from the experiment by instant CO_2 asphyxia. The weight of the AT in rats (MAT, EDAT, RPAT, and SAT) was determined by weighing on an analytical balance and its specific gravity was calculated (AT weight per 100 g of rat body weight). Two fragments were excised from each AT sample; the material was fixed in formalin solution (10%). The densification of the tissues fixed in formalin was achieved by taking through alcohols of increasing concentration, Nikiforov's liquid (96% alcohol and diethyl ether at a 1 : 1 ratio), chloroform and embedding in paraffin. Serial sections with a thickness of 4–5 × 10^6 m were prepared from the blocks which were stained with hematoxylin and eosin, van Gieson's picrofuchsin, according to Mallory, and Sudan III. The slides were studied with the Olympus BH-2 microscope (Japan) using the Baumer/optronic Type: CX05c camera and the Olympus DP-Soft (Version 3:1) software, which was used to conduct a morphometric study. The mean size of 500 fat cells was determined for each group of animals, and cell distribution by size (% of cells of small <50 μm, medium 50–100 μm and large 100> μm size) was evaluated.

For statistical analysis, all data was entered into Excel spreadsheets. The obtained digital data was processed using mathematical statistics with variance and alternative analyses. The results were analyzed using the licensed statistical programs Microsoft Excel and Statistika 6.0. At the same time, the mean (M) values of each parameter using the Student's *t*-test, the standard deviation (SD), the mean error of the arithmetic mean, as well as an estimate of the distribution of values were calculated. The mean and standard error (M ± SE) were used to describe continuous variables of changes which were normally distributed.

4. RESULTS AND DISCUSSION

4.1. Changes in the AT morphological and histological parameters in rat models of MS

During the experiment, it was shown that in subgroup 1A, 25% of young males and 27% of young females did not have significant anthropometric signs of obesity by the end of the experiment – their weights were characterized as overweight. The rest of the animals in subgroup 1A showed a significant increase in the mean body weight (mean increase in body mass index of 40% ($P < 0.01$) and the increase in body weight in males was more pronounced than in females. An increase in the AT total specific weight in females of group 1A was due to an increase in the SAT specific weight, which exceeded the control values by 1.5 times (where $P < 0.05$ compared to control), which was 1.3 times higher than the SAT specific weight in males. The values of the MAT and RPAT specific weight by the end of the experiment remained close to the control values. A 1.3-fold increase in the EDAT (P <0.01) was observed (Ta-

Table 2. Parameters of body weight and specific weight of the mesenteric, epididymal, retroperitoneal and subcutaneous adipose tissue in rats with experimental MS

Parameters	Subgroups											
	1K, n = 12		1A, n = 36		2K, n = 12		2A, n = 36		3K, n = 12		3A, n = 36	
	female n = 6	male n = 6	female n = 18	male n = 18	female n = 6	male n = 6	female n = 18	male n = 18	female n = 6	male n = 6	female n = 18	male n = 18
Mean body weight of rats, g	164.83 ± 4.87	190.5 ± 4.42	298.42 ± 3.44 [c,d,i]	439.97 ± 4.5 [f,i]	220.5 ± 7.92	265.83 ± 6.76	467.0 ± 8.07 [c,i]	513.78 ± 6.73 [c]	230.5 ± 6.16	283.0 ± 5.58	529.2 ± 11.66 [c]	545.31 ± 16.42 [c]
Total specific weight of the AT, g	2.20 ± 0.3	2.43 ± 0.41	3.16 ± 0.5	3.71 ± 0.78 [g]	2.85 ± 0.4	2.9 ± 0.6	4.06 ± 0.7	4.42 ± 0.5[a]	5.2 ± 1.1	5.9 ± 1.4	10.51 ± 2.5 [a]	11.1 ± 2.2 [a]
MAT specific weight, g	0.59 ± 0.03	0.42 ± 0.04	0.73 ± 0.05 [a,i]	1.02 ± 0.01 [h]	0.49 ± 0.03	0.50 ± 0.01	0.71 ± 0.12 [i]	0.79 ± 0.14 [a,i]	0.92 ± 0.14	1.06 ± 0.17	2.02 ± 0.28 [b]	2.08 ± 0.28 [b]
EDAT specific weight, g	0.39 ± 0.04	0.56 ± 0.07	0.64 ± 0.04 [c,i]	0.76 ± 0.1 [i]	0.58 ± 0.04	0.58 ± 0.03	0.80 ± 0.15 [i]	0.78 ± 0.19 [i]	1.04 ± 0.15	1.28 ± 0.14	2.33 ± 0.25 [c]	2.49 ± 0.25 [c]
RPAT specific weight, g	0.49 ± 0.03	0.61 ± 0.02	0.65 ± 0.03 [b,i]	0.77 ± 0.08 [i]	0.82 ± 0.03	0.85 ± 0.04	1.02 ± 0.24	1.12 ± 0.25	1.38 ± 0.20	1.59 ± 0.21	2.79 ± 0.31 [c]	3.02 ± 0.31 [c]
SAT specific weight, g	0.76 ± 0.05	0.84 ± 0.01	1.14 ± 0.07 [c,i]	1.16 ± 0.05 [c,i]	0.96 ± 0.05	0.97 ± 0.05	1.43 ± 0.28 [i]	1.53 ± 0.21[a,i]	1.86 ± 0.29	1.97 ± 0.31	3.37 ± 0.42 [b]	3.51 ± 0.42 [b]

Comments: [a] significance $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$ (compared to the corresponding control subgroup); [d] significance $P < 0.05$; [e] $P < 0.01$; [f] $P < 0.001$ (compared to subgroups 2A); [g] significance $P < 0.05$; [h] $P < 0.01$; [i] $P < 0.001$ (compared to subgroups 3A).

Table 3. Morphological characteristics of adipocytes in the adipose tissue in rats with experimental MS.

Parameters	Subgroups											
	1K $n = 12$		1A $n = 36$		2K $n = 12$		2A $n = 36$		3K $n = 12$		3A $n = 36$	
	female $n = 6$	male $n = 6$	female $n = 18$	male $n = 18$	female $n = 6$	male $n = 6$	female $n = 18$	male $n = 18$	female $n = 6$	male $n = 6$	female $n = 18$	male $n = 18$
Adipocyte section area, μm^2	6516.7 ± 88.7	6574 ± 92.2	6768.3 ± 131.6 [i]	6794.5 ± 99.11 [e,i]	6755.2 ± 99.3	6894.2 ± 103.8	7012.1 ± 98.5 [h]	7185.5 ± 101.3 [a,h]	6963.6 ± 121.8	7126.2 ± 117.3	7435.4 ± 110.5 [c]	7566.7 ± 118.8 [b]
Mean number of adipocytes in the SAT	82.1 ± 0.33	89.4 ± 0.38	95.8 ± 0.41 [c,f,i]	98.2 ± 0.4 [c,f,i]	84.7 ± 0.29	86.4 ± 0.42	44.6 ± 0.51 [c,i]	46.2 ± 0.43 [c,i]	73.2 ± 0.51	76.5 ± 0.95	40.6 ± 0.24 [c]	41.9 ± 0.28 [c]
Mean size of MAT adipocytes, μm	41.1 ± 0.23	47.2 ± 0.31	46.4 ± 0.33 [c,f,i]	56.8 ± 0.18 [c,e,i]	43.6 ± 0.21	44.4 ± 0.27	55.2 ± 0.27 [c,i]	58.2 ± 0.36 [c,i]	46.3 ± 0.31	48.8 ± 0.34	69.4 ± 0.41 [c]	72.5 ± 0.51 [c]
Mean size of EDAT adipocytes, μm	122.8 ± 0.52	123.8 ± 0.46	132.8 ± 0.43 [c,f,i]	134 ± 0.27 [c,f,i]	129.6 ± 0.54	131.4 ± 0.45	153.3 ± 0.33 [c,i]	159.1 ± 0.42 [c,i]	135.5 ± 0.43	139.8 ± 0.42	166.2 ± 0.33 [c]	168.4 ± 0.72 [c]
Mean size of RPAT adipocytes, μm	91.2 ± 0.44	93.6 ± 0.27	99.5 ± 0.54 [c,f,i]	100.1 ± 0.63 [c,f,i]	94.6 ± 0.32	96.8 ± 0.41	110.4 ± 0.54 [c,i]	118.2 ± 0.49 [c,i]	99.1 ± 0.53	102.8 ± 0.47	122.3 ± 0.75 [c]	125.4 ± 0.83 [c]
Mean size of SAT adipocytes, μm	47.8 ± 0.77	49.5 ± 0.67	53.6 ± 0.54 [c,f,i]	57.2 ± 0.74 [c,f,i]	53.1 ± 0.33	55.8 ± 0.48	50.2 ± 0.42 [c,i]	53.1 ± 0.51 [c,i]	58.4 ± 0.45	59.7 ± 0.53	66.2 ± 0.72 [c]	68.1 ± 0.65 [c]

Comments: [a] significance $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$ (compared to the corresponding control subgroup); [d] significance $P < 0.05$; [e] $P < 0.01$; [f] $P < 0.001$ (compared to subgroups 2A); [g] significance $P < 0.05$; [h] $P < 0.01$; [i] $P < 0.001$ (compared to subgroups 3A).

ble 2). In males of subgroup 1A, an increase in the total specific weight of the AT compared to the subgroup 1K occurred due to an increase in the SAT and MAT. An insignificant increase in the volume of these AT types by 1.6 times and 1.4 times, respectively, which was associated with a decrease in the volume of the RPAT (0.5 times) and the EDAT (0.9 times) (Table 2).

In group 1A, an increase in the mean number of adipocytes in the SAT was observed compared to the subgroup 1K by the end of the experiment: 1.1-fold in males and 1.2-fold in females. The same pattern was observed in other types of the AT, with the highest values in the MA in males (Table 3). The number of the RPAT and EDAT cells increased by 1.08 times with a significance from $P < 0.05$ to $P < 0.01$, without significant differences between the subgroups by gender. At the same time, with an increase in the number of adipocytes, an increase in the mean adipocyte size was recorded: 1.12-fold in the SAT in females and 1.15-fold in males compared to the subgroup 1K.

In the experimental subgroups of group 2A (sexually mature rats), an increase in animal body weight had less pronounced gender differences compared to group 1A and occurred due to an increase in the specific weight of all studied AT types, but with different intensity. In females of subgroup 2A, compared to the subgroup 2K, there was a 1.45-fold increase in the AT weight, where the MAT specific weight exceeded the initial values by 1.8 times, $P < 0.01$, and SAT – by 1.5 times, $P < 0.05$. In males of subgroup 2A, the weight of the animals increased 1.58-fold compared to the subgroup 2K, due to the MAT specific weight, its values were 2-fold higher than in the subgroup 2K and 1.6-fold higher than the SAT. An increase in body weight in males in the subgroup 2A was more intense than in females. At the same time, in the animals from group 2A on a diet, together with an increase in the AT specific weight on a diet, there was a tendency towards a decrease in the number of cells, especially in the SAT: 1.9-fold in males of subgroup 2A and 1.7-fold in females of subgroup 2A (where $P < 0.001$). In group 3A, which consisted of old animals, an increase in fat deposits occurred due to an increase in the MAT specific weight, compared to the controls: 1.2-fold in males and 1.1-fold in females ($P < 0.05$). The mean EDAT specific weight increase was 1.1-fold ($P < 0.05$), and the RPAT specific weight practically did not differ from the subgroup 2K values, without significant gender differences. In males and females of subgroup 3A, there was a tendency towards a decrease in the number of adipocytes in the SAT (1.8-fold at $P < 0.001$). Thus, by the end of the experiment, the mean number of adipocytes in the SAT in the subgroup 3A was significantly lower than the number of adipocytes in the SAT in the subgroup 3K (Table 3).

The SAT microscopy in all experimental groups showed that this type of the AT consists of fat cells (adipocytes, lipocytes), the size, shape and density of which had age and gender differences. Thus, in both subgroups of group 1 (1A, 1K), the cells were round and oval, and significantly smaller in size compared to groups 2A and 3A. In the animals in groups 2A and 3A, there was a significant increase in the size of fat cells associated with a decrease in the mean number of adipocytes in all types of the AT during the experiment (Table 3). An increase in the cell volume was heterogeneous: fat cells had shapes from a round-oval of small size to an irregular polygonal shape of a giant size. Therefore, for a more accurate characterization of the qualitative composition of the AT, all adipocytes within one fat depot were divided by us into small (up to 50 μm in diameter), medium (50–100 μm in diameter) and large (more than 100 μm in diameter). It was found that in all control groups, irrespective of the age and gender of the animals, small cells (up to 50 μm) prevailed in the MAT, large cells (more than 100 μm) – in the EDAT, medium and large cells – in the RPAT (50–100 μm) and small and medium cells – in the SAT. At the same time, the animals of groups 2A and 3A, with the development of MS, had a tendency towards a decrease in the number of medium-sized adipocytes in the SAT in both females and males. The adipocyte mean size in all 3 experimental groups of animals

increased under the influence of the main important factor – a positive energy load, and was higher than the control values by 60% in the MAT ($P < 0.005$), by 22% in the EDAT ($P < 0.01$), by 16% in the RPAT ($P < 0.01$) and by 12% in the SAT ($P < 0.05$) compared to the group of rats receiving a standard diet. Moreover, these parameters were 3%–5% higher in males, than in females (Table 3). Positive age-dependent changes in an increase in the adipocyte section area by 1.05 times were observed, $P < 0.001$ and $P < 0.01$.

4.2. The significance of the AT morphological changes in the formation of an inflammatory response in rat models of MS, taking into account age and gender aspects

Modern diagnostics of obesity and MS includes an assessment of anthropometric parameters such as body mass index (BMI), specific body weight gain and total AT weight. However, the heterogeneous contribution of the AT components to the formation of excess body weight determines the lack of objectivity of these parameters to identify MS mechanisms, its development and diagnosis of concomitant pathologies. Based on the fact that the alimentary factors result in the impaired intrasystemic regulation in the AT and trigger the mechanisms of the development of chronic low-grade inflammation (LGI), more informative and reliable qualitative methods are required for early diagnosis of LGI. Therefore, the identification of pathomorphological changes in the AT become the central pathogenetic platform for the identification of further functional disorders that can explain the mechanisms of the development of a sustained cycle: 'cell ↔ inflammatory reaction,' which is a predictor in the formation of MS.

The analysis and comparison of the obtained AT characteristics in 3 age groups showed that the increase in the AT specific weight in young rats was more restrained compared to adult and old animals. In group 1A, the increase in the AT specific weight mainly occurs due to an increase in the number of fat cells through their division: hyperplastic processes are most pronounced in the SAT in females and the MAT in males. Hyperplasia of the AT in young animals can be considered as a protective reaction of the body to a pathogenic exogenous factor – overeating, i.e. as a 'recovery mechanism' of metabolic changes in the AT in response to alimentary load.[21,22] On the one hand, SAT hyperplasia inhibits the development of obesity in young animals, as evidenced by the percentage of animals with normal body weight observed at the end of the experiment in group 1A on a high-fat and high-carbohydrate diet. On the other hand, SAT hyperplasia in young animals, especially females, may cause less pathogenicity for initiating processes aimed at reducing insulin sensitivity. It is known that the SAT activity does not play a leading role in the development of insulin resistance and no markers contribute to homeostasis model assessment of insulin resistance (HOMA-IR), while the mean adipocyte size in the VAT significantly correlates with this parameter.[23]

In the animals from group 2A (sexually mature males and females) on a high-carbohydrate and high-fat diet, an increase in almost all types of the AT prevails, with a predominant increase in the MAT specific weight in males and the SAT specific weight in females. It was noted that the severity of the increase in the specific body weight in males is consistent with the degree of an increase in the number of adipocytes, as well as with an increase in their mean size. In males of subgroups 2A, in contrast to males of subgroups 1A, both hyperplastic and hypertrophic modifications of the AT were observed. In males of group 2A, an increase in the size of adipocytes both in the SAT and MAT was observed as the body weight of the animal increased, possibly due to the deposition of fat in the cells themselves. At the same time, changes in the shape of fat cells (irregular shape, appearance of individual giant cells) were observed in the MAT. However, the RPAT and EDAT still contained a sufficient number of small- and medium-sized cells, which may indicate the presence of transitional mechanisms in the AT that contribute to the transition from the hyperplastic type of AT growth to the hypertrophic one. An increase in adipocyte size is known to be an important prerequisite for impaired glucose uptake by the insulin-dependent tissues and has an inversely proportional relationship in the development of obesity.[24] These values are independent of gender and body weight, but this parameter correlates with plasma insulin levels and insulin sensitivity.[25]

Considering the fact that subgroup 2A consisted of sexually mature animals, the features of an increase in their fat depots could largely be mediated by an active hormonal background, this could determine a pronounced gender-specific dependence of fat distribution in fat depots in the course of the experiment, compared to subgroups 1A and 3A. There is enough evidence in current literature that, in addition to genetic and exogenous factors (living conditions, diet, exercise), sex hormones are an important determinant of the regulation of fat deposition. It is known that estrogens, the receptors for which prevail in the SAT, affect the processes of adipocyte hyperplasia, stimulating the formation of young adipocytes from pre-adipocytes.[26] This means that SAT hyperplasia also plays a protective role in the development of obesity in the adult body.

Obesity developed most rapidly in animals of group 3A (old rats). It was established that hypertrophic processes prevail in the AT in the group of old animals together with significantly reduced hyperplastic ones. The biggest deviations from the control values, among the parameters studied, were observed during determination of the VAT specific weight in old females. An increase in the VAT specific weight in females was associated with a predominance of large adipocytes. It is known that in obesity, an additional increase in the size of adipocytes and an increase in their number can occur under the influence of endocrine factors synthesized by the obesity-activated adipocytes.[27,28] It is believed that with age, the density of β-adrenergic receptors, corticosteroid and androgen receptors increases, and the density of α2-adrenergic receptors and insulin receptors decreases in the VAT adipocytes.[29] This determines a particularly high sensitivity of the VAT to the lipolytic action of catecholamines and low sensitivity to the anti-lipolytic action of insulin in group 2A, providing for high susceptibility to hormonal

changes and contributing to the development of abdominal obesity with the subsequent formation of clinical and metabolic complications. However, with age, the mechanism for increasing the number of fat cells is exhausted, and the body only uses the hypertrophic pattern of increasing cell volume, which is considered more pathological. The analysis and comparison of the size of adipose tissue cells between experimental subgroups 1A, 2A and 3A showed the dependence of the increase in the size of adipocytes on the age of the animal. In group 1A of young animals (regardless of gender), small- and medium-sized cells prevailed in the SAT and MAT, and large cells (50–100 μm) prevailed in the EDAT and RPAT.

It should be noted that during the experiment, the presence of lymphoid-macrophage infiltration, signs of circulatory disorders in the vessels of the microvasculature and the appearance of sclerotic changes were observed in all age groups and their severity increased with the course of a high-calorie diet, i.e., were associated with a progressive increase in body weight. This data is a significant indicator of the triggering of the mechanisms of LGI, the severity of which can be consistent with the hypertrophic type of AT growth. In the subsequent studies, authors will provide a more detailed report on the possible age and gender differences in the AT participation in the development of LGI. Hence, this study showed that the experimentally determined changes in the integrative AT parameters in rats in the pathogenesis of MS were associated with age and gender; however, they cannot be an objective indicator of LGI which plays a key role in the development of diseases associated with MS. It was found that in a rat model of MS, changes in the AT weight, as well as histological and morphological changes in the AT under the influence of the alimentary factor, were associated with age and gender. Thus, in young rats, an increase in BMI occurred mainly due to hyperplasia of the SAT and VAT cells. An increase in the AT in a young body caused by the action of a pathogenic factor always begins with a compensatory, hyperplastic, 'protective' mechanism, which depends rather on age than on gender. With age, the hyperplastic type of AT growth turns into a hypertrophic one, especially in the MAT. An inversely proportional relationship was observed: a decrease in adipocyte resistance (especially VAT) with increasing age of the animal.

5. CONCLUSIONS

Age dependence of an increase in the AT specific weight associated with a decrease in the mean number of adipocytes and an increase in the mean adipocyte size was shown experimentally. Therefore, changes in the structural and functional characteristics of the AT stimulated by obesity, such as total volume, changes in the severity and nature of fat deposition, as well as AT qualitative characteristics, can promote the understanding of an increase in the AT metabolic activity in the form of LGI reactions as a primary pathogenetic link of MS and, subsequently, they can be used to control the condition and correct AT dysfunction in MS. The results obtained can be further considered as a biological marker that determines the 'scenario' of the development of such disorders associated with obesity, as systemic inflammatory response syndrome, insulin resistance, etc.

Conflict of interest

None declared.

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None declared.

Ethics

The experiments were carried out in accordance with the General Principles for Conducting Experiments on Animals approved by the 1st National Congress on Bioethics (20.09.01 Kiev, Ukraine) and with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes,[18] as well as in accordance with the requirements of the Regulation on Ethics of the Ministry of Health of Ukraine No. 690 of 23.09.2009.[19]

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