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Original Article

Histomorphological changes in brown adipose tissue and energy metabolism in rats of different ages with induced visceral obesity

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Abstract

The question of the mechanisms of interrelationship between age-related changes in the morpho-functional state of brown adipose tissue (BAT) with the state of energy metabolism and their role in the pathogenesis of visceral obesity (VO) remains open. The aim of this work was to compare histomorphological changes in BAT of rats of different ages with VO. The study was conducted in male Wistar rats aged 6 and 21 months. VO was modeled in the animals by 12 weeks of exposure to a high-calorie diet. Histological preparations were made from the interscapular bodies of the BAT. Blood serum concentrations of lipids and cholesterol were determined. Total oxygen consumption and rectal temperature were measured in rats. VO leads to morphological changes in the BAT, indicating its hypofunction. The increase in BAT weight is due to the hypertrophy of adipocytes and their transformation into white adipose tissue. With VO, total oxygen consumption and rectal temperature increase, they were indicating the presence of a certain degree of disruption of oxidation and phosphorylation processes. The intensity of BAT histomorphological disturbances and energy metabolism is more pronounced in young rats and depends on the degree of obesity.

Keywords: high-calorie diet, adipose tissue morphometry, alimentary obesity.

Introduction

Visceral obesity (VO) is one of the most pressing problems facing modern medicine due to its increasing prevalence in both adults and children and the inadequate effectiveness of existing treatment and prevention methods [1]. VO is a multifactorial disease that results from a long-term imbalance between high-calorie food intake and energy expenditure [2]. White adipose tissue (WAT) is known to store energy in the form of triglycerides [3]. Meanwhile, brown adipose tissue (BAT) has a high concentration of mitochondria and uniquely expresses uncoupling protein 1 (UCP1), allowing it to specialize in energy expenditure and contractile thermogenesis [4, 5].

The dissipation of chemical energy in the form of heat is one of the most important mechanisms for protecting the body not only from the effects of low temperatures but also from excessive food intake. In recent years, there has been growing interest among researchers and clinicians in harnessing the potential of BAT for weight control, energy metabolism correction and obesity treatment [6].

BAT was once thought to be necessary only in babies. However, recent morphological and imaging studies have shown that this tissue is present and active in adults [4]. BAT weight and activity are known to change with age. In newborns, increased BAT weight at birth is associated with reduced body fat accumulation during the first 6 months of life [7]. In adulthood, there is a decrease in BAT activity, which may influence the accumulation of fat deposits [8].

However, the age-related histomorphological changes of BAT in obesity have not been fully investigated. The question of the mechanisms of interrelationship between age-related changes in the morphofunctional state of BAT and the state of energy metabolism and their role in the pathogenesis of VO also remains open and requires further study. The answer to these questions may be of practical interest for developing new effective methods of prevention and treatment of VO by targeting the activity of BAT.

The aim of this work was to study and compare the histomorphological changes of BAT and energy metabolism in rats of different ages with induced VO.

Material and methods

Rats/ethical statement

Forty male Wistar rats, aged 3 months and 18 months, obtained from the vivarium of the Bogomoletz Institute of Physiology of the National Academy of Sciences of Ukraine, were selected for the experiment. The rats were individually housed in plastic cages with mesh partitions, which ensured minimal stress to the animals due to social isolation. The rats were maintained at approximately 21°C and 40–60% relative humidity, with a 12-hour light/dark cycle. Work with rats was conducted in accordance with the recommendations of the European Convention for the Protection of Vertebrate Animals for Experimental and Other Scientific Purposes (Strasbourg, 1986). The study was approved by the Biomedical Ethics Committee of the Bogomoletz Institute of Physiology, National Academy of Sciences of Ukraine (Protocol No. 5, dated 31.11.19).

Modeling of visceral obesity

The control rat received 20 g of standard chow daily (recipe K 120-1 "Rezon-1", Ukraine) with a caloric content of 66 kcal. Its composition was as follows: fats – 6%, proteins – 23% and carbohydrates – 55%. Access to water was not restricted.

The experimental rat was given 20 g of a high-calorie diet (HCD) for 12 weeks, consisting of fats – 45%, carbohydrates – 31%, and proteins – 9%. The caloric content of such a diet for one rat was 486 kJ (116 kcal). The HCD included pork lard, white bread crumbs and sunflower seeds in addition to the standard chow. In addition, the rats received a drinking ration of 1 day of water/1 day of 10% fructose solution. On the day the rat drank fructose, the caloric content of the diet was increased to 586 kJ (140 kcal). The experimental animals were given food ad libitum, and the completeness of consumption was monitored daily [9].

At the end of the experiment, the presence of VO in rats was diagnosed by determining the weight of visceral fat and its ratio to body weight (index of visceral obesity). Visceral fat was extracted mechanically from the abdominal cavity.

Histomorphological analysis of the BAT

Tissue samples were taken at random from the interscapular bodies of BAT. They were fixed in Bouin's fluid, dehydrated in alcohols of increasing concentration (from 70° to 96°) and in dioxane. The samples obtained were embedded in paraffin. Paraffin sections of 6 µm thickness were made on a sliding microtome. The sections were stained with Boehmer's hematoxylin and eosin. The Van Gieson staining method was used to visualize connective tissue elements [10]. Using a digital camera ("Levenhuk", USA), the micro preparations were photographed on a microscope "Nikon Eclipse E100" (Japan). Morphometry on digital images of micropreparations was performed using the "ImageJ" program.

The relative areas of parenchyma, connective tissue and vessels were determined in histological sections of BAT. The mean diameter and cross-sectional area of adipocytes and their nucleus and cytoplasm were measured, and the nuclear-cytoplasmic ratio was determined. The number and density of adipocytes per unit area were calculated. The number of nucleolus in the nucleus of adipocytes was counted. The number and area of lipid droplets in adipocytes were calculated. The distribution of adipocytes was made according to the number of lipid droplets. Thus, adipocytes are divided into 3 types: A1 – contains 1 large lipid droplet; A2 – contains 1 large lipid droplet and several small ones; A3 – contains many small droplets. The stromal-parenchymal index (the ratio of the relative area of connective tissue to the area of parenchyma) and the trophic index (the ratio of the relative area of vessels to the area of parenchyma and connective tissue) were determined [11].

Determination of oxygen consumption and rectal temperature

The intensity of oxidative metabolism in rats was assessed by the value of oxygen consumption (VO_2) and rectal temperature at the beginning and end of the experiment. Oxygen consumption in rats was determined on an empty stomach in a closed system to study gas exchange [12]. The VO_2 value was calculated in ml

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per 1 minute per 100 g of body weight and adjusted to standard physical conditions (STPD): dry gas at 0°C and 760 mmHg. Rectal temperature was measured using a "Beurer FT 09/1" electronic medical thermometer with an accuracy of ±0.1°C.

Evaluation of lipids in blood serum

The concentration of lipids and cholesterol in rat blood serum was determined by the colorimetric-enzymatic method using standard reagent kits ("Filisit-Diagnostika", Ukraine) on a biochemical analyzer ("Sinnowa", China). Standardized protocols were used to determine these indicators in blood serum.

Statistical analysis

The data obtained were processed by the methods of variational statistics using the software "Statistica 6.0 for Windows" (StatSoft, USA) and "Excel 2010" (Microsoft, USA). The normality of the distribution of the digital arrays was checked using the Shapiro-Wilk W test. If the distribution was normal, the Student's t-test was used to estimate the difference coefficient of reliability between the control and experimental groups. Differences were considered significant when $p<0.05$. One-way analysis of variance (ANOVA) was also used.

Results

Clear signs of VO were observed in rats of both age groups exposed to HCD for 12 weeks. This was evidenced by an increase in visceral fat weight by 140% ($p<0.05$, 6-month-old rats) and by 56% ($p<0.05$, 21-month-old rats) compared with the control. The weight of interscapular brown fat bodies in adult control rats was 34% ($p<0.05$) less than the weight of fat in young rats. This is consistent with the general biological pattern that BAT undergoes involution with age. Autophagy and inflammatory processes increase with age, leading to an age-related decrease in BAT activity. BAT weight in both 6- and 21-month-old HCD-treated rats was increased by 72% and 82% ($p<0.05$, respectively) compared with the controls.

The presence of obesity was indicated by an increase in the concentration of total lipids and cholesterol in the blood serum. The lipid and total cholesterol concentration in 6-month-old experimental rats was higher by 46 and 14% ($p<0.05$, respectively) compared with the control group. In 21-month-old rats, the concentration of lipids was 53% higher ($p<0.05$) (Figure 1).

Microscopic examination of interscapular BAT sections from experimental rats revealed that it consisted of lobules (mainly triangular in shape) separated by the thinnest layers of loose fibrous connective tissue (CT),

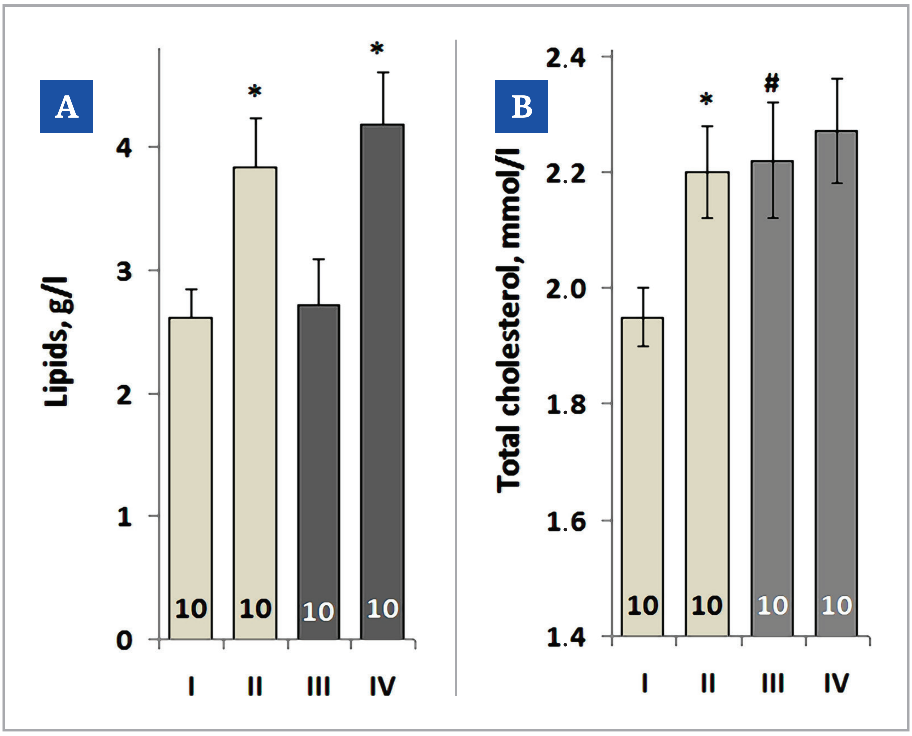

Figure 1: Concentration lipids (A) and total cholesterol (B) in blood serum control (I) and experimental (II) of 6-month-old rats, control (III) and experimental (IV) of 21-month-old rats. *$p<0.05$ – the significance of differences compared with the control; #$p<0.05$ – the significance of differences compared with the control 6-month rats.

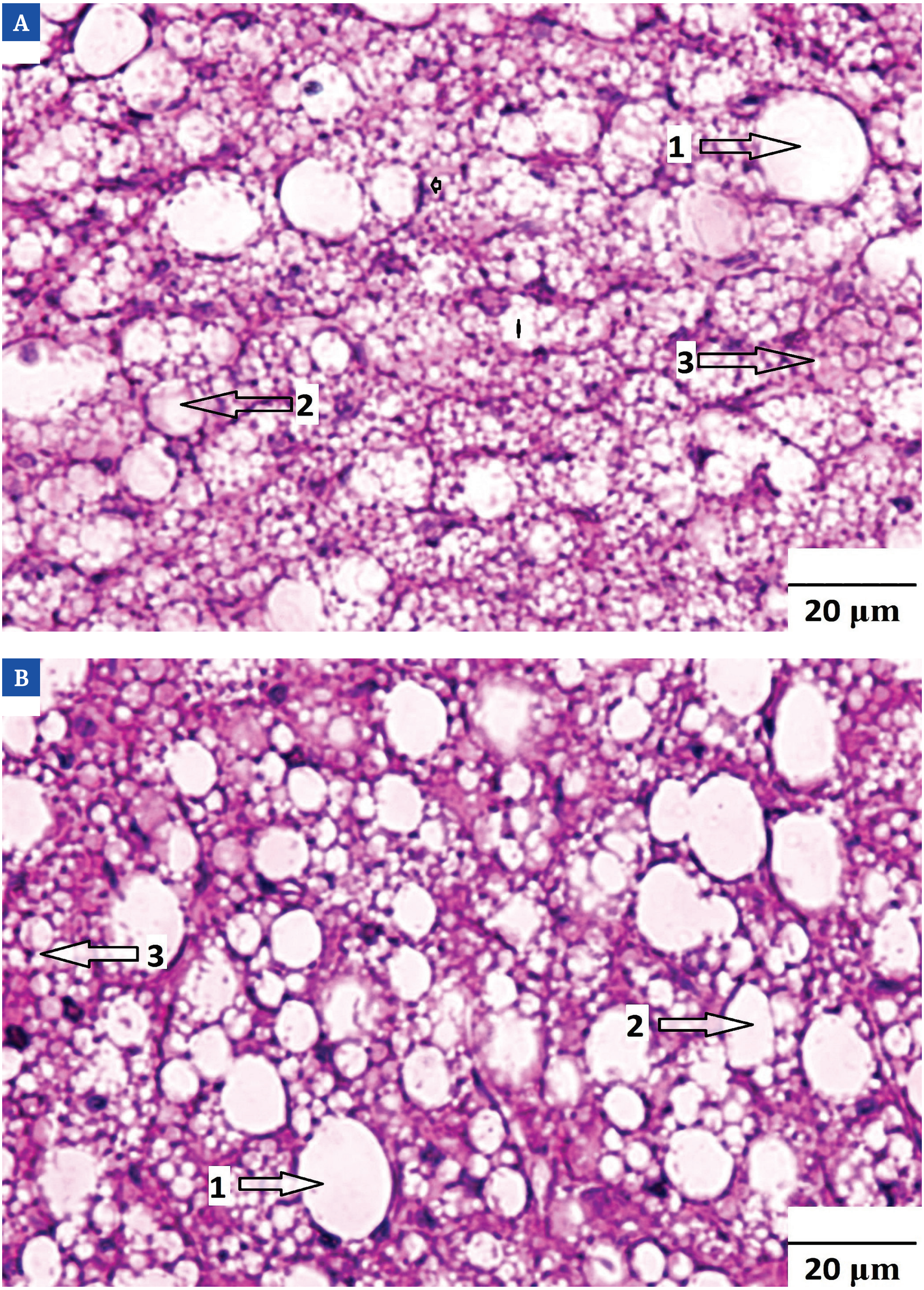

Figure 2: Micrograps of the brown adipose tissue of a 6-month-old control rat (A) and a rat with obesity (B). Hematoxylin-eosin stain. X 800. 1 – adipocyte with 1 large lipid droplet (white adipocyte); 2 – adipocyte with one large and several small drops (beige adipocyte); 3 – adipocyte with many small drops (brown adipocyte).

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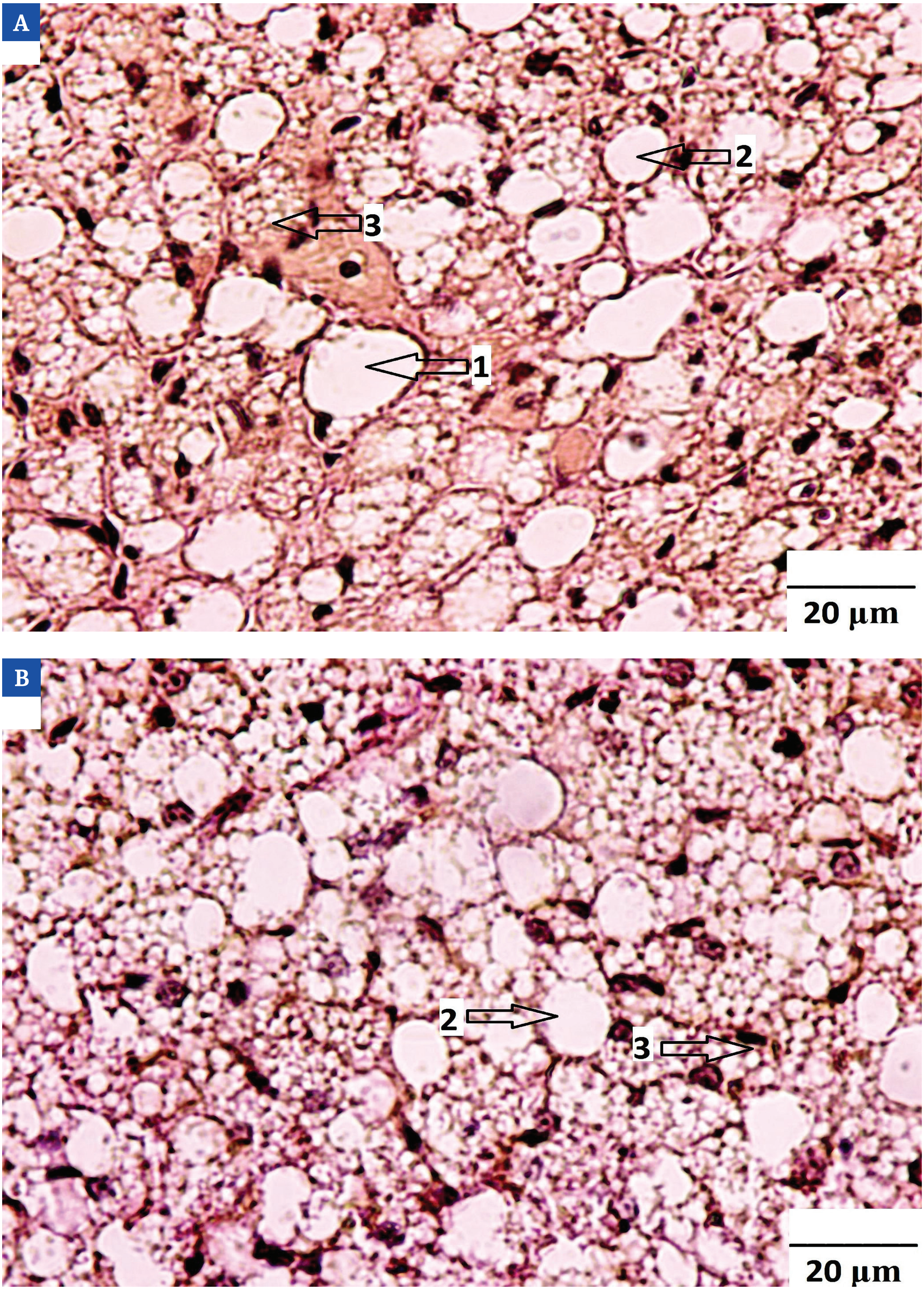

Figure 3: Micrograps of the brown adipose tissue of a 21-month-old control rat (A) and a rat with obesity (B). Van Gieson stain. X 800. 1 – adipocyte with 1 large lipid droplet (white adipocyte); 2 – adipocyte with one large and several small drops (beige adipocyte); 3 – adipocyte with many small drops (brown adipocyte).

which was weakly stained with hematoxylin and eosin (Figure 2) and picrofuchsin (Figure 3). The amount of interlobular CT is greater near large vessels entering the lobule. Layers of loose CT contain thin reticular fibers. The CT layers contain a large number of haemocapillaries, much larger than in WAT.

The higher the degree of VO of the animal, the higher the number of white adipocytes in the BAT. Brown adipocytes were smaller than white adipocytes and had a polygonal shape. The nucleus in the cells is located in the center, is rounded and contains several nucleolus. The cytoplasm contains numerous fat droplets. The nucleus in white adipocytes had an elongated shape and was pushed to the periphery of the cell by a large fat droplet. In the peripheral parts of the lobules, brown adipocytes are transformed into white adipocytes and *vice versa*. In adipocytes located near large vessels, the volume of fat droplets is smaller than in cells of other localisations (Figures 2 and 3).

Differences in histomorphometric indicators of the BAT of control rats of different age groups were observed. The 21-month-old animals had a smaller area of adipocytes (by 23%, $p<0.05$) and the area of their nucleus (by 13%, $p<0.05$) and cytoplasm (by 24%, $p<0.05$), a greater number of adipocytes by 48% ($p<0.05$) compared with the young animals. In adult rats, the number of lipid droplets in adipocytes was also 43% ($p<0.05$) lower, but their area was 47% ($p<0.05$) larger. In control, 21-month-old rats, a higher number of adipocytes with 1 large lipid droplet (by 740%, $p<0.05$) and a lower number of cells with 1 large and many small lipid droplets (by 28%, $p<0.05$) were observed than in young animals (Table 1). This means that with age, there is evidence of a decrease in BAT activity and its conversion into WAT.

A significant increase in the number of white adipocytes was observed in the BAT of 6-month-old rats on HCD. The adipocytes of these animals were found to be larger: diameter by 24% ($p<0.05$), area by 41% ($p<0.05$) and the area of their cytoplasm by 44% ($p<0.05$) compared with the control. At the same time, the nuclear-cytoplasmic ratio decreased by 24% ($p<0.05$). The number of adipocytes and their density per unit area were 32% ($p<0.05$) lower. The number of nucleolus in the nucleus of the adipocytes was 13% ($p<0.05$) lower than in the control. A lower number of adipocytes containing many small droplets (by 43%, $p<0.05$) and a greater number of cells with 1 large lipid droplet (by 420%, $p<0.05$) and the number of adipocytes containing 1 large and many small droplets (by 146%, $p<0.05$) than in control. The area of the lipid droplets was 92% ($p<0.05$) larger. There was also a tendency for an increase in the relative area of CT and a decrease in the area of blood vessels in the BAT of these rats compared with the control (Table 1). That is, the morphometric data obtained indicate a decrease in the functional activity of the BAT and its transformation into WAT.

In the BAT of 21-month-old animals on HCD, adipocytes were also found to be 20% ($p<0.05$) larger in

Table 1: Morphometric indicators of the brown adipose tissue (n=10, M±m).

Indicators	6-month-old rats		21-month-old rats	
	Control	Obesity	Control	Obesity
Adipocyte diameter, μm	18.0±0.5	22.3±0.6*	16.1±0.4	19.4±0.6*
Area, μm²				
Adipocyte	300.4±25.0	424.9±22.6*	230.3±12.3**	305.0±26.0*
Nucleus	23.2±0.9	25.5±1.1	20.1±0.5**	23.2±0.9*
Cytoplasm	277.2±25.6	399.4±22.6*	210.2±11.8**	281.8±25.2*
Nuclear-cytoplasmic ratio	0.084±0.008	0.064±0.004*	0.096±0.008	0.082±0.009
Number of adipocytes, pcs/22000 μm²	56.7±2.5	38.8±2.0*	83.7±8.9**	61.6±2.9*
Density of adipocytes placement, pcs/1000 μm²	2.58±0.11	1.76±0.09*	3.80±0.49**	2.89±0.27*
Number of nucleolus in the nucleus, pcs	1.58±0.04	1.37±0.05*	1.54±0.06	1.37±0.05
Number of lipid droplets in the adipocyte, pcs	17.6±0.8	17.4±1.2	10.1±0.4**	16.6±1.0*
Area of lipid droplets, μm²	11.6±0.6	22.3±0.4*	17.0±0.2**	19.7±0.3*

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Table 1: Continued.

Indicators	6-month-old rats		21-month-old rats	
	Control	Obesity	Control	Obesity
Distribution of adipocytes, %				
A1 – one big drop	1.0±0.4	5.2±0.4*	8.4±0.8**	5.0±0.3*
A2 – one large and several small drops	20.2±0.5	49.7±1.1*	14.6±0.4**	27.7±0.5*
A3 – many small drops	78.8±1.5	45.1±1.9*	77.0±1.9	67.3±1.6*
Relative area, %				
Parenchyma	92.3±1.1	92.0±1.7	91.3±1.7	90.8±1.5
Connective tissue	4.5±0.8	5.1±0.9	4.0±0.2	5.0±0.2
Vessels	3.2±0.4	2.9±0.8	4.7±0.1**	4.2±0.8
Stromal-parenchymal index	0.083±0.01	0.08±0.01	0.095±0.021	0.10±0.01
Trophic index	0.035±0.005	0.032±0.006	0.051±0.002**	0.046±0.01

Note: *p<0.05 – significance of differences compared with the control; **p<0.05 – significance of differences compared with the control of 6-month-old rats

diameter, 32% (p<0.05) larger in area, and 15% (p<0.05) and 34% (p<0.05) larger in the area of their nucleus and cytoplasm, respectively, compared with the control. The number and the density of adipocytes were 26% (p<0.05) lower. In the adipocytes of the experimental rats, there was a tendency for the number of nucleolus in the nucleus to decrease.

There was also a lower number of adipocytes containing many small lipid droplets (by 13%, p<0.05), a number of cells with 1 large lipid droplet (by 40%, p<0.05) and a higher number of adipocytes containing 1 large and many small lipid droplets (by 90%, p<0.05) in these animals than in the control. The average area and number of lipid droplets in cells were 16% and 64% (p<0.05, respectively) greater. There was also a tendency for an increase in the relative area of CT and a decrease in the area of blood vessels in the BAT (Table 1). That is, the morphometric data obtained indicate a decrease in the functional activity of the BAT and its transformation into WAT in adult rats with VO. However, the transformation of BAT is not as intense as it was at a young age.

It was found that in 21-month-old control rats, oxygen consumption and basal body temperature showed a clear tendency to decrease compared with the young animals (Figure 4). The presence of a reliable direct correlation between the level of oxygen consumption and rectal temperature in control rats (r=+0.53; p=0.05), as well as an inverse correlation between the age of the rats and oxygen consumption (r=-0.62 p=0.05), age and rectal temperature (r=-0.67; p=0.05).

The development of obesity led to an increase in oxygen consumption and rectal body temperature in both young and adult rats. In 6-month-old rats, oxygen consumption was 52% (p<0.05) higher and rectal temperature was 0.4°C higher than in control rats. A similar type of change in these indicators was observed in 21-month-old rats, but the magnitude was significantly less. Thus, differences in oxygen consumption did not exceed 19% (p<0.05), and rectal temperature – 0.2°C. At the same time, the differences in oxygen consumption and rectal temperature between 6-month-old and 21-month-old rats increased by 30% and 0.5°C, respectively (Figure 4).

The nature of the change in the correlation coefficient between oxygen consumption and rectal body temperature in obese rats was very revealing. Meanwhile, in young non-obese rats, r=+0.59 (p=0.05); in obese rats, it did not exceed +0.37 (p=0.05). In old rats, the correlation coefficient between the amount of oxygen consumption and the basal temperature was +0.57 (p=0.05), and with the development of obesity, it did not exceed +0.30. This means that the negative effect of obesity on energy metabolism is most pronounced in young rats.

Discussion

The balance of functional activity between BAT and WAT plays an important role in maintaining optimal metabolic levels in the body. In obesity, WAT is

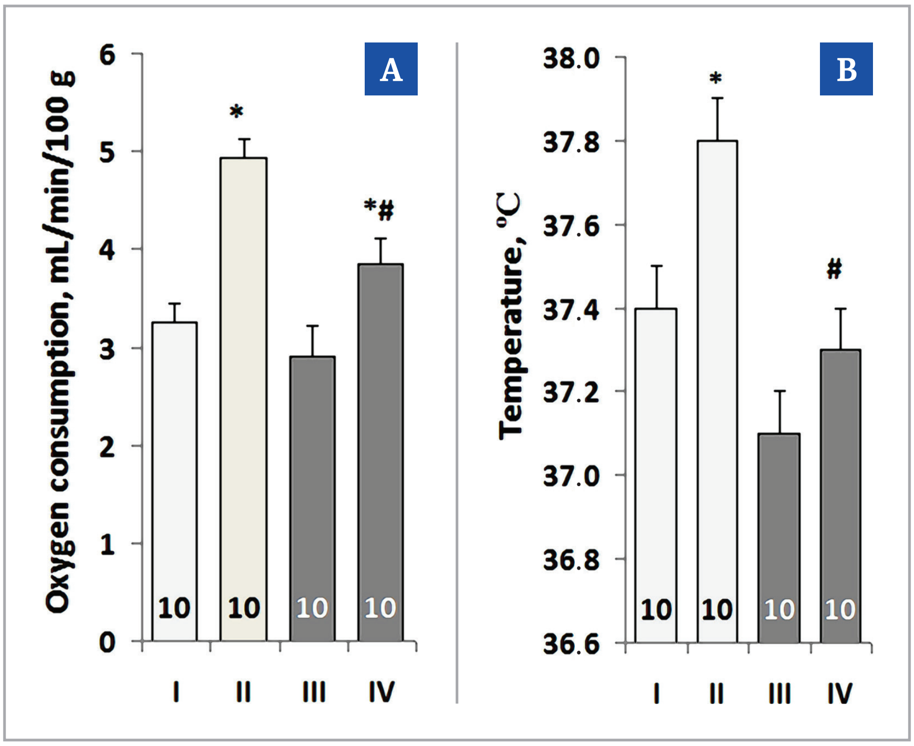

Figure 4: Oxygen consumption (A) and rectal temperature (B) of control (I) and experimental (II) rats aged 6 months, control (III) and experimental (IV) rats aged 21 months. *p<0.05 – significance of differences compared with the control; #p<0.05 – significance of differences compared with the experimental 6-mounth-old rats.

able to secrete pro-inflammatory cytokines and other biologically active substances that reduce the activity of BAT and its thermogenic capacity. At the same time, lipids can accumulate in BAT, similar to what happens in WAT, contributing to the development of insulin resistance and metabolic dysfunction [13]. Chronic inflammation of low-intensity adipose tissue, including BAT, is a hallmark of obesity. In obesity, there are signs of oxidative stress, chronic inflammation and fibrosis, which reduce the functional activity of mitochondria and impair the ability of BAT to produce heat and efficiently burn stored fat [8].

The results of our research show that exposing rats to HCD for 12 weeks leads to the development of clear signs of VO: the visceral fat weight, the concentration of total lipids and cholesterol in the blood serum were significantly increased. In young animals, lipid metabolism disorders and the degree of obesity were more pronounced.

The increase in BAT weight in experimental animals is due to an active increase in the amount of WAT, the transformation of brown adipocytes into white. Unidirectional changes in BAT structure were observed in rats of different ages consuming HCD. However, changes in BAT were more pronounced in younger animals. In addition, the BAT of 6-month-old rats showed more evidence of transformation into WAT. This is evidenced by a probable greater number of white and beige adipocytes than in 21-month-old experimental rats. As you know, the less active brown adipocyte, which does not participate in thermogenesis, adopts a morphology more similar to that of a white adipocyte [14]. In adult experimental rats, however, the number of white adipocytes in the BAT decreased. An increase in the amount of CT and a decrease in the area of blood vessels in the BAT of experimental animals indicates inhibition of oxygen transport to parenchymal elements and a deterioration in the conditions for metabolic processes.

It is known from the literature that BAT activity decreases with obesity. A “whitening” process takes place, in which BAT acquires similar characteristics to WAT. Whitening of BAT is a complex metabolic complication of obesity and depends on various factors: high-calorie diet, age, species of animals, genetics etc. [8]. At the same time, accumulation of large lipid droplets in the cytoplasm, mitochondrial dysfunction, decreased blood supply, autophagy, and inflammation are observed in BAT [15]. It has been studied that the process of BAT whitening is most common in dietary obesity. However, the mechanism of this phenomenon is still unknown and requires further research.

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Aging is strongly associated with BAT dysfunction and an increased risk of obesity [16]. A significant decrease in BAT and beige adipose tissue activity with age leads to an increase in the percentage of visceral fat [17]. In general, aging is strongly associated with low-grade systemic inflammation and changes in the endocrine system, leading to BAT dysfunction [18]. The work of Pan *et al*. investigated BAT whitening due to an increase in adipocytes with 1 large lipid droplet occurring through the secretion of interferon (IFN)-g in 18-month-old mice. In addition, IFN-g inhibits brown preadipocyte differentiation, which promotes adipose tissue remodeling in aged mice [19].

The development of VO is closely related to the state of energy metabolism processes. The amount of oxygen consumption reflects the state of metabolic processes and the amount of energy consumed by the body. Body temperature is also an important indicator of the rate of oxidation processes occurring in the body. We have shown that obesity increases rats' oxygen consumption and basal body temperature. At the same time, the most pronounced changes were observed in young rats. This indicates the presence of signs of dissociation of oxidation and phosphorylation processes, as a result of which proton energy, instead of ATP formation, goes to heat release [20]. The transmembrane uncoupling protein thermogenin is thought to play an important role in these processes, but the specific mechanisms of its involvement in uncoupling oxidation and phosphorylation processes in obesity, as well as the reasons for age-related differences in the degree of expression of these processes, are not yet fully understood [21]. Further research is needed to fully understand the mechanisms of the interrelationship between VO, the state of BAT, the processes of oxygen consumption and thermoregulation.

Conclusion

Exposure of rats to HCD leads to the development of VO and the appearance of morphological changes in BAT, indicating its hypofunction. The increase in BAT weight is due to the hypertrophy of adipocytes (due to an increase in the amount of fat in them) and their transformation into WAT. In VO, total oxygen consumption and basal body temperature increase, indicating the presence of a certain degree of disruption of oxidation and phosphorylation processes. The intensity of BAT histomorphological disturbances and energy metabolism is more pronounced in young rats and depends on the degree of obesity. The obtained results are not only of theoretical importance but are also of interest for practical medicine in solving problems of preventing a decrease in BAT function and preventing its whitening in patients of various ages with the presence of VO. In the future, we plan to study possible ways of activating the BAT in obesity and determine the possibility of using them for preventive purposes.

Conflict of interest

The authors declare no conflict of interest.

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