

Morpho-functional Features of the Pancreas in Different Ages Rats with Alimentary Obesity

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Abstract

Aim: To compare of histo-morphological disorders of the pancreas in rats of different ages with alimentary-induced obesity.

Materials and Methods: The study was conducted on the male Wistar rats aged 6 and 21 months. Alimentary obesity was modeled by feeding the animals a high-calorie diet (580 kcal/100 g) with an excess of fats and carbohydrates for 12 weeks. The control animals received the standard diet (330 kcal/100 g). Histological preparations were made from the pancreas tissue. Histo-morphometry was carried out using the computer program "ImageJ". The concentration of glucose, lipids and cholesterol in the blood serum of rats was determined.

Results: It was revealed that a high-calorie diet led to the development of alimentary obesity and the appearance of pronounced signs of pancreatic hypofunction in experimental rats. The intensity of histo-morphological disorders of the pancreas depended on the age and degree of animal's obesity. These changes were more pronounced in young rats. The endocrine function of the pancreas underwent more negative changes in obesity than exocrine.

Conclusion: The results obtained are important for practical medicine in resolving the treatment and prevention of pancreas diseases in obesity patients of different ages.

Keywords: Alimentary Obesity; High-calorie Diet; Pancreas; Histo-morphometry; Age

Abbreviations

AO: Alimentary Obesity; CT: Connective Tissue; HCD: High-calorie Diet; NAFLD: Non-alcoholic Fatty Pancreas Disease

Introduction

Obesity is a growing global health problem. It is associated with a high risk of developing a number of comorbidities, including cardiovascular and gastrointestinal diseases, type II diabetes, joint and muscle disorders, respiratory and psychological problems, which significantly affect the quality of life, and increase the risk of mortality [1].

The first works devoted to the study of pancreas diseases in obesity date back to 1927, when Schaefer revealed a direct dependence of gland mass on body weight. Olsen studied 394 autopsy materials of the pancreas and established the relationship of its damage with body weight, diabetes and atherosclerosis [2]. In the last decade, researchers have been paying close attention to pancreas diseases in obesity [3]. It was established that the consumption of fatty food leads to fatty liver steatosis, an increase in the level of triglycerides, free fatty acids, interleukin-1 β and tumor necrosis factor α [4]. All this is accompanied by the development of β -cells dysfunction of the pancreas [5].

In the process of ontogenesis, the pancreas reacts differently to the same adverse factors. The age-related susceptibility of this organ to the influence of obesity is no exception. At present, there is no unanimous opinion regarding the specifics of the influence of alimentary obesity (AO) on histo-morphological changes of the pancreas in animals of different ages. Therefore, in order to compare and analyze the nature and degree of expressiveness of age-related structural changes of the pancreas in AO, we used in the experiment two groups of rats of different ages.

The aim of the study is to compare the morpho-functional changes of the pancreas in rats of different ages with alimentary-induced obesity.

Materials and Methods

For the experiment, 48 male Wistar rats were selected at the age of 3 months (weighing 250 ± 10 g) and 18 months (weighing 450 ± 10 g). AO was modeled by keeping animals on a high-calorie diet (HCD) with an excess of fat (45%) and carbohydrates (31%). Each rat received 6 g of specially prepared granulated feed (70% standard compound feed with the addition of 30% pork lard); 6.8 g of pork lard; 3.6 g of white breadcrumbs; 3.6 g of sunflower seeds, which totaled 116 kcal. Experimental animals received feed ad libitum under daily monitoring of the completeness of its consumption. A day later, instead of water, the rats received a 10% fructose solution. Control animals were on a standard diet. The rat of the control group received 20 g of balanced compound feed daily, the caloric content of which was 66 kcal. The duration of the experiment was 12 weeks [6].

The presence of AO in rats, after the end of the experiment, was diagnosed by determining the mass of visceral fat and its ratio to body weight (index of visceral obesity). Visceral fat was isolated by the dissection method. Rats were removed from the experiment by decapitation under ether anesthesia. Research was conducted in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986). The research protocol (No. 5 dated November 31, 2019) was also approved by the Biomedical Ethics Commission of the Bogomoletz Institute of Physiology of the National Academy of Sciences of Ukraine.

Tissue samples were taken from pancreas body, from which histological preparations were made according to the standard

method. They were fixed in Bouin's liquid, dehydrated in alcohols of increasing concentration (from 70° to 96°) and dioxane. The obtained samples were embedded in paraffin. Paraffin sections 6 μ m thick were made on a sled microtome. Staining of the obtained sections was carried out with Boehmer's hematoxylin and eosin. To visualize elements of connective tissue, the Van Gieson staining method was used [7]. With the use of a digital camera ("Levenhuk", USA), micropreparations were photographed on a microscope "Nikon Eclipse E100" (Japan). Morphometry on digital images of micropreparations was performed using the "ImageJ" program.

Histo-morphometric analysis of exo- and endocrine parts of pancreas was performed on histological sections tissue. The diameter and cross-sectional area of acinus, the height and area of exocrinocytes, their nucleus and cytoplasm were measured in the exocrine part of gland. The number of nucleolus in the nucleus of exocrinocytes and the average number of cells in the acinus were counted. The average number of pancreatic islets per unit area (0.25 mm^2) and the number of endocrinocytes were counted in the endocrine part of gland. The cross-sectional area and diameter of the islets were measured and the density of the cells was determined. The width of the interlobular and interacinous connective tissue layers was measured to determine the state of the connective tissue elements in the gland. The relative area of the exo- and endocrine pancreas, as well as the connective tissue in the gland was determined by the method of superimposing point morphometric grids [8,9].

The concentration of glucose, lipids and cholesterol in the blood serum of rats was determined by the colorimetric-enzymatic method using standard sets of reagents ("Filisit-Diagnostika", Ukraine) on a biochemical analyzer ("Sinnova", China).

The obtained data were processed by the methods of variational statistics using the software "Statistica 8.0 for Windows" (StatSoft, USA) and "Excel 2010" (Microsoft, USA). The normality of the distribution of digital arrays was checked using the Shapiro-Wilk W-test. In case of normality of the distribution, the Student's t-test was used to estimate the difference coefficient of the reliability of the difference between the control and experimental groups. Differences were considered significant at $P < 0.05$. One-way analysis of variance (ANOVA) was also used. Multiple pairwise comparison of groups was performed using Tukey's HSD test with a significance level of 0.05.

Results and Discussion

Visceral fat mass in both 6- and 21-month-old rats fed a HCD was found to be 145% and 58% greater, respectively, compared to age-matched control rats. The ratio of visceral fat mass to body weight in experimental animals was significantly higher than

control values by 122% (6-month-old) and 56% (21-month-old). Changes in these parameters indicate the presence of pronounced AO in rats. The development of obesity was more intense in young animals. The mass of the pancreas was less by 9% in 6-month-old rats, which were on HCD. The mass of the gland in 21-month-old experimental rats did not differ from the control (Table 1).

Indicators	6 month old rats		21 month old rats	
	Control	Experiment	Control	Experiment
Visceral fat weight, g	19.0 ± 1.1	46.6 ± 1.7*	23.9 ± 1.6	37.7 ± 1.0*
Visceral fat/body weight ratio	0.046 ± 0.005	0.102 ± 0.01*	0.052 ± 0.006	0.081 ± 0.006*
Pancreas weight, g	0.57 ± 0.03	0.52 ± 0.03	0.63 ± 0.05	0.63 ± 0.07

Table 1: Weight of the pancreas and visceral fat (Mean ± SD).

Note: here and table. 2-3 *P < 0.05 - significance of differences compared to the control.

The presence of AO in experimental rats was evidenced by an increase in the concentration of lipids and cholesterol in the blood serum. Thus, the concentration of lipids in 6- and 21-month-old experimental rats was probably higher by 47 and 54%, respectively, compared to the control. The concentration of total cholesterol in blood serum was significantly higher (by 13%) only in young

experimental rats (Table 2). That is, in young animals that were on HCD, disorders of fat metabolism were more pronounced. This is consistent with our data on a more significant increase in visceral fat mass and obesity index in 6-month-old rats. That is, in young animals, the probability of developing AO is much higher than in old ones.

Indicators	6 month old rats		21 month old rats	
	Control	Experiment	Control	Experiment
Glucose, mmol/l				
At the beginning of the experiment	7.70 ± 0.42	7.71 ± 0.33	7.64 ± 0.19	7.89 ± 0.21
At the end of the experiment	7.61 ± 0.36	9.50 ± 0.71*	7.51 ± 0.35	8.15 ± 0.32
Lipids, g/l	2.61 ± 0.23	3.84 ± 0.40*	1.36 ± 0.37	2.09 ± 0.43*
Total cholesterol, mmol/l	1.95 ± 0.05	2.20 ± 0.08*	2.22 ± 0.10	2.27 ± 0.09

Table 2: Concentration of glucose, lipids and cholesterol in blood serum (Mean ± SD).

The rat's pancreas of the experimental groups had a preserved physiological structure, which was divided into exo- and endocrine parts. The exocrine part was the bulk of the gland and was represented by acinuses and ducts. The shape of the acinuses is quite diverse: round, oval and elongated. Acinus from the middle are lined with exocrinocytes of different shapes. One pole, more narrowed (top), they are directed to the center of the acinus, opposite expanded (base) - outside. The cytoplasm of cells had granularity, especially on direction to the apical pole. The nucleus

was located at the base where the granularity was expressed to a lesser extent and contained nucleolus. The acinuses were combined into slices, externally covered with a connective tissue, which is represented by loose weaves of thin bundles of elastic and collagen fibers. A local accumulation of large fat droplets (average area 2200 μm²) was revealed in the exocrine part of the experimental rat's pancreas (Figure 1).

In the exocrine part of the pancreas of 6-month-old rats that were on HCD, the area of acinus, exocrinocytes, their nucleus

Figure 1: Photomicrograph of the pancreas of a 6- (A) and 21-month-old (C) control rat and a 6- (B) and 21-month-old (D) rat fed a high-calorie diet. x200. Van Gieson staining. Note: 1 - Langerhans islet; 2 - interlobular connective tissue; 3 - acinus; 4 - collagen fiber; 5 - fat drop; 6 - pancreas duct.

and cytoplasm was less by 16, 11, 10, and 11%, respectively, compared with the control. The number of nucleolus in the nucleus of exocrinocytes was probably by 16% less than in the control (Table 3; Figure 2). Hypoplasia of the nucleolus may indicate a suppression of the protein synthesis function of cells, or a decrease in the processes of physiological regeneration at the intracellular level [10].

Figure 2: Photomicrograph of the pancreas of a 6-month-old control rat (A) and an animal that was on a high-calorie diet (B). x800. Van Gieson staining. Note: 1 - exocrinocyte; 2 - interacinous connective tissue.

Indicators	6 month old rats		21 month old rats	
	Control	Experiment	Control	Experiment
Exocrine part				
Relative area, %	77.7 ± 1.5	72.3 ± 2.1	69.3 ± 3.0	67.0 ± 1.2
Acinus diameter, μm	30.6 ± 0.8	29.1 ± 0.3	31.4 ± 0.6	29.2 ± 1.1
Acinus area, μm ²	933 ± 14	787 ± 19*	966 ± 15	812 ± 23*
Area, μm ²				
Exocrinocyte	123.9 ± 4.4	110.6 ± 1.8*	129.4 ± 5.9	128.1 ± 7.3
Nucleus	18.8 ± 0.5	16.9 ± 0.2*	20.1 ± 1.5	20.6 ± 1.5
Cytoplasm	105.1 ± 4.0	93.7 ± 1.8*	109.3 ± 4.7	107.5 ± 6.0
Nuclear-cytoplasmic ratio	0.18 ± 0.004	0.18 ± 0.004	0.18 ± 0.01	0.19 ± 0.01
The number of nucleolus in the exocrinocyte, pcs	1.56 ± 0.04	1.31 ± 0.06*	1.56 ± 0.07	1.42 ± 0.05
The height of the epithelium of the acinus, μm	12.2 ± 0.29	11.3 ± 0.3	12.7 ± 0.6	12.3 ± 0.36

The number of exocrinocytes in the acinus, pcs	7.5 ± 0.1	6.9 ± 0.2	7.6 ± 0.1	6.7 ± 0.1*
Endocrine part				
Relative area, %	5.1 ± 0.4	3.8 ± 0.5*	3.7 ± 0.7	3.6 ± 0.6
The number of islets (by 0.25 mm ²), pcs	1.12 ± 0.1	1.11 ± 0.5	1.03 ± 0.13	1.07 ± 0.09
Islet area, μm ²	14653 ± 153	8250 ± 195*	14673 ± 110	13800 ± 215
Islet diameter, μm	111.2 ± 9.1	84.7 ± 6.3*	118.3 ± 4.6	110.0 ± 8.1
The number of endocrinocytes in the islet, pcs	189.2 ± 28.1	117.8 ± 5.7*	190.8 ± 10.7	183.4 ± 12.1
Density of placement of endocrinocytes in the islet, pcs./μm ²	0.013 ± 0.0005	0.014 ± 0.005	0.013 ± 0.0005	0.013 ± 0.003
Connective tissue				
Relative area, %	17.2 ± 1.7	24.0 ± 1.1*	27.0 ± 1.2	29.4 ± 1.4
Stromal-parenchymal index	0.21 ± 0.02	0.32 ± 0.03*	0.37 ± 0.05	0.42 ± 0.02*
The width of the layers connective tissue				
Interlobular				
Interacinous				
	9.0 ± 0.6	12.2 ± 0.7*	11.9 ± 0.4	11.6 ± 0.8
	0.84 ± 0.02	0.9 ± 0.01	0.79 ± 0.03	0.89 ± 0.04*

Table 3: Histo-morphometric indicators of the pancreas (Mean ± SD).

The less pronounced changes in the structure of the exocrine pancreas were observed in 21-month-old rats, after exposure to HCD. These rats had a significantly smaller area of acinus (by 16%) and the number of exocrinocytes in them (by 12%). Other morphometric indicators did not differ from the control (Table 3). Thus, the development of AO was accompanied by the appearance of clear histomorphometric signs of the pancreas exocrine activity inhibition.

The endocrine part occupied a much smaller area of pancreas tissue. It was formed by Langerhans islets, which are dispersed in the gland. The islets are separated from acinus with a thin connective tissue layer and were pierced by a thick grid of capillaries of the endocrinocytes accumulation of rounded shape (Figure 1).

The significant signs of functional state inhibition of the endocrine part of pancreas were observed in 6-month-old rats after exposure to HCD. The relative area of the endocrine part of the pancreas in experimental animals was by 25% less than in control. The cross-sectional area, the diameter of the Langerhans islets and the number of endocrinocytes in them, were less by 44, 24 and 38%, respectively. Significant histo-morphometric changes in the structure of the endocrine pancreas of 21-month-old experimental animals were not detected (Table 3).

The functional activity of the endocrine part of pancreas in young experimental rats was reduced. The concentration of glucose in the blood serum significantly increased by 23% compared with the initial level. However, in 21-month-old rats, the concentration of glucose in the blood serum remained practically unchanged

(Table 2). Thus, in young rats under the influence of a HCD, more significant violations of carbohydrate metabolism developed, which can cause the development of diabetes. AO had a lesser effect on glucose metabolism in adult rats. This may be due to the fact that with age, the mechanisms of blood glucose levels regulation become more stable.

Violation of the glucose level regulation in the blood leads to the development of hyperglycemia and diabetes. Glucose homeostasis is maintained by the synthesis of insulin and glucagon, which is regulated by pancreas β - and α -cells, respectively. With obesity, a vicious circle develops, which is based on hyperinsulinemia and insulin resistance. Constant hyperinsulinemia leads to the depletion of β -cells of the pancreas and the development of type II diabetes [11].

The composition of the pancreas connective tissue (CT) formations includes the capsule and the stroma of the organ. In the latter, acinous, islet and interacinous CT are divided; CT membranes of lobar and lobular; interlobar and interlobular CT, as well as CT, which surrounds blood vessels and excretory ducts. All the listed formations have a similar structure and merge into each other without sharp boundaries. But each of the elements of the CT framework has its own architectural features; differences in the qualitative and quantitative composition of fibrous structures; the amount of the basic substance, the number and shape of fibroblasts [12].

An increase in the number of CT was revealed in the pancreas of 6-month-old rats with AO. This is evidenced by the probably greater relative area of CT (by 40%) and the stromal-parenchymal index (by 52%) and the greater width of interlobular CT layers (by 36%) compared to the control. The relative area of CT in the pancreas of 21-month-old experimental rats was larger by 9%, the stromal-parenchymal index by 14% ($P < 0.05$), the width of interacinous CT layers by 13% ($P < 0.05$) (Table 3). The CT is the most important component of the histo-hematic barrier. An increase in the thickness of CT layers can inhibit the transport of oxygen to the parenchymal elements of the gland, worsen the conditions for the course of metabolic processes, and reduce the penetration of hormones through the barrier into the blood.

Relatively recently, the term “Non-Alcoholic Fatty Pancreatic Disease (NAFPD)” was introduced, which leads to the development

of steatopancreatitis, and subsequently to pancreatic oncology [13]. In studies on animals that have been consuming foods with a high fat content for a long time, the accumulation of fat in the pancreas with the development of inflammation, fibrosis and insulin resistance has been revealed [14]. In a study conducted on pigs, which were on HCD for 24 weeks, steatosis was observed. It was accompanied by an increase in the amount of fat in the total area of the tissue. An increase in the area of Langerhans islets was also detected. At the same time, there was no damage to the cells of the gland [15]. It is believed that pancreatic steatosis is combined with its fibrosis [16].

Thus, obesity accompanied by hyperlipidemia contributes to the development of fat infiltration of the pancreas. The consumption of fatty foods leads to excess production of pancreas enzymes, an increase in the outflow of pancreatic juice, with the subsequent depletion of its exocrine function. This increases the risk of developing both acute and chronic pancreatitis, as well as the appearance of pancreas cancer [17,18]. Therefore, the study of the pathophysiological mechanism, clinical symptoms and ways of preventing the development of NAFPD is an urgent area of research.

Conclusion

The results of our studies have shown that a 3-month stay of rats on a high-calorie diet led to the development of alimentary obesity and the appearance of pronounced signs of pancreatic hypofunction. The intensity of histo-morphological disorders of the gland depended on the age and degree of obesity of the animals. These changes were more pronounced in young rats than in adults. At the same time, the endocrine function of the gland underwent greater negative changes from obesity than the exocrine function. The results obtained are not only of theoretical importance, but are also of interest for practical medicine in addressing the issues of treatment and prevention of pancreatic diseases in obese patients of different ages.

Conflict of Interest

The authors declare no conflict interest.

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Author Contributions

RY, ML, OC designed and performed the research. RY, ML, OC analyzed the data. RY wrote the initial manuscript. RY, ML and OC revised the final manuscript.

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