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БИОЛОГИЧЕСКИЕ НАУКИ КАЗАХСТАНА

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CONTENT

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INFLUENCE OF ALIMENTARY DEPRIVATION ON MORPHOFUNCTIONAL STATE OF THE RAT'S PANCREAS

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Summary

Literature data on the effect of alimentary deprivation (AD) on the morphological and functional changes in the pancreas, especially its exocrine part, are rare. The study of morphological changes in the exo- and endocrine pancreas of rats, after exposure to AD, was the aim of our research. Experimental rats received a diet that was reduced in weight by 30%. Access to water was free. The duration of the experiment was 28 days. Rats were decapitated under light ether anesthesia. For histological studies of the pancreas, samples were taken from its central part (body). Morphometric measurements of the gland were carried out using the computer program "Image J". It was revealed that in the exocrine pancreas of adult rats that were on AD, the area of acinus and nucleus of exocrinocytes increased, the number of nucleolus increased, the nuclear-cytoplasmic ratio and the height of the epithelium of the acinus increased. The increase in the activity of the endocrine pancreas, after exposure to AD, indicated by an increase in its following parameters: area (by 108%), number of Langerhans islets (by 34%), their size and number of endocrinocytes (by 55%). Also in the gland of these animals a decreased in the width of the layers of interlobular and interacinus connective tissue by 28%, which improves conditions for the course of metabolic processes. Thus, the effect of AD is accompanied by the appearance of morphological signs of an increased in the activity of both the exocrine and endocrine (to a greater extent) pancreas in adult rats.

Key words: *alimentary deprivation, pancreas, morphometry.*

Nutritional restriction is known to improve glucose regulation, increases resistance to stress and suppresses inflammation. During starvation, cells activate pathways that enhance internal defenses against oxidative and metabolic stress, as well as those that remove or repair damaged molecules [1, 2]. During the fasting period, cells participate in tissue-specific growth and plasticity processes. Animal studies have consistently shown a reliable effect of restricted nutrition on a wide range of chronic diseases, including obesity, diabetes, cardiovascular and endocrine disorders, tumors and neurodegenerative brain diseases [3-5].

However, despite the well-studied effect of alimentary deprivation (AD) on the body, there is insufficient literature on its effect on the morpho-functional activity of the pancreas, and the results are often contradictory. Most studies have examined the effects of AD on the endocrine pancreas [6, 7]. It is known that starvation causes physiological changes in the endocrine pancreas, namely - changes in insulin secretion, metabolism of Langerhans islets and redox state of β-cells [8]. We have not found any literature data on how the histomorphological structure of the exocrine pancreas changes in AD.

Therefore, the study of morphological changes in the exo- and endocrine pancreas of rats, after exposure to food deprivation, was the aim of our research.

Materials and methods. The experiment was made on 24 wistar rats. The age of the animals was 15 months, weight 420 ± 10 g. Rats were divided into 2 groups (12 animals each): I – control animals, II – experimental rats, which received a reduced weight (30%) diet. The daily ration for control rats was 20 g (65 kcal) of specialized feed, and for experimental rats exposed 14 g (45 kcal). This level of calorie reduction in the diet according to the classification of McKay C.M. belong to the "soft" AD, able to prolong life, increase the efficiency of molecular and cellular systems, increase the adaptive capacity of the organism. Access to water was free. The duration of the experiment was 28 days. Rats were decapitated under light ether anesthesia. Rats were done according to the provisions of the Helsinki Declaration of 1975 and its revision in 1983.

Histological, morphometric and statistical methods of research were used. From the pancreas of each rat took 5 samples of tissue, from which histological preparations were made according to the standard technique: fixed in the liquid Bowen, dehydrated in alcohols of increasing concentration (from 70 to 96 °) and dioxane. The obtained samples were poured into paraffin. Paraffin sections, 5-6 μm thick, were made on the sleigh microtoma, stained with Bemer's hematoxylin and eosin and Van Gizon method [9]. Photography of micropreparations was carried out on the microscope "Nikon Eclipse E100" (Japan). The morphometric dimensions of the gland were performed using the computer program "Image J".

In the exocrine pancreas, such morphological structures as: diameter and area of acinuses, height and area of exocrinocytes, their nucleus and cytoplasm were measured, and the number of nucleolus in cell nucleus and their amount in acinus were considered. The activity of the endocrine pancreas was evaluated by: the number of pancreatic islets (per unit area of 0.25 mm2), the number of endocrinocytes in them, the area and diameter of the islets and the density of cell location. To determine the state of connective tissue elements in the gland, the width of the interlobular and interacinus connective tissue layers was measured. The method of imposing point morphometric nets determined the area (relative) of the exo- and endocrine part, as well as stroma in the gland [10, 11].

For statistical research was used software "Statistica 6.0 for Windows" (StatSoft, USA) and "Exel 2010" (Microsoft, USA). The normality of the distribution of digital arrays was checked using Pearson's test. All research results were subject to the law of normal distribution. The criterion for the Student's t-test was used to evaluate the difference between the control and the experimental group. The differences in the value $p<0.05$ were considered reliable.

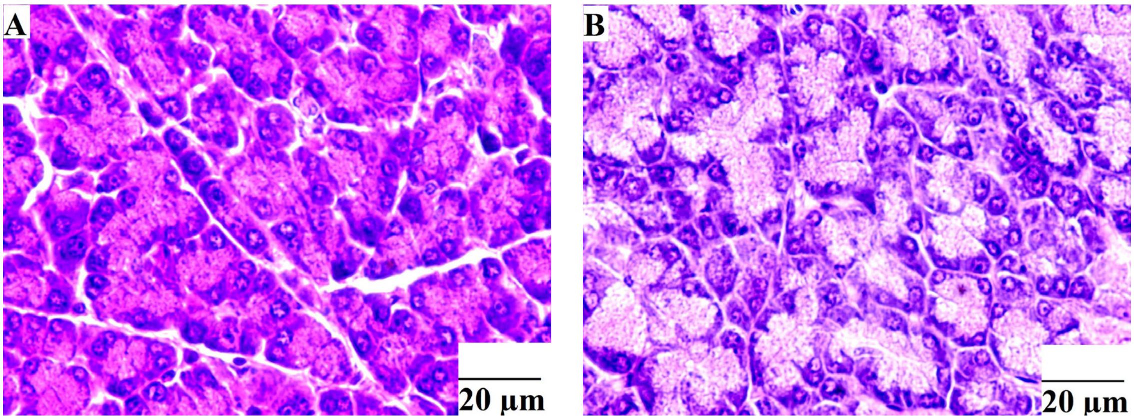
Results and discussion. The body weight of control rats during the experiment had a slight tendency to increase, and animals that were on AD, by contrast, decreased by 11%. In experimental rats, the absolute weight of the pancreas remained at the control level, while its relative weight was probably higher by 23% ($p<0.05$) (Table 1).

It was found that in the exocrine pancreas of rats exposed to AD, the acinus had a rounded, oval and elongated shape. Inside the acinus is lined with exocrinocytes, which are narrowed part (top) directed to the center of the acinus, and the opposite, expanded (base) – outward. The cytoplasm of the cells had a well-defined granularity. The nucleus was located at the base, where the grain size was less pronounced, and contained nucleolus. Acinuses were united in lobes which are externally covered with a connective tissue cover (Fig. 1).

Table 1. Body and pancreas mass (n = 12; M ± m)

Indicators	Body mass, g		Pancreas mass	
	Start of the experiment	End of the experiment	Absolute, mg	Relative (mg/g body weight)
Control	430±7	440±8	795±7	1,81±0,08
Experience	426±13	379±13*	860±10	2,23±0,10*

Note: here and in table 2 *p<0,05 – compared with the control



Picture 1. Microphotograph of the exocrine pancreas of control (A) and experimental (B) rats. Hematoxylin and eosin stain. X800

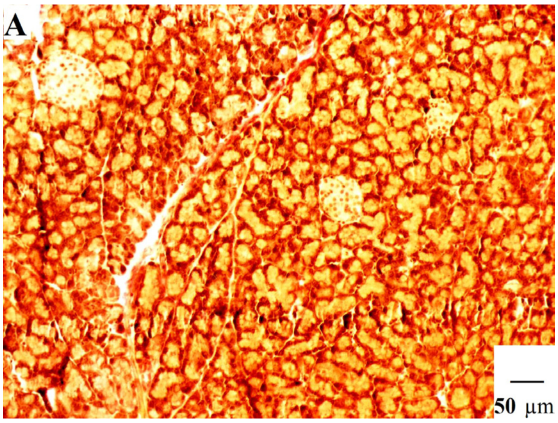
The influence of AD led to an increase in the average area of acinus by 33% compared to the control. At the same time, the area of exocrinocytes and their cytoplasm did not change. The area of the nucleus increased by 10%, which led to a probable increase in the nuclear-cytoplasmic ratio by 12%. The number of nucleolus in the nucleus of exocrinocytes of experimental rats was by 37% ($p<0.05$) greater than in the control. Hyperplasia of nucleolus indicates the activation of the protein-synthetic function of cells, or increased physiological regeneration at the intracellular level [12]. In the pancreas of animals affected by AD, found an increase in the height of the acinus epithelium by 11% ($p<0,05$) and the number of exocrinocytes placed in them (by 9%) compared with the control (Table 2). The change in these parameters indicates the activation of the functional state of the exocrine pancreas after exposure to AD.

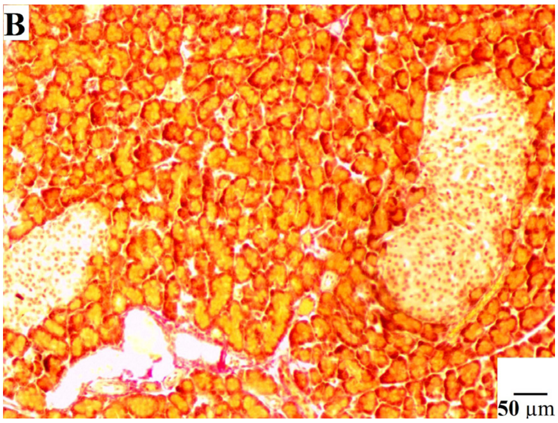
Table 2. Morphometric parameters of the pancreas (n = 12; M ± m)

Indicators	Control	Experience
Exocrine part		
Relative area, %	73,2±1,7	71,3±1,5
The diameter of the acinus, µm	27,7±0,71	30,6±0,5
Acinus cross-sectional area, µm²	692±16	922±27*
Area, µm²:		
exocrinocyte	120,6±2,5	120,6±4,8
nucleus	17,6±0,4	19,4±0,5*

cytoplasm	103±2,6	101,2±4,5
Nuclear-cytoplasmic ratio	0,170±0,006	0,190±0,008*
The number of nucleolus in the exocrinocyte, pcs	1,48±0,05	2,03±0,11*
Height of the acinus epithelium, μm	11,2±0,2	12,4±0,3*
The number of exocrinocytes in the acinus, pcs	7,8±0,2	8,5±0,4
Endocrine part		
Relative area, %	2,6±0,5	5,4±0,5*
Amount of the islets (by 0.25 mm^2), pcs	0,09±0,10	1,21±0,05*
Area of the islets, $μm^2$	9538±92	15070±149*
Diameter of the islets, μm	93,8±3,9	129,2±6,2*
The number of endocrinocytes in the islets, pcs	119,8±16,8	185,3±9,3*
Endocrinocyte density in the islets, pcs / $μm^2$	0,013±0,001	0,012±0,001
Connective tissue		
Relative area, %	24,2±1,0	23,3±1,5
Stromal-parenchymal index	0,32±0,02	0,30±0,02
Width of layers of connective tissue, μm		
interlobular	3,81±0,32	2,73±0,15*
interacinus	0,96±0,02	0,69±0,02*

The endocrine pancreas occupies a much smaller part of the pancreatic tissue. It is formed by the Langerhans islets (LI), which are dispersed in the gland. LI are separated from the acinus by a thin connective tissue layer and are clusters of endocrinocytes permeated by a dense network of capillaries [13]. The LI shape of the experimental animals is mostly round and oval, elongated islands are less often visualized (Fig. 2).





Picture 2. Microphotograph of the endocrine pancreas of control (A) and experimental (B) rats. Van Gieson stain. X200

Significant structural changes were found in the endocrine pancreas of experimental animals after exposure to AD. Thus, they observed a probable increase the area of the endocrine pancreas by 108%. The average number of LI (0,25 mm2) was 1.21, which was 34% more than the control level. The area and diameter of the LI probably increased by 58% and 38% respectively. The number of endocrinocytes in the LI of experimental rats was by 55% higher ($p<0.05$) compared with controls (Table 2; Fig. 2). The change in these parameters indicates a significant activation of the endocrine pancreas of adult animals after exposure to AD.

The capsule and stroma of the organ are part of the connective tissue formations of the pancreas. In the latter, acinar, islet and interacinous connective tissue (CT) is divided; connective tissue membranes of particles and lobules; interlobar and interlobular CT, as well as CT, which surrounds blood vessels and excretory ducts. All these formations have a similar structure and pass into each other without sharp boundaries. But each of the elements of the CT framework has the features of architecture, qualitative and quantitative composition of fibrous structures, the amount of basic substance, the number and shape of fibroblasts [13, 14].

We found that in the pancreas of rats, after exposure to AD, the number of stroma decreased. This is evidenced by the probably smaller width of the interlobular and interacinus CT layers by 28% (Table 2). Connective tissue is included in the histo-hematic barrier, and reducing its number and thickness of layers facilitates the transport of oxygen to the parenchymal elements of the gland, improves the conditions for metabolism, promotes better penetration of hormones through the histohematological barrier into the blood.

The positive role of starvation in pancreas has been identified by other researchers. In experiments on mice with induced diabetes mellitus, which were on a restricted diet, a decrease in the symptoms of this pathology was found. Thus, in these animals β-cell proliferation increased, symptoms of diabetes were eliminated, insulin secretion and glucose homeostasis were restored [6]. However, the mechanism by which restricted nutrition affects β-cell function remains unclear. Another study showed that 8-week-old Sprague-Dawley rats fed a 30% reduced calorie diet had higher β-cell activity, as evidenced by early insulin secretion in an intra-abdominal glucose tolerance test than in control animals. In addition, animals after exposure to restricted nutrition had a greater mass of β-cells and their proliferation in the pancreas [15]. It was found that AD can inhibit the development and delay the progression of pancreatic intraepithelial tumors [16]. Other authors have investigated that interval starvation has a significantly greater anti-cancer effect in the pancreas of genetically modified mouse models than the constant effect of restricted nutrition. Other authors observed that exposure to 40% of restricted nutrition alters β-cell dysfunction and insulin resistance, restores glucose homeostasis, activates β-cell autophagy in mice [17]. However, another study in one-month-old female rats showed that interval fasting (1 day complete fasting / 1 day regular diet) for 12 weeks reduced the weight of the pancreas, activated apoptosis in LI, promotes dysfunction β-cells [7].

Thus, the analysis of the literature once again confirms the ambiguity of the data on the morpho-functional state of the endocrine pancreas of animals that received limited nutrition. It depends on the type of starvation, the duration of the experiments, the age and sex of the animals, etc. All this leads to the continuation of research in this area.

Conclusion. Thus, the 28-day effect of a reduced weight diet (by 30%) has morphological signs activation of exocrine and endocrine (to a greater extent) pancreas in adult rats. In the exocrine part, the area of acinus, cell nucleus and the number of nucleolus increased, the nuclear-cytoplasmic ratio and the height of the epithelium of acinus increased. The increase in the activity of the endocrine pancreas, after exposure to AD, indicated by an increase in its following parameters: area, number of LI, their size and number of endocrinocytes. Reducing the amount of connective tissue in the gland facilitates the transport of oxygen to its parenchymal elements and improves the conditions for metabolism. These data can have both theoretical and practical role when using AD to improve the function of pancreas.

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Тамақтанудың азаюының негізі егеуқұйрықтардың морфофункционалдық жағдайына әсері

Аңдатпа

Ұйқы безіндегі морфофункционалды өзгерістерге, әсіресе оның экзокриндік бөлігіне тамақ өнімдері әсері туралы әдеби деректер Біздің жұмысымыздың мақсаты тамақ өнімдері ұшырағаннан кейін ересек егеуқұйрықтардың эксо мен эндокриндік бөлігіндегі морфологиялық өзгерістерді және эндокриндік бөлігін зерттеу болды. Науқастың егеуқұйрықтары азық-түлік диетасын алды, бұл салмақпен 30% төмендеді. Суға қол жеткізу тегін болды. Тәжірибенің ұзақтығы 28 күн болды. Егеуқұйрықтар жеңіл эфир анестезиясы бойынша жарияланды. Ұйқы безінің орталық бөлігінен (бездің денесі), гистологиялық препараттар стандартты әдістеме бойынша жүргізілді. Гландың морфометриясы j компьютерлік бағдарламасын пайдаланып сандық суреттерге жасалды. Тамақ өнімдері, акиналдың ауданы, экинустың ауданы, экинустың ауданының экзокриндік бөлігінде экзокриннің экзокриндік бөлігінде экзокриноциттер ядролары көбейді, ядролардың саны, ядролық-цитоплазмалық қатынас саны және акин эпителийінің биіктігі , ұлғайды. Тәжірибелік егеуқұйрықтардың бездерінің эндокриндік бөлігінде, атап айтқанда, морфологиялық өзгерістер болды, атап айтқанда: оның салыстырмалы ауданы (108% -ға) Сондай-ақ, олар өздеріне орналастырылған, эндокриноциттердің жоғарылауы (55%). Сондай-ақ, осы жануарлардың бездерінде Интернационалдтық және күшейтілген тіндердің енінің төмендеуі 28% -ға төмендеді, бұл метаболизмнің шығуы үшін жағдайды жақсартады. Осылайша, температурадан тысқұйрықтың әсері ұйқы бездерінің егеуқұйрықтарының экзокринді және эндокринінің (үлкен дәрежеде) белсенділігін арттырудың морфологиялық белгілерінің пайда болуымен қатар жүрді.

***Түйінді сөздер:** тамақ өнімдері, ұйқы безі, морфометриялық.*

Влияние алиментарной депривации на морфофункциональное состояние поджелудочной железы крыс

Аннотация

Литературные данные о влиянии алиментарной депривации на морфофункциональные изменения в поджелудочной железе, особенно ее экзокринной *части, единичны. Цель нашей работы состояла в том, чтобы изучить морфологические изменения экзо- и эндокринной части поджелудочной железы взрослых крыс после воздействия алиментарной депривации. Подопытные крысы получали пищевой рацион, который был снижен по массе на 30%. Доступ к воде был свободным. Продолжительность эксперимента составила 28 дней. Крыс декапитировали под легким эфирным наркозом. Для гистологических исследований поджелудочной железы брали образцы из ее центральной части (тела). Морфометрические измерения железы проведены с помощью компьютерной программы «Image J». Обнаружено, что в экзокринной части поджелудочной железы взрослых крыс, которые находились на алиментарной депривации, возросла площадь ацинусов, ядер экзокриноцитов, увеличилось количество ядрышек, ядерно-цитоплазматическое соотношение и высота эпителия ацинусов. В эндокринной части железы подопытных крыс происходили более интенсивные морфологические изменения, а именно: достоверно возросла ее относительная площадь (на 108%), увеличилось среднее количество островов Лангерганса на единицу площади (на 34%), их линейные размеры, а также количество размещенных у них эндокриноцитов (на 55%). Также в железе этих животных было обнаружено снижение ширины прослоек междолевой и межацинусной соединительной ткани на 28%, что улучшает условия для протекания метаболизма. Таким образом, влияние алиментарной депривации сопровождалось появлением морфологических признаков повышения активности как экзокринной, так и эндокринной (в большей степени) функции поджелудочной железы крыс.*

Ключевые слова: *алиментарная депривация, поджелудочная железа, морфометрия.*

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