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L-tryptophan effectively prevents fatty degeneration of rat pancreas

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Alimentary obesity is a risk factor for the development of many pathophysiological conditions in various organs, including the pancreas. Thus, the study of mechanisms, clinical symptoms and ways to prevent the development of fatty degeneration of pancreas at obesity is a current direction of research. The aim of our work was to study the influence of L-tryptophan on the morphofunctional changes of the pancreas of rats with diet-induced obesity and to evaluate the possibility of its use for the prevention of the development of the gland fatty degeneration. The study was conducted in male Wistar rats, which were 3 months old at the experiment beginning. Histologic preparations were made from pancreas tissue samples using a standard method. Morphometric measurements were performed on digital images using "Image J" software. In biochemical studies, we determined concentration of glucose in blood serum and of triglycerides, lipids and cholesterol in pancreas tissue samples. It was found that rats fed a high-fat, high-carbohydrate diet showed marked sings of developing alimentary obesity. This was evidenced by a significant increase in the weight of visceral fat (by 147%) and obesity index (by 129%). The exposure of rats to a high-calorie diet resulted in the emergence of distinct signs indicating hypofunction in both the exocrine and, to a greater extent, endocrine sections of the pancreas. The administration of L-tryptophan reduced the intensity of accumulation of visceral fat and fat in the gland itself. This was evidenced by lower concentrations of lipids (by 53%) and triglycerides (by 32%) in the pancreatic tissue compared to high-calorie diet rats. In addition, L-tryptophan prevented an excessive decrease in the function of both the exocrine and endocrine parts of the gland from the harmful effects of dietary obesity. This may be of practical interest when using tryptophan and its derivatives in the clinic to prevent a decrease in gland activity in this pathology.
Key words: L-tryptophan; alimentary obesity; pancreas.

INTRODUCTION

Obesity is considered an epidemic of the 21st century and is a metabolic risk factor for non-communicable diseases such as: cardiovascular disease, type 2 diabetes, metabolic syndrome, hypertension, some cancers, etc. [1, 2]. Therefore, the prevention and treatment of obesity represent an important societal problem. In the modern era, obesity is mainly associated with various factors, primarily, such as consumption of foods with a high glycemic index, a sedentary lifestyle, chronic stress, changes in sleep patterns, etc. [3].

Alimentary obesity (AO) is a risk factor for the development of many pathophysiological conditions in various organs, including the pancreas [4]. Recently, the term "non-alcoholic fatty pancreas disease" (NAFPD) has been introduced, which leads to the development of steatopancreatitis, and subsequently to pancreatic oncology [5, 6]. Histologically, NAFPD is a heterogeneous process characterized by increased intracellular accumulation of lipids and adipocytes infiltration of pancreatic tissue. Under conditions of oxidative stress, cytokines are released from fat, leading to local inflammation and dysfunction of the pancreas. NAFPD may increase the risk of developing pancreatitis (and increase its severity), β-cell dysfunction, and type II diabetes [7, 8]. It is believed that steatosis of the pancreas is combined with fibrosis of this organ [9]. Therefore, investigating the pathophysiological mechanisms, clinical symptoms, and preventive

strategies for fatty degeneration of the pancreas with AO is a current direction of research.

The literature analysis and our previous studies' results lead to the assumption that one of the methods of preventing the decrease in the functional activity of the pancreas at AO can be the use of the essential amino acid tryptophan. As it is known, tryptophan is a precursor of many biologically active substances, such as: kynurenine, serotonin, niacin, melatonin, indoles, etc., which play an important role in the regulation of the function of the main organs and systems of the human and animal body in normal and various pathological conditions [10]. In this context, it is appropriate to study the overall effect of the influence of these different tryptophan metabolites on the development of NAFPD and the mechanisms underlying the dominance of one or another effect. Taking into account the fact that under natural conditions a person consumes products containing the amino acid tryptophan, and not its individual metabolites, we thought it logical to start the research with this amino acid.

There is information in the literature that tryptophan has a protective effect on the pancreas, stimulates its secretory activity, and has an antitumor effect. Thus, it has been found that intravenous administration of L-tryptophan leads to a significant increase in pancreatic amylase synthesis and activation of duodenal-pancreatic reflexes. Tryptophan has been shown to activate the apoptosis signal pathway and stimulate heat shock proteins in pancreas cancer cells. L-tryptophan reduced the severity of acute pancreatitis and protected pancreatic tissue from damage caused by acute inflammation [11]. However, the effect of tryptophan on the histomorphologic changes of the pancreas and on the indicators of its lipid metabolism at AO remains little studied. All this requires a more detailed study of the role and mechanisms of the tryptophan effect on the pancreas with existing signs of obesity.

The aim of our work was to study the effect of L-tryptophan on the morphofunctional changes of the pancreas of rats with diet-induced obesity and to evaluate the possibility of its use in the prevention of the development of fatty degeneration of the gland.

METHODS

The study was conducted in 36 male Wistar rats (12 animals in each group), which were taken into the experiment at the age of 3 months. The rats were divided into 3 groups: I - control; II - rats fed a high-calorie diet (HCD) for 12 weeks; III - animals fed HCD and additionally received L-tryptophan ("Ajinomoto Eurolysine S.A.S", France) in a dose of 80 mg/kg of body weight. AO in rats was modeled by placing them on a diet high in fat (45%) and carbohydrate (31%) for 12 weeks. Each rat received 6 g of a specially prepared granulated diet (70% standard compound feed with the addition of 30% pork lard); 6.8 g of pork lard; 3.6 g of white bread crumbs; 3.6 g of sunflower seeds; this totaled 116 kcal. The experimental animals received the food *ad libitum* under daily monitoring of the completeness of its consumption. One day later, experimental rats received a 10% fructose solution instead of water [12]. Rats of the control group received 20 g of standard feed daily with a caloric content of 66 kcal. At the experiment end, rats were sacrificed by decapitation under light ether anesthesia on the day after the last dose of L-tryptophan. Visceral fat and pancreas were isolated from rats and their weight was determined using a gravimetric method.

The work with rats was performed in accordance with the terms of the "European Convention on the Protection of Vertebrate Animals Used for Experimental and Other Scientific Research" (Strasbourg, 1986) and the requirements of the Biomedical Ethics Committee of Bogomoletz Institute of Physiology.

For histomorphologic investigations, tissue samples were randomly extracted from the pancreas body. Histologic preparations were made according to the standard method: fixation in Buen's liquid, dehydration in

progressively concentrated alcohols and dioxane. The obtained samples were then embedded in paraffin. Subsequently, paraffin sections with a thickness of 6 μm were prepared using a sliding microtome. These sections were later stained with hematoxylin and eosin using the Van Gieson method [13].

Photographs of the micropreparations were taken using a "Levenhuk" digital camera (USA) mounted on a "Nikon Eclipse E100" microscope (Japan). In the gland exocrine part, the diameter and cross-sectional area of the acinus, the height and area of exocrinocytes, their nucleus and cytoplasm were measured. The number of nucleolus (per 100 nucleuses of exocrinocytes) and the average number of cells in the acinus were counted. In the gland endocrine part, the average number of pancreas islets per unit area (0.25 mm^2) and the number of endocrinocytes placed in them were calculated, the cross-sectional area and diameter of the islets were measured, and the cells density was determined. The relative area of the exo- and endocrine part, as well as the gland connective tissue was determined by the method of superimposing point morphometric grids [14]. Morphometry was performed using "Image J 1.34p" software.

Tissue samples (100 mg) were taken from the pancreas, cleaned from visceral fat, washed with distillate, homogenized, poured with a chloroform-methanol mixture. The filtered extract was used to determine the concentration of triglycerides, lipids and cholesterol by the colorimetric-enzymatic method with standard sets of reagents ("Filisit-Diagnostika", Ukraine) on a biochemical analyzer ("Sinnowa", China). Also, we determined glucose concentration in blood serum.

The data obtained were statistically analyzed processed using "Statistica 6.0 for Windows" (StatSoft, USA) and "Excel 2010" (Microsoft, USA) software. Groups were analyzed by one-way analysis of variance followed by the Bonferroni correction with a significance level of $P < 0.05$. The data had normal distribution and are presented as mean ± SEM.

RESULTS AND DISCUSSIONS

Subjecting rats to HCD for 12 weeks (group II) resulted in the onset of AO. This was evidenced by a substantial increase in visceral fat weight by 147% and the visceral obesity index (the ratio of fat to body weight) by 129% compared to the control (group I). The pancreas weight in these rats exhibited a marked tendency to decrease. With the addition of L-tryptophan (group III), the weight of visceral fat was significantly by 39% lower than that observed in group II (Table 1).

In the experimental groups, the pancreas had a preserved physiological structure divided into the exocrine and endocrine parts. The exocrine part formed the main part of the gland and was represented by acinuses and ducts. The acinuses shape was quite diverse: round, oval, oblong-elongated. Acinuses from the middle were lined with exocrinocytes of various shapes. The cells cytoplasm was granular, especially towards the apical pole. The nucleus was located at the base, where the granularity was less pronounced and contained nucleolus. The acinuses were grouped into lobes, externally covered by a connective tissue membrane, which was a loose interweaving of thin bundles of elastic and collagen fibers. In the gland exocrine part of

Table 1. Weight of visceral fat and pancreas

Indicators	Control	High-calorie diet	High-calorie diet + L-tryptophan
Visceral fat weight, g	18.9 ± 1.4	46.7 ± 2.6*	28.5 ± 1.3*,**
Index of visceral obesity	0.045 ± 0.005	0.103 ± 0.010*	0.075 ± 0.006*,**
Pancreas weight, g	0.73 ± 0.03	0.66 ± 0.03	0.65 ± 0.03

Note: here and in Table. 2 *$P < 0.05$ compared with the control, **$P < 0.05$ compared with the rats that were on a high-calorie diet.

group II rats, we observed a local accumulation of large fat droplets (with an average area of 2200 μm^2). In L-tryptophan treated rats, the number of fat droplets was significantly smaller than in group II animals and they were localized individually (Fig. 1).

In the pancreas exocrine part of HCD rats, the mean diameter and area of acinuses were significantly smaller by 16 and 33%, respectively, than in the control. The area of exocrinocytes, their nucleus and cytoplasm were also significantly by 19, 18 and 19% smaller, respectively. The height of the acinar epithelium was significantly lower by 21%. The number of nucleolus in the nucleus of exocrinocytes was by 16% lower ($P < 0.05$) than in the control (Table 2). Hypoplasia of the nucleolus may be one of the signs of inhibition of the cell protein synthetic function or a decrease in physiological regeneration at the intracellular level [15]. Taken together, these morphometric changes indicate a decrease in the activity of the pancreas exocrine part.

Obesity, accompanied by hyperlipidemia, contributes to the development of fat infiltration of the pancreas [16]. Consumption of fatty foods leads to overproduction of pancreatic enzymes, increased leakage of pancreatic juice with subsequent exhaustion of the pancreas exocrine function. This increases the risk of developing both acute and chronic pancreatitis and pancreatic cancer [9].

Less pronounced changes in the pancreas exocrine part structure were observed in group III. In these rats, the area of acinus was by 22% larger, the area of exocrinocytes and their cytoplasm located in them by 16 and 18%, respectively, and the number of nucleolus in the nucleus of cells was by 46% higher than in group II animals (Table 2). That is, L-tryptophan reduced the negative effects of HCD on the gland exocrine part morphofunctional state.

A decrease in the activity of the pancreas exocrine part in AO rats can be indicated by an increase in the amount of connective tissue (CT). This is evidenced by a significant increase in the relative area of CT in the gland (by 39%), the stromal-parenchymal index (by 48%), and the width of the interlobular CT layers (by 36%) compared to the control. The administration of

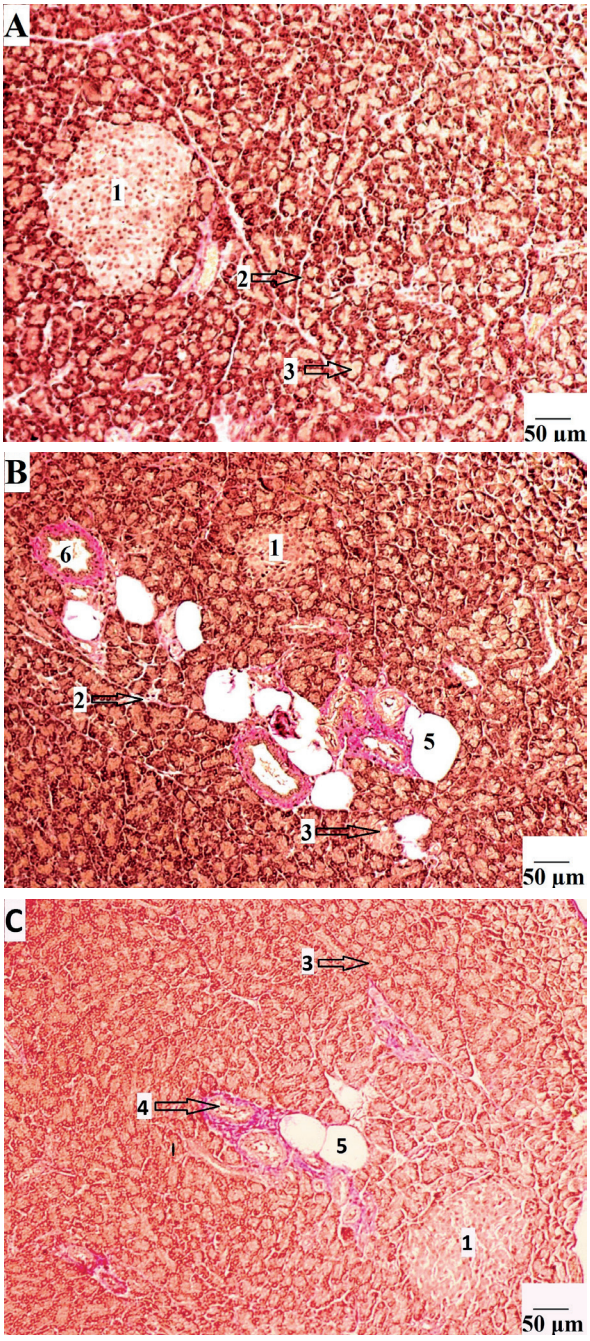

Fig. 1. Sections of the pancreas of a control rat (A), after exposure to a high-calorie diet (B) and administration of L-tryptophan at high-calorie diet (C). Van Gieson staining. 200×. 1 - Langerhans islet; 2 - interlobular connective tissue; 3 - acinus; 4 - blood vessel; 5 - fat drop; 6 - duct

L-tryptophan did not lead to significant changes in the pancreas CT state compared to animals in group II (Table 2). CT is the main component of the histohematic barrier, and the increase in the thickness of its layers inhibits the transport of oxygen to the parenchymal elements of the gland, worsens the conditions for metabolic processes, and reduces the penetration of hormones through the histohematic barrier into the blood.

The pancreas endocrine part in the control animals was much smaller, occupying about 5% of the total area of the gland tissue. It was formed by Langerhans islets scattered throughout the gland. The islets were separated from the acinuses by a thin layer of CT and were clusters of round-shaped endocrinocytes penetrated by a dense network of capillaries (Fig. 1).

In the gland endocrine part in group II rats, significant signs of suppression of its functional state were observed. The relative area of the pancreas endocrine part was by 25% less than in the control. Also, the cross-sectional area and diameter of the Langerhans islets and the number

Table 2. Morphometric indicators of the pancreas structure

Indicators	Control	High-calorie diet	High-calorie diet + L-tryptophan
Exocrine part			
Relative area, %	77.7 ± 1.5	72.3 ± 2.1	70.4 ± 2.0
Acinus diameter, μm	30.6 ± 0.8	25.7 ± 0.3*	7.6 ± 1.1
Acinus area, μm^2	933 ± 14	629 ± 22*	773 ± 15*,**
Area, μm^2			
exocrinocyte	123.9 ± 4.4	100.7 ± 3.5*	116.6 ± 4.9**
nucleus	18.8 ± 0.5	15.5 ± 0.7*	16.1 ± 0.9*
cytoplasm	105.1 ± 4.0	85.2 ± 2.1*	100.5 ± 2.2**
Nuclear-cytoplasmic relationship	0.179 ± 0.004	0.182 ± 0.004	0.160 ± 0.005
Number of exocrinocytes in the acinus	7.5 ± 0.1	7.0 ± 0.2	7.1 ± 0.2
Number of nucleolus in the nucleus	1.56 ± 0.04	1.31 ± 0.06*	1.91 ± 0.08*,**
Epithelium height of the acinus, μm	12.2 ± 0.2	9.6 ± 0.5*	10.5 ± 0.5*
Endocrine part / Langerhans islets			
Relative area, %	5.1 ± 0.4	3.8 ± 0.5*	4.8 ± 0.5**
Number of islets / 0.25 mm^2	1.12 ± 0.10	1.11 ± 0.50	1.08 ± 0.07
Islets area, μm^2	14653 ± 153	8250 ± 105*	10858 ± 189*,**
Islets diameter, μm	111.2 ± 9.1	84.7 ± 6,3*	99.4 ± 3.7**
Number of endocrinocytes in the islet	189.2 ± 18.1	117.8 ± 5.7*	143.8 ± 10.5*,**
Density of endocrinocytes placement in the islets / 1000 μm^2	12.9 ± 0.5	14.3 ± 0.4	13.2 ± 0.5
Connective tissue			
Relative area, %	17.2 ± 1.7	23.9 ± 1.8*	24.8 ± 1.1*
Stromal-parenchymal index	0.21 ± 0.02	0.31 ± 0.01*	0.33 ± 0.04*
Width of the layers, μm			
interlobular	9.0 ± 0.6	12.2 ± 0.7*	9.5 ± 0.4**
interacinus	0.84 ± 0.02	0.9 ± 0.01	0.86 ± 0.02

of endocrinocytes located in them were smaller by 44, 24, and 38%, respectively (Table 2).

A decrease in the functional activity of the pancreas endocrine part in AO rats was indicated by a significant increase in the glucose concentration in blood serum to 6.8 ± 0.2 mmol/l in comparison with 5.5 ± 0.2 mmol/l in the control (increased by 25%). Thus, rats exposed to the HCD influence experienced notable disruptions in carbohydrate metabolism, resulting in the development of pronounced hyperglycemia.

The disturbance of blood glucose levels is a well-known reason for hyperglycemia and diabetes. Glucose homeostasis is maintained by the synthesis of insulin and glucagon, regulated by the pancreatic β- and α-cells, respectively. In obesity, a vicious cycle emerges, characterized by hyperinsulinemia and insulin resistance. This persistent hyperinsulinemia contributes to the depletion of pancreatic β-cells, leading to the onset of type II diabetes [17].

It was found that L-tryptophan prevented the excessive decrease in the pancreas endocrine function by HCD consumption. This was evidenced by a significantly larger relative area of the endocrine part by 26%, the area and diameter of the Langerhans islets by 32 and 17%, respectively, and the number of endocrinocytes placed in them by 22% as compared to group II (Table 2). In group III rats, the concentration of glucose in blood serum was 15% ($P < 0.05$) lower than in group II animals.

It was found that the concentration of total lipids, triglycerides, total cholesterol, and high-density cholesterol in the pancreas of HCD rats was significantly higher by 235, 32, 90, and 155%, respectively, compared to the control. This indicates a violation of lipid metabolism and the development of fatty liver steatosis. In group III, a significantly lower concentration of lipids (by 53%), triglycerides (by 32%) and high-density cholesterol (by 67%) was found compared to group II animals (Fig. 2). That is, tryptophan reduces the severity of lipid metabolism disorders in AO, this prevents excessive accumulation of fat in the gland.

In the studies of other authors, conducted in animals fed a high-fat diet for a long period of time, the accumulation of fat in the pancreas with the development of inflammation, fibrosis and insulin resistance was found [18]. In a study of pigs fed HCD for 24 weeks, steatosis of the pancreas was also observed, as evidenced by an increase in the amount of fat in the total area of the tissue. At the same time, there was no significant damage to the cells of the gland and the number of Langerhans islets increased [5].

Previously, we studied the effect of L-tryptophan on the histomorphometric indicators of the pancreas in healthy young rats. We observed

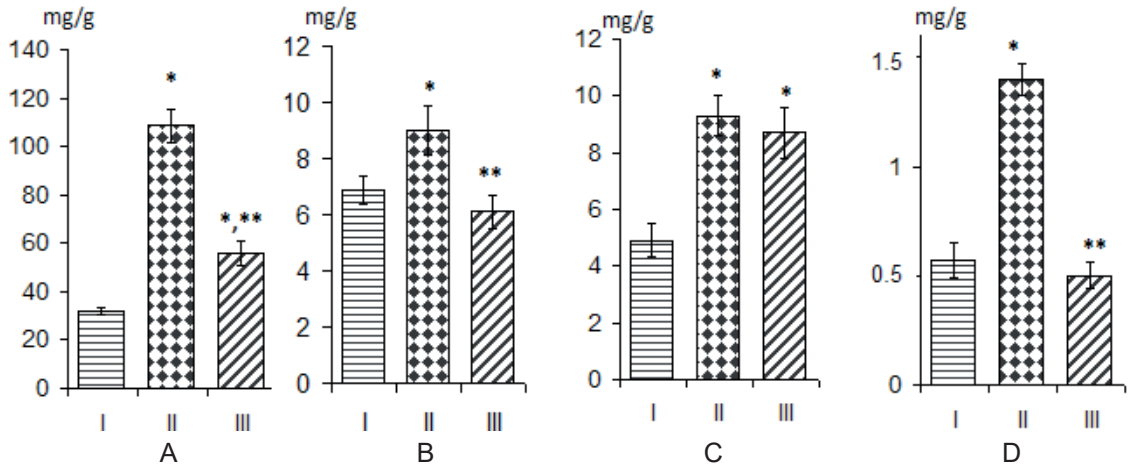

Fig. 2. Concentration of total lipids (A), triglycerides (B), total cholesterol (C) and high-density cholesterol (D). I - control; II - high-calorie diet; III - high-calorie diet + L-tryptophan. *$P < 0.05$ compared with the group I, **$P < 0.05$ compared with the group II

slight morphometric signs of decreased activity of its exocrine part. However, after the exposure to L-tryptophan, the endocrine activity of the pancreas did not undergo significant changes. At the same time, the administration of L-tryptophan significantly reduced the amount of CT in the pancreas, which is relevant for practical medicine, regarding the use of this amino acid in the prevention and complex treatment of glandular fibrosis [19].

The main effects of tryptophan in NAFPD are determined by the specific physiological properties of its metabolites, mainly serotonin and melatonin. Unlike tryptophan, they do not penetrate the blood-brain barrier. In this regard, the influence of serotonin, produced in the central nervous system and in the gastrointestinal tract, on the processes of obesity development and on individual links of the pathogenesis of NAFPD differs significantly [20].

Currently, there remains no consensus regarding the mechanism underlying the beneficial impact of tryptophan on the pancreas. Consequently, it is believed that the effect of tryptophan in preventing fatty degeneration of the pancreas and reducing its functional activity in AO may be attributed to multiple factors. These factors include the reinforcement of antioxidant protection within pancreatic tissue through the direct elimination of toxic radical oxygen and nitrogen, preservation of the activity of antioxidant enzymes such as superoxide dismutase, catalase, or glutathione peroxidase, reduction in the production of the pro-inflammatory cytokine tumor necrosis factor α, accompanied with the stimulation of the anti-inflammatory IL-10, enhancement of peripheral blood flow, reduction in neutrophil infiltration, reduce of apoptosis and necrosis in the inflamed tissue of the gland, and promotion of the regenerative process [11, 21-23].

CONCLUSIONS

In conclusion, L-tryptophan may be considered as one of natural agents for protecting the pancreas from the negative consequences of visceral obesity and accelerating its regeneration. It has protective properties and helps to reduce the degree of manifestation of pancreatic dysfunction and the accumulation of fat in the pancreas with AO. The beneficial effect observed in our experimental studies suggests the perspective of further investigations into the viability of employing tryptophan in clinical practice for the correction and prevention of NAFPD in diet-induced obesity.

The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of co-authors of the article.

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ЕФЕКТИВНІСТЬ ЗАСТОСУВАННЯ L-ТРИПТОФАНУ ДЛЯ ЗАПОБІГАННЯ РОЗВИТКУ ЖИРОВОГО ПЕРЕРОДЖЕННЯ ПІДШЛУНКОВОЇ ЗАЛОЗИ ЩУРІВ

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Аліментарне ожиріння є фактором розвитку багатьох патофізіологічних станів різних органів, у тому числі підшлункової залози (ПЗ). Тому актуальним напрямом досліджень є вивчення механізмів, клінічних симптомів та шляхів запобігання розвитку жирової дистрофії ПЗ при ожирінні. Метою роботи було дослідження впливу L-триптофану на морфофункціональні зміни ПЗ щурів з аліментарноіндукованим ожирінням та оцінювання можливості його використання для профілактики розвитку жирового переродження залози. Дослідження проведено на щурах-самцях лінії Вістар, вік яких на початку експерименту становив 3 міс. З тканини ПЗ виготовляли гістологічні препарати за стандартною методикою. Морфометрію здійснювали на цифрових зображеннях за допомогою комп'ютерної програми «Image J». Біохімічними методами в сироватці крові визначали концентрацію глюкози, а в тканині залози – концентрацію тригліцеридів, ліпідів та холестерину. Виявлено, що щури, які перебували на дієті з високим вмістом жирів і вуглеводів, мали виражені ознаки розвитку аліментарного ожиріння. Про це свідчило вірогідне збільшення маси вісцерального жиру (на 147%) та індексу ожиріння (на 129%). Було відмічено гіпофункцію екзокринної та ендокринної (більшою мірою) частини

ПЗ. Введення L-триптофану зменшувало інтенсивність накопичення вісцерального жиру та жиру в самій залозі. Слід відмітити також меншу концентрацію ліпідів (на 53%) та тригліцеридів (на 32%) у тканині ПЗ порівняно зі значеннями у щурів, які знаходилися на висококалорійному раціоні. Також дія L-триптофану перешкоджала надмірному зниженню функціонування як екзокринної, так і ендокринної частини залози від пагубного впливу аліментарного ожиріння. Це може мати практичний інтерес при використанні такої кислоти та її похідних у клініці для запобігання зниження активності залози при цій патології.
Ключові слова: L-триптофан; аліментарне ожиріння; підшлункова залоза.

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