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Research Article

Age-related Differences in the Effect of L-Methionine on Histo-Morphometric Indicators of Pancreas Activity in Rats

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Abstract

Introduction: With age, the pancreas, like most other organs, undergoes a decrease in functional activity. Therefore, the search for methods that could activate the pancreas, if necessary, is an actual direction of research. One of these methods can be the use of the amino acid methionine, or preparations based on it.

Aim: To investigate the histo-morphological changes of the rat's pancreas of various ages after the administration of L-methionine to them and to evaluate the prospects of its use as a means to correct pronounced age-related decreases in its function.

Materials and Methods: Experiments were performed on 48 male rats aged 3 and 15 months. Experimental animals, in addition to the standard diet, received L-methionine at a dose of 250 mg/kg of body weight every day for 21 days. Histological preparations were made from pancreas tissue according to the standard method. Morphometry was carried out using the computer program "Image J". 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).

Results: At the end of the experiment, 15-month-old rats receiving L-methionine showed morphometric signs of increased exocrine functional activity (the area of acinus and the height of their epithelium increased, the nuclear-cytoplasmic ratio of exocrinocytes, and the number of nucleolus in cell nucleus increased, the amount of connective tissue decreased), as well as the endocrine part of the pancreas (the size of the Langerhans islets and the number of endocrinocytes placed in them increased). In 3-month-old animals, on the contrary, methionine reduced the activity of the endocrine part of the gland and almost did not change the histo-morphometric indicators of the exocrine part.

Conclusion: The obtained data can be used for the correction of pronounced age-related decreases in the function of the pancreas in adults and the elderly, as well as in the prevention of age-related chronic diseases of the gland. At the same time, receiving additional doses of methionine at a young age is not desirable.

Keywords: Pancreas; Histo-morphometry; Methionine; Age

Introduction

Aging of the body is a hereditarily programmed biological phenomenon that leads to the limitation of the adaptive capabilities of the body and the development of age-related pathology. Aging is accompanied by serious involutional reorganization of organs, including the pancreas [1]. Age-related destruction of the pancreas begins already after 40 years and consists in the development of changes in the vessels of the organ with their narrowing or obliteration, sclerosis of the ducts walls, their obstruction, proliferation of the epithelium, replacement of secreting cells by connective tissue with the appearance of interlobular and interacinus fibrosis [2]. The total amount of acinar tissue of the pancreas decreases to 30-40%. Involutional changes lead to a decrease in the enzymatic activity of the pancreas and the volume of pancreatic juice. The inten-

sity and quality of digestion deteriorates: incomplete digestion of proteins, fats, and carbohydrates occurs [3]. In addition, with age, there is a decrease in the number and activity of β-cells of the islet apparatus of the pancreas and the number of insulin receptors, which leads to the development of insulin resistance with subsequent metabolic disorders [4,5]. Therefore, the search for methods and ways that could, if necessary, stimulate the activity (lost with age) of pancreas is an actual direction of research. One of the methods of normalizing the physiological functions of the pancreas can be the use of sulfur-containing compounds, primarily methionine.

The influence of methionine on the state of the pancreas in pathology, as well as the effectiveness of its use for the correction of already existing disorders, were mainly studied [6,7]. At the same time, the question of the use of methionine in healthy individuals with age-related reduced pancreas function remains poorly researched. Until now, the question of how pronounced the effect of is using methionine to increase the functional activity of the pancreas in the elderly remains open.

The aim of the study is to investigate the histo-morphological changes of the rat's pancreas of various ages after the administration of L-methionine to them and to evaluate the prospects of its use as a means to correct pronounced age-related decreases in its function.

Materials and Methods

The study was conducted on 48 male Wistar rats aged 3 and 15 months. Rats were divided into 4 groups (12 animals each): I and III – control animals 3 and 15 months old, respectively; II and IV – experimental rats of young and adult age, which received an additional oral dose of L-methionine at the rate of 250 mg per 1 kg of body weight. Such a dose of methionine can be considered preventive, as it does not lead to a significant increase in its content in the body and the occurrence of homocysteinemia. At the same time, it is sufficient to correct a possible amino acid deficiency in the body under the influence of various adverse factors of the external environment to the values of the physiological norm. In order to avoid the occurrence of stress during forced administration of methionine to animals, the drug was injected into food (cheese mass) with visual control of its complete consumption. Rats of the control group received a similar portion of cheese mass without methionine. The animals of both groups were housed in uniform conditions with a standard diet and free access to water. The duration of the experiment was 21 days.

Research with animals was conducted in accordance with the national «Common Ethical Principles of Animal Experiments» (Ukraine, 2001), which are consistent with the provisions of the «European Convention for the Protection of Vertebrate Animals Used 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. 17 dated 31/10/17).

Histological, morphometric, biochemical and statistical research methods were used. 5 tissue samples were taken from the body of the pancreas of each rat, from which histological preparations were made according to the standard method: 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, 5-6 μm thick, were made on a sled microtome, stained with Boehmer's hematoxylin and eosin. To visualize the elements of connective tissue, the Van-Gieson staining method was used [8]. With the use of a digital camera, micropreparations were photographed on a "Nikon ECLIPSE E100" microscope (Japan). Morphometry was carried out using the computer program "Image J".

Histo-morphometry of exo- and endocrine part was performed on the histological sections of the pancreas tissue. In the exocrine part of the gland, the diameter and cross-sectional area of acinus, the height and area of exocrinocytes, their nucleus and cytoplasm were measured, the number of nucleolus in the nucleus of exocrinocytes and the average number of cells in the acinus were counted. In the endocrine part of the gland, the average number of pancreas islets per unit area (0.25 mm^2) and the number of endocrinocytes were calculated, the cross-sectional area and diameter of the islets were measured, and the density of the cells was determined. The relative area of the exo- and endocrine part, as well as the connective tissue in the gland was determined by the method of superimposing point morphometric grids [9,10].

The concentration of glucose 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 ("Sinnowa", China).

Statistical processing was carried out by methods of variational statistics using the computer program "Statistica 6.0". The normality of the distribution of digital arrays was checked using the Pearson test. Data are reported as the mean ± SD when normally distributed. Groups were analyzed by one-way analysis of variance followed by Bonferroni t-tests with a significance at $p < 0.05$.

Results and Discussion

It was found that both the absolute and the relative weight of the pancreas of 3-month-old rats receiving L-methionine tended to decrease, which may indicate a ingibition in its secretory activity [11]. The pancreas weight of 15-month-old experimental rats remained unchanged.

The structure of the pancreas of rats receiving L-methionine preserved the physiological structure and was well differentiated into exo- and endocrine parts. The exocrine part made up the bulk of the gland and was represented by ducts and acinus, which had a variety of shapes: rounded, oval or elongated. The composition of acinus included exocrinocytes of various shapes, and their cytoplasm had pronounced granularity. The nucleus was located at the base and contained a nucleolus. The endocrine part of the pancreas occupied a small area and was represented by Langerhans islets, mostly round and oval in shape (Figure 1).

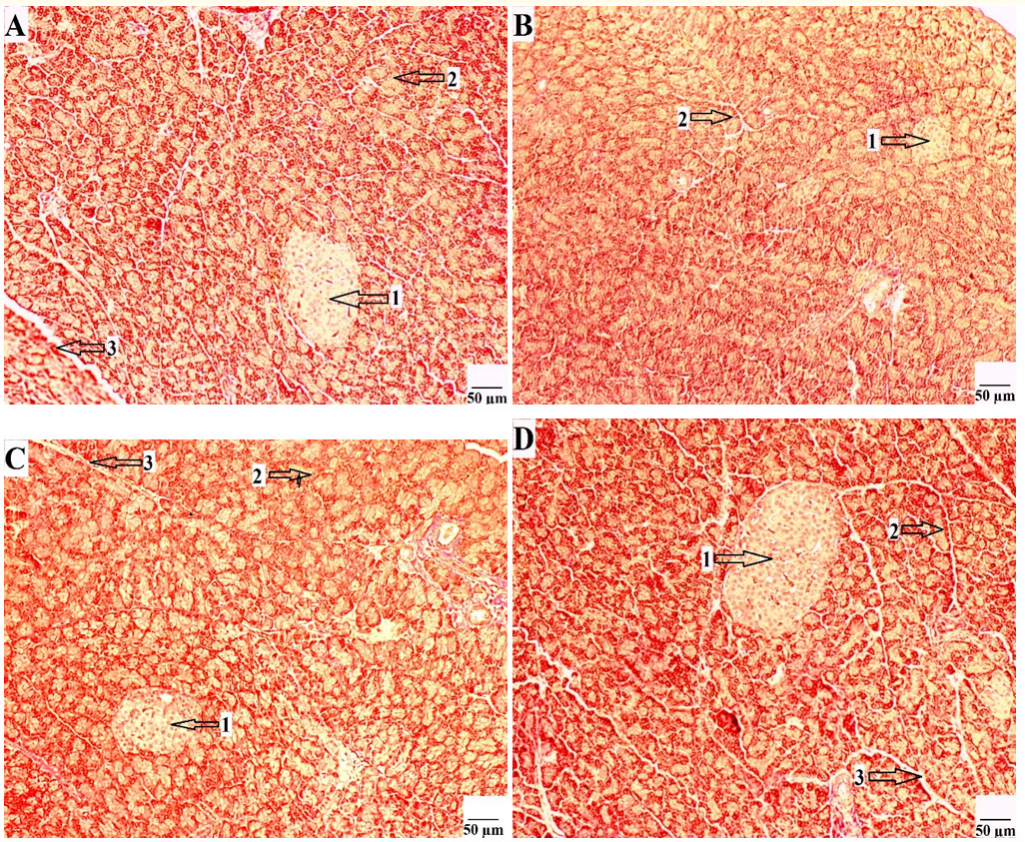

Figure 1: Photomicrograph of the rat's pancreas of the control group (A-3 months old, C-15 months old) and the experimental group after exposure to L-methionine (B-3 months old, D-15 months old).

Note: 1-Langerhans islet; 2-acinus; 3-interlobular connective tissue. Van Gieson staining. x200.

When comparing the histo-morphometric indicators of the pancreas of control rats, a decrease in its activity with age was revealed. Thus, in the exocrine part of the gland of 15-month-old rats, the area of acinus was smaller by 27% ($p < 0.05$) and the area of exocrinocytes by 13% ($p < 0.05$) than in 3-month-old rats. The endocrine part of the pancreas underwent even greater age-related changes. Thus, in adult animals, a smaller relative area of the endocrine part (by 32%, $p < 0.05$), a less average number of Langerhans islets (by 22%, $p < 0.05$), their area (by 31%, $p < 0.05$) and the number of endocrinocytes placed in them (by 18%, $p < 0.05$) was noted compared to young rats. Also, in the pancreas of adult rats, a larger amount of connective tissue was observed, namely: a greater its relative area by 20% ($p < 0.05$) and a stromal-parenchymal index by 28% ($p < 0.05$), a greater width of interlobular and interacinus connective tissue layers by 21 and 30% ($p < 0.05$), respectively, than in young animals (Table 1). This corresponds to the general regularity of a decrease in the activity of pancreas with age [2,3].

Indicators	3 month old rats		15 month old rats	
	Control	Experiment	Control	Experiment
Exocrine part				
Relative area, %	76.1 ± 1.5	78.3 ± 1.4	73.2 ± 1.7	76.7 ± 1.5
Acinus diameter, **µm**	30.3 ± 0.9	29.9 ± 0.6	27.7 ± 0.7	28.5 ± 0.5
Acinus area, **µm**^{2}	952 ± 20	854 ± 29	692 ± 16^	788 ± 30*
The height of the epithelium of the acinus, **µm**	12.4 ± 0.3	12.2 ± 0.3	11.2 ± 0.2	12.6 ± 0.2*
Area, **µm**^{2}:				
exocrinocyte	139 ± 7	126 ± 5	121 ± 6^	126 ± 5
nucleus	19.1 ± 0.9	18.5 ± 0.7	17.0 ± 0.4	20.6 ± 0.9*
cytoplasm	119.9 ± 3.0	107.5 ± 4.5	104 ± 3^	105.4 ± 4.5
Nuclear-cytoplasmic ratio	0.159 ± 0.003	0.172 ± 0.002	0.163 ± 0.003	0.195 ± 0.004*
The number of nucleolus in the exocrinocyte, pcs	1.57 ± 0.05	1.74 ± 0.06	1.48 ± 0.05	1.78 ± 0.05*
The number of exocrinocytes in the acinus, pcs	8.7 ± 0.3	8.1 ± 0.1	7.8 ± 0.2	7.6 ± 0.1
Endocrine part				
Relative area, %	3.8 ± 0.8	3.4 ± 0.4	2.6 ± 0.5^	2.7 ± 0.3
The number of islets (by 0.25 mm^2), pcs	1.15 ± 0.01	0.97 ± 0.01*	0.90 ± 0.02^	0.99 ± 0.09
Islet area, µm^2	13816 ± 121	11170 ± 195*	9538 ± 92^	11621 ± 182*
Islet diameter, **µm**	114.1 ± 5.1	108.7 ± 6.8	93.8 ± 2.9^	105.1 ± 2.6*
The number of endocrinocytes in the islet, pcs	145.9 ± 9.6	158.2 ± 14.5	119.8 ± 6.6^	165.0 ± 10.8*
Density of placement of endocrinocytes in the islet, pcs./1000 µm^2	10.6 ± 0.9	14.2 ± 0.8*	12.6 ± 0.9	14.2 ± 0.6
Connective tissue				
Relative area, %	20.1 ± 0.9	18.3 ± 1.1	24.2 ± 0.5^	20.6 ± 0.6*
Stromal-parenchymal index	0.25 ± 0.03	0.22 ± 0.02	0.32 ± 0.02^	0.26 ± 0.03*
The width of the layers connective tissue, **µm**	3.16 ± 0.21	2.73 ± 0.36*	3.81 ± 0.12^	3.15 ± 0.31*
interlobular	0.74 ± 0.02	0.63 ± 0.01*	0.96 ± 0.02^	0.67 ± 0.01*
interacinous				

Table 1: Histo-morphometric indicators of the pancreas (Mean ± SD).

Note: * $p < 0.05$-probability compared to the control; ^ $p < 0.05$- probability compared to the control of 3-month-old rats.

In 15-month-old rats that received L-methionine, the area of pancreas acinus was 14% ($p < 0.05$) larger than in control animals. The area of exocrinocyte nucleus was also larger (by 17%, $p < 0.05$), which led to an increase in the nuclear-cytoplasmic ratio by 12% ($p < 0.05$). Hypertrophy of the nucleus and an increase in the nuclear-cytoplasmic ratio indicate, first of all, on stimulate in the functional activity of the cell [12]. The average height of the acinus epithelium in adult experimental animals was 13% ($p < 0.05$), and the number of nucleolus in the nucleus of exocrinocytes was 20% greater ($p < 0.05$) than in control animals (Table 1). Hyperplasia of nucleolus indicates an increase in the protein synthetic activity of exocrinocytes. An increase in the number of nucleolus in the nucleus may also indicate the activation of the processes of physiological regeneration of exocrinocytes at the intracellular level [13]. In the exocrine part of the pancreas of 3-month-old rats treated with L-methionine, histo-morphometric indicators remained close to the control values. However, in these animals, a clearly expressed tendency to decrease the area of both acinus and exocrinocytes was observed (Table 1). Thus, the nature and degree of expressiveness of the changes in the studied morphometric indicators of the pancreas, after the administration of L-methionine, may indicate an increase in the activity of its exocrine part in 15-month-old animals.

Age-related differences in the histo-morphometric indicators of the endocrine part of the pancreas of rats treated with L-methionine were revealed. Thus, in 15-month-old experimental animals, a clearly expressed tendency to an increase in the average number of Langerhans islets, a larger cross-sectional area of the islets (by 22%, $p < 0.05$) and their diameter (by 12%, $p < 0.05$) was noted. The total number of endocrinocytes in the islets was 38% higher ($p < 0.05$) compared to the control. In 3-month-old experimental rats, on the contrary, a decrease in the activity of the endocrine function of the gland was observed. This was evidenced by a decrease in the average number of islets by 16% ($p < 0.05$) and their area by 15% ($p < 0.05$) and a tendency to decrease the relative area of the endocrine pancreas (Table 1, Figure 1). This nature of changes in histo-morphometric parameters may indicate the activation of the endocrine part of the pancreas in adult animals, and a decrease in its activity in young rats after the administration of L-methionine.

A decrease in the functional activity of the endocrine part of the pancreas of young experimental rats was indicated by a increase in the concentration of glucose in the blood serum by 17% ($p < 0.05$) compared to the initial level. In 15-month-old rats, the glucose concentration, on the contrary, decreased by 17% ($p < 0.05$) (Figure 2).

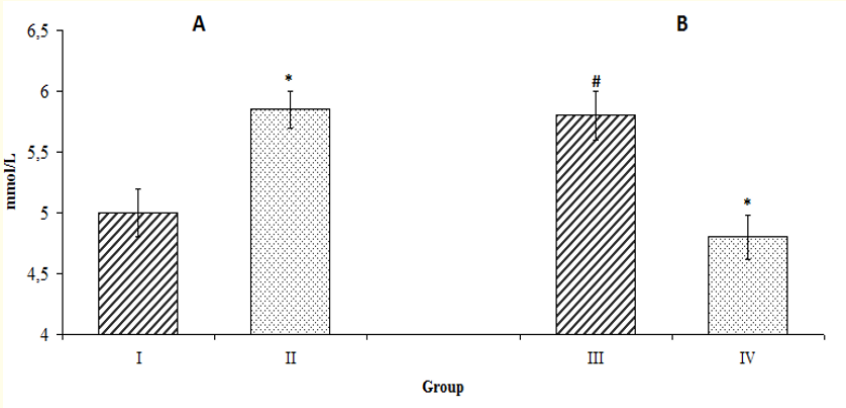

Figure 2: Glucose concentration in blood serum of 3-month-old (A) and 15-month-old rats (B). I and III-control animals 3 and 15 months old, respectively; II and IV-experimental rats of young and adult age, which received a L-methionine. *$p < 0.05$ compared with the control group, #$p < 0.05$ compared to 3-month-old control rats.

The results of our research showed a decrease in the amount of connective tissue in both 3-month-old and 15-month-old (to a greater extent) rats that received L-methionine. Thus, in the tissue of the pancreas of experimental adult rats, the area of the connective tissue and the stromal-parenchymal index were smaller than in the controls, by 14 and 19% ($p < 0.05$), respectively. The width of interlobular and interacinus connective tissue layers in these animals was less by 17 and 30% ($p < 0.05$), respectively. In 3-month-old experimental animals, a decrease in the width of interlobular and interacinus connective tissue layers was also noted by 14 and 15% ($p < 0.05$), respectively, compared to the control (Table 1). A decrease in the number of connective tissue elements in the pancreas tissue can be considered as one of the signs of activation of its function and increase in regenerative capabilities, as well as one of the important factors contributing to the improvement of metabolism between acinus.

In the research of other authors, it was shown that the structure of the pancreas of rats that were on diets with a content of 2 to 4% methionine underwent significant pathological changes, namely: the loss of basophilia by exocrinocytes, vacuolization of the cytoplasm and pyknosis of their nucleus, as well as degeneration and loss of individual exocrinocytes. The structure of the Langerhans islets, however, did not undergo significant changes. It was noted that damage to the structure of the pancreas of rats receiving a diet with a 4% methionine content was more pronounced [14]. Boquist L. conducted research on hamsters, which were administered L-methionine intraperitoneally at a dose of 0.5 g/kg every day for 12 days. When using the method of light microscopy, it was shown that 1 day after the administration of the drug, the structure of the pancreas did not change significantly. After 2 days, only small changes were observed in individual animals. From the 4th day, changes were detected in most hamsters. They were expressed in the swelling of exocrinocytes and a decrease in basophilia. From the 7th day, cytoplasmic vacuoles and eosinophilic bodies appeared. The structure of the acinus changed, which gradually atrophied and were replaced by fatty and fibrous tissue. From the 10th day, cells with large nucleus were detected, goblet cells were mixed in the cells of the ducts, and the number of β-cells decreased in the islets of the pancreas. Electron microscopy showed that from the 4th day there was a loss of the orderly arrangement of the endoplasmic reticulum with expanded cisternae and a decrease in the number of ribosomes and zymogen granules in some cells. Changes in cristae, their swelling and damage were observed in mitochondria. Cytoplasmic bodies appeared in exocrinocytes, mainly of fibrillar nature [15].

In other works, on the contrary, a positive effect of methionine on pancreas was obtained. It was found that the additional administration of methionine into the diet of animals has a stimulating effect on the proteolytic activity of the exocrine part of the pancreas. Thus, it was noted that a small amount of methionine added to feed can increase the secretion of pancreatic amylase [16]. It was shown that the administration of methionine inhibits proliferation and inhibits the cell cycle of BxPC-3 and HPAC cancer cells in pancreas cell culture [7]. This confirms the conclusion of a number of authors that increasing the consumption of methionine can help reduce the risk of developing pancreatic cancer [6,17]. Methionine in combination with selenium has also been investigated to improve markers of insulin function and resistance, such as glucose tolerance, plasma adiponectin, and liver glycogen, demonstrating insulin-mimetic activity in mice with induced diabetes [18]. It was found that methionine can influence the growth, differentiation and viability of pancreas cells. Thus, the study of Parsa., *et al*. on rat pancreas cell culture, it was found that methionine is required for the differentiation of exocrinocytes of the gland [19].

Such ambiguity of the results obtained by different authors in studies with methionine is associated with both the use of animals of different ages in the experiments, and a wide range of differences in the dosage and duration of methionine administration, which determines the feasibility of further research in this direction.

Conclusion

Thus, we found that additional administration of prophylactic doses of L-methionine (250 mg/kg) to healthy adult rats leads to the appearance of clearly expressed morpho-functional signs of increased activity of the exo- and endocrine part of the pancreas. In young animals, on the contrary, methionine reduced the activity of the endocrine part of the gland and almost did not change the histo-morphometric indicators of the exocrine part. The obtained data can be used for the correction of pronounced age-related decreases in the function of the pancreas in adults and the elderly, as well as in the prevention of age-related chronic diseases of the gland. At the same time, receiving additional doses of methionine at a young age is not desirable.

Conflict of Interest

The author declare no conflict interest.

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Bibliography

1. JafariNasabian P., *et al.* "Aging human body: changes in bone, muscle and body fat with consequent changes in nutrient intake". *Endocrinology* 234.1 (2017): R37-R51.

2. Möller K., *et al.* "Pancreatic changes with lifestyle and age: What is normal and what is concerning?" *Endoscopic Ultrasound* 12.2 (2023): 213-227.

3. Löhr JM., *et al.* "The ageing pancreas: a systematic review of the evidence and analysis of the consequences". *Journal of Internal Medicine* 283.5 (2018): 446-460.

4. Tudurí E., *et al.* "The pancreatic β-cell in ageing: Implications in age-related diabetes". *Ageing Research Reviews* 80 (2022): 101674.

5. Kehm R., *et al*. "Age-related oxidative changes in pancreatic islets are predominantly located in the vascular system". *Redox Biology* 15 (2018): 387-393.

6. Larsson SC., *et al.* "Methionine and vitamin B6 intake and risk of pancreatic cancer: a prospective study of Swedish women and men". *Gastroenterology* 132.1 (2007): 113-118.

7. Benavides MA., *et al.* "L-Methionine inhibits growth of human pancreatic cancer cells". *Anticancer Drugs* 25.2 (2014): 200-203.

8. Rehfeld A., *et al.* "Histological Methods. In: Compendium of Histology". *Springer, Cham* (2017).

9. Hossain MA., *et al.* "Histomorphological and morphometric studies of the pancreatic islet cells of diabetic rats treated with aqueous extracts of Momordica charantia (karela) fruits". *Asian Pacific Journal of Tropical Disease* 4.2 (2014): S698-S704.

10. Yanko R., *et al*. "Morpho-functional features of the pancreas in different ages rats with alimentary obesity". *Acta Scientific Gastrointestinal Disorders* 5.11 (2022): 03-09.

11. Capurso G., *et al.* "Exocrine pancreatic insufficiency: prevalence, diagnosis, and management". *Clinical and Experimental Gastroenterology* 12 (2019): 129-139.

12. Koda M. *et al.* "Nuclear hypertrophy reflects increased biosynthetic activities in myocytes of human hypertrophic hearts". *Circulation Journal* 70.6 (2006): 710-718.

13. Dubois ML., *et al.* "The Nucleolus: Structure and function". *The Functional Nucleus*. 23 (2016): 29-49.

14. Kaufman N., *et al.* "Pancreatic damage induced by excess methionine". *Archives of Pathology* 70 (1960): 331-337.

15. Boquist L. "The effect of excess methionine on the pancreas. A light and electron microscopic study in the Chinese hamster with particular reference to degenerative changes". *Laboratory Investigation* 21.2 (1969): 96-104.

16. Hara H., *et al.* "Supplementation of methionine to a low soybean protein diet strikingly increases pancreatic amylase activity in rats". *Journal of Nutritional Science and Vitaminology (Tokyo).* 43.1 (1997): 161-166.

17. He D., *et al. "*Methionine oxidation activates pyruvate kinase M2 to promote pancreatic cancer metastasis". *Molecular Cell* 82.16 (2022): 3045-3060.

18. Ueno H., *et al.* "Effect of seleno-L-methionine on oxidative stress in the pancreatic islets of a short-term induced diabetic mouse model in insufficient selenium status". *Biological and Pharmaceutical Bulletin* 41.1 (2018): 80-85.

19. Parsa I., *et al.* "Pancreas acinar cell differentiation. 3. Importance of methionine in differentiation of pancreas anlage in organ culture". *The American Journal of Pathology* 59.1 (1970): 1-22.