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THE CHARACTERISTIC OF QUALITATIVE COMPOSITION OF THE MATRIX METALLOPROTEINASES IN HOMOGENATES OF TISSUES DURING THE DEVELOPMENT OF EXPERIMENTAL OBESITY IN THE RATS

Obesity is the complex and multifactorial disease that develops with influence of genetic, physiological, socio-economic and environmental factors. This disearse could increase the risk of comorbidities and mortality. Economic growth, automation of many technical processes and urbanization are the reasons of the sedentary lifestyle over the past 30 years, which has led to the widespreading of obesity among the population in 2 or even 4 times. In general, obesity is associated with excessive growth of adipose tissue, the development of which depends on cellular processes related to the population of cells such as adipocytes and their precursors. However, the accumulation of fat mass causes dysfunction of other tissues, which leads to a number of pathological processes. Our study was focused on the enzymatic activity of liver, kidney, and muscle tissue in obese rats. We have determined the changes the enzymatic activity matrix metalloproteinases (MMP) such as MMP-7 and MMP-9 in the experimental tissues. We found the manifestation of the enzymatic activity of MMP-7 in tissues of rats suffered with obesity, while in the healthy rats is not observed such the process. We suggest that this is due to the development of tissue fibrosis. An addition, we also recorded an increase in the enzymatic activity of MMP-9 in muscle tissue rats suffered with obesity. Probably, it links with the regenerative process in the experimental tissue. Further researches of the activity of matrix metalloproteinases in different tissues must provide knowledge about the biochemical processes during obesity which help to create the several right medicines and the development of new methods / approaches to the diagnostic and treatment of this pathology.

Keywords: obesity, metalloproteinases, enzymography.

Introduction. Obesity is a serious chronic disease that is a serious problem for human health. Over the last 30 years, the world has seen an exponential increase in obesity among the population, with obesity among adults and children (6-11 years) doubling, and rates of adolescent (12-19 years) obesity tripling. The accumulation of fat mass depends on the interaction between genetic, metabolic, physiological and environmental factors. Obesity is the cause of diseases such as type 2 diabetes, fatty liver disease, hypertension, myocardial infarction, stroke, dementia, osteoarthritis, obstructive sleep apnea, some cancers. Obesity is also associated with unemployment, social ills, and declining socio-economic productivity. [1,2]. Obesity causes the development of inflammatory processes – circulating concentrations of many inflammatory markers in obese people are much higher than in healthy people. It is believed that the development of inflammatory processes during obesity plays a significant role in the emergence and spread of insulin resistance and other metabolic disorders [3]. Obesity causes the growth of adipose tissue, stimulated by the accumulation of cells – adipocytes. At the same time, adipocytes not only store fat, but are also an active endogenous and paracrine organ that secretes various bioactive mediators that affect body weight homeostasis, insulin resistance, circulating lipid content, blood pressure and coagulation [4,5].

Obesity mainly affects the remodeling of the extracellular matrix due to the growth of adipose tissue, fibrosis, development and rupture of atherosclerotic plaques. A number of regulatory factors and proteolytic enzymes are involved in the process of remodeling the extracellular matrix. Matrix metalloproteinases (MMPs) are major participants in extracellular matrix degradation, and the regulation of their expression and activity plays a crucial role in tissue homeostasis. In general, MMPs are multifunctional proteases that are primarily involved in the cleavage of extracellular matrix components with the subsequent release of bioactive fragments and proteins, are involved in membrane exfoliation, play an important role in chemokine processing and affect the activity of others. This means that changes in MMP expression affect the structure and function of the extracellular matrix, as well as basement membranes, which can lead to the development of pathological conditions [6,7,8]. During obesity, the inflammatory adipokine leptin induces the expression of MMP-2 and MMP-9 in various tissue cells. These MMPs are considered markers of obesity, because they are found in high concentrations in the blood of patients suffering from fatty disorders. In addition, the role of MMPs in the degradation of extracellular matrix components during invasion and metastasis of cancer cells is known. Moreover, the increase in MMP expression is associated with inflammation due to their stimulation of the activity of various stromal cells, such as fibroblasts and leukocytes [9,10,11]. There has also been an increase in MMP-9 in patients with obesity associated with cardiovascular disease. There are a large number of studies that confirm the importance of MMP in the development of atherosclerosis. Even in some scientific literature, MMPs are considered markers of vascular disease. The inflammatory process in obesity also affects muscle remodeling by reducing the activity of MMP-2 and inhibiting the cells responsible for hypertrophy [12].

Thus, obesity is a progressive disease that causes a number of problems in the body. Given the above, MMPs play a significant role in the development of pathological conditions during obesity. The relationship between the increase / decrease in the concentration of different types of MMP and the stimulation of dysfunction of the cardiovascular system, muscle tissue, gastrointestinal tract, nervous system.

Objective: analyze the qualitative composition of matrix metalloproteinases in the tissues of the liver, kidneys and muscles of obese rats.

Materials and methods. The experiments were performed on white nonlinear male rats with an initial weight of 115-150 g. During 1 week of the experiment, all animals received standard food "Purina rodent chow" and water ad libitum. On day 8, the animals were randomly divided into

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2 groups. Rats of the 1st group ("Control") continued to receive standard food for the next 10 weeks. Animals of the 2nd group ("High-calorie diet") consumed high-calorie food, which consisted of standard food (60 %), lard (10 %), chicken eggs (10 %), sucrose (9 %), peanuts (5 %), milk powder (5 %), vegetable oil (1 %) and received water for 10 weeks. The liver, kidneys, and muscles were excised, washed in chilled 0.9 % NaCl, cleared of connective tissue and blood vessels, shredded with scissors, and homogenized in chilled buffer. The obtained homogenates were centrifuged at 600 g for 15 min at + 4 °C. The precipitate was discarded and the supernatant was centrifuged again at 15,000 g for 15 minutes. Aliquots of supernatant were packaged in eppendorf-type microtubes and frozen at -20 °C until further use. Enzyme-electrophoretic analysis was performed according to the method [13]. The separating gel was polymerized in the presence of gelatin at a rate of 1 mg/ml. The concentration of the separating gel was 12 %, which prevented the migration of polymerized gelatin into the separating gel. Separation of the samples was performed at a current of 19 mA. After electrophoretic separation, the gels were washed in 2.5 % Triton X-100 for one hour to remove residual sodium dodecyl sulfate. The gels were then filled with 0.05 M Tris-HCl buffer, pH 7.4, containing 0.13 M NaCl, and incubated for 12 hours. Fixation and staining of gels was performed according to a standard electrophoresis protocol.

The paper adhered to international recommendations for conducting medical and biological research using animals in accordance with the European Convention (Strasbourg, 1986). Experimental work with rats was carried out in the vivarium of the Taras Shevchenko National University of Kyiv (2018 – 2021). Work with animals was regulated by the rules of experimental work with experimental animals, which were approved by the Academic Council of this institution, which in turn was consistent with current legislation of Ukraine adopted at that time.

Results and discussion. We analyzed enzymograms of the tissues of the liver, kidneys and muscles of obese rats. Enzymograms of non-obese rat liver, kidney, and muscle tissues were used as controls.

The enzyme diagram of liver tissues in obese rats clearly shows high enzymatic activity in the area with a molecular weight of 21 kDa, whereas in obese rats **(Fig. 1)** this was not observed, which can be explained as an increase in enzymatic activity of MMPs molecular weight of about 19-21 kDa, which corresponds to the active form of MMP-7, during the development of obesity in the body. According to the literature, the growth of MMP-7 in liver tissue is a biomarker of the spread of non-alcoholic fatty liver disease, as well as the development of biliary atresia, which stimulates remodeling of liver tissue and progression of fibrosis of this organ. In addition, MMP-7 may be a target for therapeutic approaches to prevent liver damage by fibrosis [13–15].

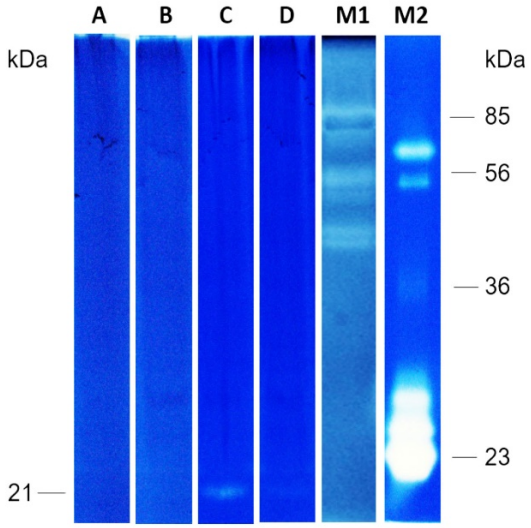

Fig. 1. Typical gelatin zymograms of liver tissue from control (A, B) and experimental rats (C, D).
Lanes M1 and M2 = molecular-weight markers (active proteolytic enzymes with known molecular mass).
In all cases samples (10μL each) were electrophoresed in 12 % SDS-PAGE with gelatin and treated as described in "Methods"

In addition, differences were found between the zymogrames of the kidney tissues of the rats healthy and suffered with obesity **(Fig. 2)**. It was the enzymatic activity in the range of 19-21 kDa, which appropriates of the active form of MMP-7 in rats suffered with obesity. MMP-7 is known to be expressed in the low concentrations in adults and only in some tissues, but MMP-7 is activated during various diseases. The significant enzymatic activity of MMP-7 in the kidneys indicates the acute inflammation and chronic kidney disease. MMP-7 causes the injury to the renal tubules and the progression of renal fibrosis. We suggest that the increase in the enzymatic activity of MMP-7 causes obesity and affects adversely to the kidney tissue [16, 17].

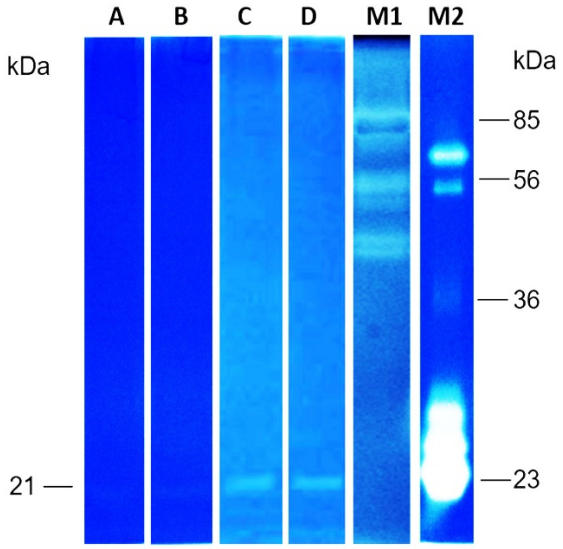

Fig. 2. Typical gelatin zymograms of kidney tissue from control (A, B) and experimental rats (C, D). Lanes M1 and M2 = molecular-weight markers (active proteolytic enzymes with known molecular mass). In all cases samples (10μL each) were electrophoresed in 12 % SDS-PAGE with gelatin and treated as described in "Methods"

After analyzing the enzymograms of muscle tissues, we recorded enzymatic activity in the range of 19-21 kDa and 85 kDa in obese rats **(Fig. 5)**, while in non-obese rats this was not observed **(Fig. 3)**. According to the literature, 19-21 kDa is the molecular weight of the active form of MMP-7, and 85 kDa is of the active form of MMP-9. Manifestation of the enzymatic activity of MMP-7 may indicate skeletal muscle hypertrophy. While MMP-9 is considered an important protease in the regenerative process, because they are strongly expressed in muscle damage and affect tissue remodeling. Thus, we associate the development of obesity with a negative impact on muscle tissue of the body through increased expression of MMP-7 and MMP-9 [18,19].

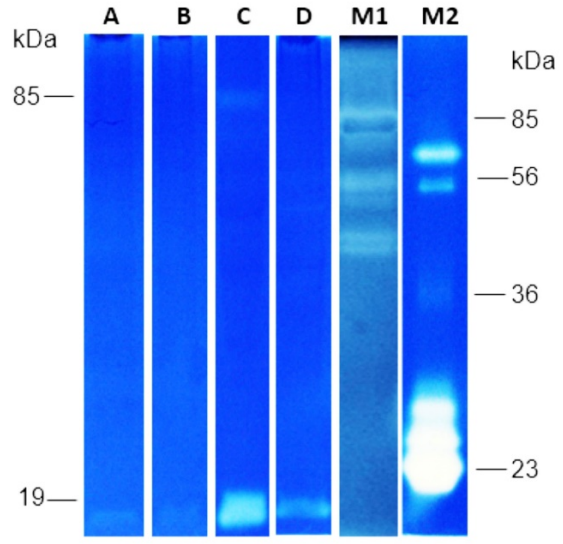

Fig. 3. Typical gelatin zymograms of muscle tissue from control (A, B) and experimental rats (C, D). Lanes M1 and M2 = molecular-weight markers (active proteolytic enzymes with known molecular mass). In all cases samples (10μL each) were electrophoresed in 12 % SDS-PAGE with gelatin and treated as described in "Methods"

As result, Table 1 is described the enzymatic activity of MMPs in experimental tissues. The enzymatic activity of MMP-7 was observed in liver, kidney and muscle tissues of rats suffered with obesity. Moreover, the enzymatic activity of MMP-9 was detected in the muscle tissue of experimental rats.

***Table* 1. Enzymatic activity of MMPs in rat tissues**

Type of tissue	Control group		Experimental group	
	Enzymatic activity of MMP-7	Enzymatic activity of MMP-9	Enzymatic activity of MMP-7	Enzymatic activity of MMP-9
Liver tissue	-	-	+	-
Kidney tissue	-	-	+	-
Muscle tissue	-	-	+	+

Conclusion. Enzymograms of liver, kidney, and muscle tissues from control and experimental rats were studied. It was found that under the conditions of induced obesity in rats there are changes in the composition of the MMP of the previously mentioned tissue types. Obesity of the kidneys, liver and muscles of obese rats showed an increase in the enzymatic activity of MMP-7, which is usually normal or not manifested at all, or poorly functioning. We hypothesize that MMP-7 affects the development of liver, kidney, and muscle fibrosis. Interestingly, the enzymatic activity of MMP-9, which affects the degradation of extracellular matrix components, increases in muscle tissue. Further studies of the function of matrix metalloproteinases in liver, kidney and muscle tissues in normal and in pathology will provide an understanding of biochemical processes in obesity and the development of new methods / approaches to treatment of this pathology.

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ХАРАКТЕРИСТИКА ЯКІСНОГО СКЛАДУ МАТРИКСНИХ МЕТАЛОПРОТЕЇНАЗ У ГОМОГЕНАТАХ ТКАНИН ЗА РОЗВИТКУ ЕКСПЕРИМЕНТАЛЬНОГО ОЖИРІННЯ У ЩУРІВ

Ожиріння є складним і багатофакторним захворюванням, що розвивається під впливом генетичних, фізіологічних, соціально-економічних та екологічних факторів. Ожиріння може збільшити ризик вияву супутніх захворювань та летальних наслідків. Економічне зростання, автоматизація багатьох технічних процесів та урбанізація є причинами малорухливого способу життя за останні 30 років, що призвело до стрімкого зростання розвитку ожиріння серед населення у 2, а то й у деяких регіонах у 4 рази. Загалом ожиріння характеризується надмірним розростанням жирової тканини, розвиток якої залежить від перебігу клітинних процесів, пов'язаних із популяцією таких клітин, як адипоцити та їх попередники. Зазначаємо, що накопичення жирової маси викликає дисфункцію інших тканин, яке призводить до небезпеки поширення інших патологічних процесів. Наше дослідження зосереджено на ферментативній активності печінки, нирок і м'язової тканини у щурів з ожирінням. Визначено зміни ферментативної активності матричних металопротеїназ (ММР), таких як ММР-7 і ММР-9, в експериментальних тканинах. Виявлено ферментативну активність ММР-7 у тканинах щурів, які страждали на ожиріння, тоді як у здорових щурів такого процесу не спостерігалося. Ми припускаємо, що це пов'язано з розвитком фіброзу тканин. Також зафіксовано підвищення ферментативної активності ММР-9 у щурів м'язової тканини, які страждали від ожиріння. Імовірно, це пов'язано з регенеративним процесом в експериментальній тканині. Подальші дослідження активності матриксних металопротеїназ у різних тканинах можуть дати інформацію, яка дозволить розробити нові методи/підходи до діагностики та лікування цієї патології.

Ключові слова: ожиріння, металопротеїнази, ензимографія.