MORPHOLOGICAL CHANGES IN THE LIVER AND INTESTINE IN THE EXPERIMENTAL METABOLIC SYNDROME IN RATS


Institute «Medical Academy named after S. I. Georgievsky» of V. I. Vernadsky Crimean Federal University, Simferopol, Russian Federation

Metabolic syndrome (MS) is a complex of disorders closely associated with developing the non-alcoholic fatty liver disease (NAFLD) accompanied by a violation of the intestinal microbiota. An assessment of morphological changes in the liver and intestines during the development of experimental metabolic syndrome in rats was carried out. The study was performed on 32 Wistar white rats aged 10–12 months using a 60 % fructose feeding pattern for 16 weeks as the MS model. It has been established that the fructose feeding model in rats causes the development of NAFLD with the result of a violation of the bar structure of the lobules, moderate intralobular inflammation, and the formation of hepatocellular granular dystrophy and perisinusoidal fibrosis. In the intestinal villi, destruction of apical epithelial cells and changes in cell nuclei are observed. Thus, the development of MS in rats is accompanied by the formation of signs of non-alcoholic steatohepatosis and damage to intestinal cells, which confirms the involvement of these organs as the primary target organs in MS, and contributes to the development of metabolic, dysbiotic, and inflammatory disorders.

Keywords: metabolic syndrome, liver, intestines, non-alcoholic fatty liver disease, microbiota

A critical risk factor for the development of cardiovascular diseases, the problem of metabolic syndrome (MS) is one of the highest priorities and relevant in modern fundamental and clinical medicine [1]. This is due to the fact that MS contains several metabolic, inflammatory, hormonal and vascular disorders, which significantly increase the risk of developing second-type diabetes mellitus and various cardiovascular complications [2].

Of particular interest is the study of morphological changes in the histological structure of the liver and intestines during the development of MS. Non-alcoholic fatty liver disease (NAFLD) affecting about 25 % of the world's population. According to various studies, the increase in the prevalence of NAFLD occurs in parallel with the epidemic of obesity and MS [3]. The liver gradually passes through the stages of non-alcoholic steatosis, which progresses to alcoholic steatohepatitis and non-alcoholic cirrhosis or hepatocellular carcinoma [4].

In the study of MS, microbiota plays a significant role in determining the effective absorption of nutrients in the gastrointestinal tract. In this case, there are changes in the number of bacteria, both increasing and decreasing, depending on the type of bacteria [5]. At the moment, scientists are conducting research on the relationship between the problem of constipation with the development of metabolic syndrome. Constipation-induced endogenous intoxication is recognized as one of the proven risk factors for many diseases, including metabolic syndrome and colorectal cancer. Endotoxemia can also form in liver lesions, resulting in increased production of proinflammatory cytokines and expression of their receptors (tumor necrosis factor-α, interleukin-6 and interleukin-8), inhibition of antioxidant liver function, increase in altered forms of lipoproteins in the blood [6].

This study aims to evaluate morphological changes in the liver and intestines during the development of experimental fructose-induced metabolic syndrome in rats.

**Material and Methods.** The experimental study was carried out on 32 white Wistar rats of the SPF category, aged 16 weeks, with body weights of 400–440 g. The animals were divided into two groups equally – the control group and the comparison group with the experimental MS.

The MS uses a fructose-based model with 60 % fructose content based on standard solid feed (Research Diet, USA) [7]. The duration of the feeding was 16 weeks. The experimental study was performed using the Aperio Image Scope program (Aperio Technologies Inc, USA), followed by statistical processing using the MedStat and Statistica 10.0 (StatSoft, USA) The study a parametric Student’s t-test, the distribution does not differ from regular was used. Values above 0.05 were considered reliable.

**Results and Discussion.** The development of MS characteristic features accompanied experimental modeling of MS by feeding rats with 60 % fructose feed. A significant increase in visceral fat mass was found in the group of rats with experimental MS by 2.5 times (11.31±0.28 g, p<0.001). Biochemical studies of rat blood showed that by the 24th week of the experiment, the average blood glucose level in rats with induced MS was significantly higher by 12.5 % (p<0.01) than the average glucose level in the group of intact animals. Changes in the lipid profile accompanied experimental modeling of MS in rats: an increase in total cholesterol by 15.8 % (p<0.01), an increase in TG levels by 39.2 % (p<0.01), and a decrease in HDL by 36.9 % (p<0.001). There was also a significant increase in the level of uric acid by 44.8 % (p<0.001) in the blood plasma of rats with experimental MS (174.44±16.89 mmol/l) compared with the control group (120.48±15.12 mmol/l).

Morphology of the liver of rats in the control group and the simulation of MS. The histological structure of the liver of rats in the control group corresponds to the normal (Fig. 1 A). Modeling of experimental MS revealed the typical characteristics of NAFLD in the liver. Liver cells slightly increase in size, binuclear cells are found both in the central and peripheral zones, and the cellular composition is heterogeneous. In the central zone of the lobules, lipid inclusions in the cytoplasm of hepatocytes are more significant and tend to merge, while on the periphery, they are smaller, dust-like in areas (Fig. 1 B). A violation of the beam structure of the lobules with a chaotic, random arrangement of cells was also revealed. Moderately pronounced intralobular inflammation, cell edema, hepatocellular granular degeneration, there is periportal fibrosis (Fig. 1 C).

In the peripheral parts of the cytoplasm, the ER cisterns undergo fragmentation agrER predominates. The number of free ribosomes is sharply reduced compared to the control. Mitochondria react most acutely; the integrity of the inner and outer membranes is violated, cristae cooled and then centrifuged for 15 minutes at 3000 rpm (604g). The analysis of biochemical markers was carried out according to the standard methodology. Blood plasma was frozen in liquid nitrogen and stored at −80°C for further analysis.

The presence of visceral obesity was confirmed by weighing visceral fat (VLT 500) after autopsy. Euthanasia was performed at the end of the experiment by inhalation of 96 % diethyl ether to study the histological structure of the liver and intestine.

The material for light microscopy was fixed in formalin and then embedded in paraffin blocks. Sections were stained with hematoxylin-eosin. For electron microscopy, the material was fixed with osmium oxide, followed by poured into epoxy resin. Fixation, and pouring, for light microscopy, was carried out according to the generally accepted method. Ultrathin sections were studied using a FEM-125K electron microscope (Industrial Union LLC, Moscow). In morphometric counting was performed using the Aperio Image Scope program (Aperio Technologies Inc, USA), followed by statistical processing using the MedStat and Statistica 10.0 (StatSoft, USA) The study a parametric Student’s t-test, the distribution does not differ from regular was used. Values above 0.05 were considered reliable.
are not visualized, and lipid inclusions are found in the cytoplasm near mitochondria. Accumulation of chromatin in the nuclei is characteristic, which may be a sign of polyploidy as a compensatory tissue reaction aimed at activating regenerative processes. The cytoplasm is filled with fine-grained contents; the ER is poorly developed with signs of dystrophy. Mitochondria decrease in size, and the electron density of the matrix increases (Fig. 1 C, D).

The accumulation of lipids is associated with their excessive synthesis, impaired TG secretion, and impaired protein metabolism during a carbohydrate diet. An essential factor in such a biochemical transformation of cells is the disruption of redox processes, which is confirmed by ultramicroscopic examination. This may also be associated with damage to mitochondrial membranes and increased oxidation of fatty acids (FA) due to excessive accumulation in intact mitochondria and peroxisomes. At the same time, the emerging toxic forms of oxidizing agents, in this case, hepatotropic (due to their localization and specific action) link. This triggers necrosis, the release of cytokines that activate a nonspecific aseptic inflammatory response and fibrosis involving Ito stellate and Kupffer cells [8, 9]. This pathogenetic chain is realized when the compensatory processes developing in the tissue are turned on as opposed to damage. Attention is drawn to the narrowing of the lumen of the sinusoidal capillaries, expressed over the entire area of the lobule on the cut; only in the central vein region do the final segments of the sinusoids have a visible lumen. Such a violation of the cytoarchitectonics of the liver affects its function, which is reflected in the results of biochemical studies.

According to the results of morphometric analysis of liver sections, the area of the parenchyma on liver preparations in rats of the experimental group is 2.97 % (p=0.002) higher than in rats of the control group. However, the average values of the diameter and area of vessels and ducts in the hepatic lobules in rats of the control group are significantly higher (p<0.001) than in the group of rats with MS: central vein – by 49.47 % and 72.93 %; interlobular vein – by 15.94 % and 59.13 %; interlobular bile duct – by 29.65 % and 55.18 %, and interlobular artery – by 65.37 % and 87.77 % for the diameter and area, respectively (Fig. 2).

Fig. 1. Fragments of the liver of male rats of the control and experimental groups at the age of 10–12 months: light microscopy, stained with hematoxylin and eosin (A, B); electronic micrographs (C, D). A – Peripheral part of the lobule, triad, control group; ×400; B – Central zone of the hepatic lobule, MS group. Violation of the histotopographic structure of the lobules, narrowing of the lumen of the sinusoidal capillaries, perisinusoidal fibrosis; ×400; C – A fragment of a hepatocyte in the initial stage of necrosis, MS group; ×6400; D – Hepatocyte fragment, MS group; ×8000
This confirms the descriptive characteristics of micropreparations that narrow the perisinusoidal space and the development of perisinusoidal fibrosis described in the experimental MS group. This indicates the development of initial fibrotic changes in the liver against the background of prolonged inflammation and damage.

Even though the human and white rat livers are significantly similar in some morphological and functional parameters (poor contouring of the lobules, moderate development of the perilobular stroma, the presence of border plates, many binuclear cells and pronounced polyplody, weak accumulation of lipids in the norm), the reaction to damage has its characteristics. So, talking about our experiment, an increase in the number of binuclear cells characteristic of a person as a compensation for a damaging alimentary factor was not revealed (Fig. 3). On the contrary, the number of binuclear cells in rats became less; there was hypertrophy of mononuclear hepatocytes with the development of dystrophic processes.

Morphology of the intestines of rats in the control group and MS group.

The histological structure of the intestinal villi of rats in the control group corresponds to the normal. In the intestinal villi of rats with fructose-induced metabolic syndrome, the following morphological changes were revealed: at low magnification, the destruction of the apical part of the villus epithelial cells and the release of the contents into the intestinal lumen were noticeable.

On a large increase, the lymphatic capillaries along the long axis of the villi, epitheliocytes and their nuclei become more elongated in shape, their cytoplasm, and the connective base more eosinophilic (Fig. 1 B). The cytoplasm of the goblet cells is still transparent and slightly eosinophilic.

In the crypts, goblet cells are practically not expressed; the severity of Paneth cells and undifferentiated epitheliocytes does not change.

Synthesis organelles (EPS, Golgi complex, ribosomes) are developed in the Panet cell, and secretory granules are arranged in the apical part (Fig. 1 B). It is known that the epithelial stratum of the small intestine is completely renewed in 3 days, the epitheliocytes of the crypt – in 5 days, except for Panet cells [10]. The lymphatic system was only found on the intestinal wall slices of the control group. Identified as solitary follicles with an area of 582 739.1 nm² to 322 278.201 nm² and grouped lymphoid formations with an area of 1 252 639 nm².

Morphometry showed that the mean thickness of the villus epithelium in the experimental rat group was 4.48 % higher than the control group (p=0.784), as well as the thickness of the mucous membrane was 7.76 % (p=0.621), but these changes were not statistically reliable. The muscle layer of the intestinal wall of the control group is 6.18 % thicker than the experimental group (p=0.782). There is a clear separation of the vividly eosinophilic lymph capillaries. The percentage of capillaries is 38 % higher in the control group (p=0.529). Control group rats’ villi are thicker than rat vortices experienced at 4.73 % (p=0.577).

When the membranes are damaged, mitochondria initiate necrosis and fibrosis with increasing liver size and histoarchitectonic impairment, moderate intralobular inflammation, hepatocellular grainy dystrophy, cell edema, contraction of sinusoid capillaries, chromatin build-up in nuclei. However, in our experiment, we have found an unnatural reduction in the number of bi-nuclear cells. These changes have developed against the background of disturbances in protein and lipid exchange during a fructose diet. Liver dystrophy is caused by accumulation in hepatocytes of TH due to an increase in the intrahepatic pool of free LCD released from adipose tissue as a result of insulin resistance resulting from a fructose diet, as well as an excessive synthesis of TH and violation of their disposal from hepatocytes [11]. This is based on the disruption of intracellular biochemical processes. Due to membrane damage, mitochondria in the surviving organelles (mitochondria and peroxisomes) compensate for increased oxidation of the LCD, resulting in hepatotoxic oxidant forms, which induce the initiation of necrosis, and release of cytokines and fibrosis involving perisinusoidal Ito and Kupfer cells [11, 12]. This indicates the development of compensatory-adaptive reactions under pathobiologically altered protein-lipid metabolism [13, 14].

Morphometric measurements of the intestine were not reliable. However, it has been shown that the form of epitheliocytes of villi of the intestine of rats and the nuclei of these cells becomes more elongated, and the cytoplasm of epitheliocytes becomes more elongated. The connective detached base is more eosinophilic. There is a destruction of apical epitheliocytes of villi and the exit of part of them into the lumen of the intestine. Zhou X. and so on. have received similar morphological changes in their experiment in studying the gut structure of high carbohydrate and high lipid rats. Still, their results were also unreliable [15–17].

Conclusions. Thus, histological examination of the liver of rats with simulated MS showed signs of NAFLD and steatosis. However, the above changes indicate that the intestinal wall has been damaged, resulting in the development of intestinal autoimmune toxemia, which exacerbates the endogenous endotoxemia characteristic of MS. Since the mechanisms of MS development and organ damage due to its development are not yet fully disclosed by researchers, this topic represents a wide field for further study and discovery.

Informed consent. The basic rules for the maintenance and care of experimental animals corresponded to...
the standards given in the Order of the Ministry of Health of the Russian Federation No. 708n of 23.08.2010 «On Approval of the Rules of Laboratory Practice in the Russian Federation», the ethical principles established by the European Convention for the Protection of Vertebrates Used for Experimental and Other Scientific Purposes (adopted in Strasbourg on 18.03.1986 and confirmed in Strasbourg on 15.06.2006). The Ethics Committee approved the experiment.

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**References**


**About authors:**

Petrenko Vitalina Igorevna, CMSc, Assistant of the Department of basic and clinical pharmacology; tel.: +79780661792; e-mail: petrenko-vitalina@mail.ru; https://orcid.org/0000-0001-9451-1757

Saenko Yulia Sergeevna, student; tel.: +73652554940; e-mail: julia.saenko@list.ru; https://orcid.org/0000-0002-5304-6112
The results of an X-ray assessment of the dynamics of bone regeneration of postoperative defects in an experiment on rats using a stress-inducing device to form non-physiological occlusion of the jaws are presented. More effective and complete restoration of the alveolar ridge after tooth extraction was shown in animals treated intraperitoneally with ethylmethylhydroxypyridine succinate under chronic stress conditions. X-ray analysis of the bone regeneration confirmed the stimulation of bone healing by the drug in terms of 30 and 60 days in the form of filling the defect with trabecular bone, which, in terms of quantitative and qualitative composition, can provide the function of a scaffold for newly formed vessels and nerves.

Keywords: radiography, experiment, osseointegration, chronic stress

The emergence of new imaging techniques in clinical and experimental practice has significantly expanded the possibilities for studying the mechanisms of osseointegration [1, 2]. At the same time, the possibilities of reparative bone formation of postoperative jaw defects, including modeling of bone loss processes under conditions of chronic stress or osteoporosis, are far from being...