



Structural and Functional Changes in the Liver and Spleen of Rats Exposed to a Hypercaloric Diet

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Abstract: The paper presents the results of an experimental study of morpho-functional changes in the liver under the influence of a calorie-dense diet enriched with a high-fat, high-carbohydrate (HFHCD) formulation. The aim of the study was to model metabolic disorders similar to fatty liver disease (FLD), followed by an analysis of structural and functional changes in the organ. Sexually mature Wistar rats receiving HFHCD for a fixed period were used as the model. The morphological and biochemical studies conducted revealed signs of fatty hepatosis (FH), disruption of hepatic tissue architecture, inflammatory changes, and functional abnormalities, confirmed by changes in liver enzyme levels. The data obtained provide deeper insight into the mechanisms underlying the pathogenesis of metabolic liver disorders and confirm the effect of HFHCD on the structural and functional state of the organ's parenchyma.

Keywords: high-fat high-carbohydrate diet (HFHCD), fatty hepatosis, non-alcoholic fatty liver disease (NAFLD), liver morphology, metabolic syndrome, hepatocytes, lipid metabolism, Wistar rats.



1. INTRODUCTION

In recent decades there has been a rapid increase in the prevalence of metabolic disorders, including obesity, insulin resistance, and non-alcoholic fatty liver disease (NAFLD), largely associated with changes in the population's eating habits [1]. Increased consumption of calorie-dense food rich in fats and simple carbohydrates has a significant impact on the functional state of internal organs, especially the liver as the central organ of metabolism [2].

Prolonged consumption of exclusively dispersed (finely ground) food, according to Kuznetsova T.I., affects the development of hepatocytes in the early postnatal period; this is expressed as an increase in hepatocyte volume, while in the later period it is characterized by persistent hypotrophic changes in liver cells.

It is known that the liver plays a key role in lipid, carbohydrate and protein metabolism, as well as in detoxification and energy storage [3]. Dietary disturbances lead to the development of morphological changes in hepatocytes, disruption of intrahepatic microcirculation, and deterioration of functional parameters, which may contribute to the progression of pathologies such as steatosis, steatohepatitis and fibrosis [4].

Experimental exposure of rats to HFHCD for 12 weeks, alongside metabolic disturbances, allows reproduction of the early signs of systemic inflammation characteristic of metabolic syndrome (MS) and obesity [5].

According to Michurina S.V., alimentary obesity leads, on the one hand, to the development of fatty degeneration, and on the other, stimulates the functional activity of hepatocytes, which can be regarded as a compensatory response to increased consumption of animal fat. According to the authors, structural changes in parenchymal cells are accompanied by functional tension of capillary-connective-tissue structures and impaired hepatic blood circulation [6].

High-fat load leads to the development of destructive-dystrophic changes in the liver; this is because liver macrophages, engulfing lipids, do not manage to biotransform them in time. As a result, the number of altered macrophages and the amount of fat in the liver increase [7]. This leads to reorganization of the liver parenchyma against the background of hepatocyte hypertrophy [8].

The study by Yanko R.V., is devoted to a model of fatty hepatosis (FH) based on a combined (fat-carbohydrate) high-calorie diet in rats, leading to the development of pronounced morphological and biochemical signs of this pathology. The most pronounced manifestations of FH are observed in young animals. However, according to Mustafina L.R., HFHCD causes significant damage to both the stroma and the parenchyma of the liver, promoting the development of dystrophic, fibrotic and inflammatory changes. The less pronounced morphological changes in the liver after administration of HFHCD to young rats are probably related to its high regenerative capacity [9].

The study established that use of the proposed high-fat and high-carbohydrate diet in rats reproduces most of the typical features of MS: obesity, elevated blood pressure, hyperglycemia, reduced glucose tolerance, insulin resistance, and dyslipidemia with a predominance of triglyceridemia. The diet-induced MS model may be useful for studying the causes of development and progression of metabolic and hemodynamic disorders in MS, as well as for investigating potential approaches to its prevention and treatment [10]. Regarding the capacity for NAFLD development in rats receiving a fructose-enriched diet [11].

In the course of studying the effect of a fructose-induced diet on the development of an inflammatory process in the rat liver, increased activity of specific liver enzymes biochemical markers of hepatocyte cytolysis was observed, as a result of possible steatosis and inflammatory infiltration of the liver, consequent to destructive-dystrophic changes in hepatocytes against the background of carbohydrate and lipid metabolism disorders [12]. Use of a fructose-enriched diet for 35 days promoted the development of hyperglycemia, insulin resistance, dyslipidemia and increasing lipid metabolism disorders [13]. This is evidenced by increases in body weight, adipose tissue mass, insulin resistance and accumulation of triglycerides in the liver of rats [14]. There is evidence of the development of features characteristic of metabolic syndrome (MS) and hemostatic system dysfunction: hypercholesterolemia, hypertriglyceridemia, hyperglycemia, an

increase in excess body weight, reduced anticoagulant potential of the blood, and promotion of thrombotic conditions [15].

Thus, the leading factor in the formation of destructive changes in the liver is excessive sucrose consumption, with concomitant dietary protein deficiency aggravating the functional changes in hepatocytes [16].

In order to gain a deeper understanding of the mechanisms underlying pathological liver changes under the influence of a calorie-dense diet, this study used an experimental model in laboratory animals [17]. This makes it possible to study, under controlled conditions, the dynamics of morphofunctional changes, which is important for developing effective preventive and therapeutic approaches to preventing metabolic changes in the liver [18].

2. MATERIALS AND METHODS OF THE STUDY

The study was conducted at the laboratory of Navoi Innovation University, in compliance with ethical norms and standards for working with laboratory animals, in accordance with the recommendations of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes.

Rats are the optimal test system for modeling NAFLD, with males being more susceptible to dietary disturbances and developing this pathology more easily and quickly, by analogy with humans (more often in men than in women). Outbred animals were chosen for ease of population extrapolation and translation of data. The control group included 10 rats, the experimental group included 20 animals receiving HFHCD. The total duration of the experiment was 12 months.

The study used 30 sexually mature white Wistar rats weighing 180–220 g. All animals were randomized by the random-number method into two groups: the control group ($n = 10$), kept under standard vivarium conditions on a normal food ration with free access to water, received a standard laboratory diet; the experimental group ($n = 20$) received HFHCD for 12 weeks. FH was modeled using a hypercaloric diet obtained by adding to standard feed 20% rendered beef fat, 5% fructose, 5% isolated soy protein, 2% choline, and 0.5% cholesterol. Animals were kept under standard conditions (temperature 22–24°C, 12-hour light regime, free access to food and water). During the study, body weight of all animals was measured monthly using electronic scales.

The outcome of modeling with HFHCD depends on the nature of the fat added to it. Animal fats form MS more effectively than vegetable fats; pork fat produces steatohepatitis, while beef fat produces steatosis. For this reason, our study gave preference to rendered beef fat.

At the end of the experimental period, all animals were euthanized, followed by collection of biological material. The study included:

Biochemical blood analysis: levels of ALT, AST, total bilirubin, cholesterol, triglycerides and glucose in blood serum were measured using portable analyzers [Accu-Chek Active (Roche) and Accutrend Plus (Cobas)] after preliminary 8-hour fasting of the animals, by collecting blood from the lateral tail vein at control points. For histological examination, organs with pre-measured mass were placed in 10% formalin solution for 24 hours, followed by preparation of sections and staining with hematoxylin and eosin. In the liver, the following features were assessed: severity of necrosis, lobular inflammation, and ballooning degeneration.

Macroscopic and histological examination of the liver: histological sections were stained with hematoxylin-eosin and Sudan III to detect FH;

Morphometric analysis: parameters of the cellular composition of the liver, the presence of fatty degeneration, inflammatory infiltrates and other morphological changes were determined.

Statistical processing of the obtained data was performed using GraphPad Prism 7 software. The normality of distribution of quantitative traits was tested, given the small number of observations, using the Shapiro–Wilk W -test; significance of differences under normal distribution of quantitative traits was assessed using Student's t -test (for independent samples), and for distributions not meeting normality criteria, the nonparametric Mann–Whitney U -test (for comparing two independent, unpaired variation series) was used.

Numerical data presented in the tables are given as: arithmetic mean (M) $\pm$ standard error of the mean (m). A value of $p < 0.05$, generally accepted in medicine, was used as the criterion of statistical reliability of the conclusions obtained.

3. STUDY RESULTS AND DISCUSSION

Macroscopic examination of the liver of rats in the experiment showed that, as in the control group, the surface of the animals' liver was smooth and uniformly dark red in color, the capsule thin and transparent, and the liver tissue on section plethoric and moderately dense. However, under HFHCD, histological examination revealed some change in the overall architecture of the liver in the rats: sinusoids were dilated, and their radial arrangement was noted in certain areas. Polygonal-shaped hepatocytes with a well-defined, undisturbed membrane and nuclei of varying sizes were observed.

As a result of 12 weeks of feeding laboratory animals a calorie-dense diet, pronounced morphofunctional changes in the liver were revealed in the experimental group compared to the control group.

Biochemical parameters in the experimental group showed a statistically significant increase in the levels of: alanine aminotransferase (ALT) 2.1 times higher than in the control ($p < 0.05$); aspartate aminotransferase (AST) 1.8 times higher ($p < 0.05$); total cholesterol and triglycerides 1.5–2 times higher ($p < 0.05$); as well as an increase in blood glucose levels, indicating the development of insulin resistance. These data indicate impaired liver function and the development of metabolic disturbances.

Table 1. Blood biochemical parameters in rats (M $\pm$ m)

No.	Parameter	Control group	Experimental group
1	ALT (U/L)	42.5 $\pm$ 3.1	88.5 $\pm$ 5.7
2	AST (U/L)	52.2 $\pm$ 4.5	95.7 $\pm$ 6.2
3	Total bilirubin (μ mol/L)	7.9 $\pm$ 0.6	11.9 $\pm$ 0.9
4	Glucose (mmol/L)	5.5 $\pm$ 0.3	7.9 $\pm$ 0.4
5	Cholesterol (mmol/L)	3.0 $\pm$ 0.2	5.3 $\pm$ 0.3
6	Triglycerides (mmol/L)	1.2 $\pm$ 0.1	2.7 $\pm$ 0.2

Note: p-values were obtained by comparing the experimental group with the control group. Significant differences were noted at $p < 0.05$.

The development of hyperglycemia has been described in numerous HFHCD models using a fructose-enriched hypercaloric diet. Thus, addition of 10% fructose to drinking water in a 12-week experiment in rats led to the onset of hyperglycemia against a background of concomitant obesity and lipid metabolism disorders. Moreover, when steatohepatitis predominates, glucose levels tend to rise, while with predominant steatosis the changes are insignificant.

A decrease in serum albumin concentration may indicate impairment of the liver's protein-synthetic function and is confirmed by the presence, in most animals, of numerous inflammatory-necrotic changes in the liver according to morphological examination results. A decrease in blood albumin is not a characteristic sign of NAFLD, but may appear at a late stage of steatohepatitis or in cirrhosis. All this suppresses the free-radical (reactive oxygen species) formation response caused by the inflammatory process. According to the authors, the decrease in blood albumin in NAFLD is a kind of vicious circle.

Table 2. Pathomorphological liver changes in animals (% of the total number of animals in the group) (M $\pm$ m)

No.	Histological feature	Control group (%)	Experimental group (%)
1	Fatty degeneration	0	96
2	Hepatocyte ballooning degeneration	0	72
3	Inflammatory infiltrates	10	86
4	Disruption of hepatic lobule architecture	0	71

Adipose tissue in rats is represented by subcutaneous and visceral adipose tissue (including mesenteric, retroperitoneal and epididymal). In rodents, epididymal adipose tissue normally has a greater mass than at other locations. An increase in the mass fraction of visceral fat is a characteristic feature of metabolic syndrome in both humans and animals and is one of the most important prognostic factors for the development of obesity-associated diseases.

Table 3. Morphometric parameters of the rat liver (M ± m)

No.	Histological feature	Control group	Experimental group
1	Mean liver weight, g	7.2 ± 0.5	9.4 ± 0.7 *
2	Hepatocyte nuclear displacement (%)	1.1 ± 0.5	36.0 ± 4 *
3	Disruption of trabecular structure, % of fields of view	0	28 ± 3 *
4	Hepatocytes with lipid inclusions (%)	12.4 ± 1.7	45.3 ± 3.2
5	Nuclear-to-cytoplasmic ratio	0.52 ± 0.04	0.41 ± 0.03
6	Density of hepatic cords (units/mm ²)	86.7 ± 4.1	64.5 ± 3.8

*Note: p-values were obtained by comparing the experimental group with the control group. Significant differences marked * — at p < 0.05.*

Macroscopic changes in the liver showed that, on macroscopic examination, the control group of animals had no pathological changes: the liver surface was smooth, uniformly dark red in color, the capsule thin and transparent. The liver tissue on section was plethoric and moderately dense. On dissection of animals receiving the high-calorie diet, the liver was enlarged, had a dull capsule, a yellowish tint and a friable consistency, characteristic of fatty infiltration.

Histological examination revealed that the lobular structure of the organ's parenchyma was not visualized. Disruption of the radial arrangement of sinusoids was noted, which were in places dilated. Hepatocytes had a polygonal shape and predominantly small nuclei. Pronounced fatty degeneration of the organ was found small and medium-sized lipid droplets in all hepatocytes. Lipids often occupied nearly the entire cell, pushing the nucleus and organelles to the periphery and forming so-called "signet-ring" cells. After the death of hepatocytes, the fat droplets merged, leading to the formation of fatty cysts, around which an inflammatory reaction generally developed. A vascular-mesenchymal reaction of the organ was observed. In the parenchyma, within the sinusoids and along the portal tracts, a moderate diffuse and small-focal polymorphocellular infiltrate was found; varying blood filling of the central veins and plethora of a number of portal-tract vessels were revealed. Sudan III staining revealed significant accumulation of lipids in the cytoplasm of hepatocytes. In the adipose tissue, large adipocytes filled with neutral fats, stained bright red, are visible. Thin layers of connective tissue, stained light pink, pass between the cells. The background of the specimen is pale, which emphasizes the intense staining of the lipid droplets. On the micrograph, hepatocytes with pronounced accumulation of neutral fats, stained bright red, are visible. The lipid droplets are distributed in the cytoplasm of the cells and vary in size and shape. Thin layers of connective tissue can be seen between the cells, and the dark violet nuclei of the hepatocytes contrast well with the background. Normally, in the organ parenchyma stained with hematoxylin and eosin, the typical trabecular structure of the liver is visible: hepatocytes form cords (trabeculae), between which sinusoidal capillaries are located. The cytoplasm of hepatocytes is stained pink (eosin), and their nuclei dark violet or blue (hematoxylin). The nuclei are round, clearly distinguishable, often with visible nucleoli. Flattened endothelial cells are noticeable in the sinusoids. The overall tissue structure is clear, which is characteristic of a normal liver.

On van Gieson staining, large round adipocytes with unstained (white) fat vacuoles are visible. The intercellular substance contains bundles of collagen fibers, stained reddish, forming a thin network between the cells. The cytoplasm and muscular elements of the organ are stained yellow, and the cell nuclei dark blue. This staining well emphasizes the connective-tissue framework of the liver's adipose tissue.

Morphometric data showed that with hematoxylin-eosin staining, the cytoplasm of most hepatocytes had a uniform color, and no vacuoles were observed. Necrotic changes in hepatocytes

occurred predominantly at the periphery of the lobules. Central veins and portal tracts were more often of unchanged shape. In places, the central veins showed a small accumulation of blood cells. Some central veins were dilated. In the parenchyma, within the sinusoids and along the portal tracts, a moderate diffuse and small-focal infiltrate was noted. Small lipid inclusions were present in certain areas.

Morphological examination also revealed no disturbances in the general architecture of the liver parenchyma; in certain areas the sinusoids were dilated, and their radial arrangement was noted. Hepatocytes were polygonal in shape, with a well-defined, undisturbed membrane, nuclei of varying size, and light karyoplasm. The cytoplasm of hepatocytes had a granular coloration; in certain areas of the organ, cells with cytoplasmic vacuolization were found. Binucleate hepatocytes were often found. Central veins and portal tracts were generally of unchanged shape. In places, the central veins showed a small accumulation of blood cells. Single central veins were slightly dilated. In the parenchyma, within the sinusoids and along the portal tracts, a weak diffuse cellular infiltrate was found.

Sudan III staining showed the presence of numerous small lipid inclusions in hepatocytes. The number of hepatocytes containing lipid inclusions was 3.5 times higher in the experimental group compared to the control ($p < 0.01$). An increase in the nuclear-to-cytoplasmic ratio and a decrease in the density of hepatic cords were also observed.

The FH model in rats is characterized not only by similarity of the formation conditions (hypercaloric nutrition and the development of visceral obesity), but also by morphological (signs of steatohepatitis) and biochemical (dyslipidemia, hyperglycemia) manifestations. This potentially increases the adequacy of translating data obtained on such a model from preclinical to clinical practice. However, a significant drawback of the modified model is the long duration of pathology formation and, as a consequence, its labor intensity.

A prolonged high-calorie diet leads to the development of fatty hepatosis in rats. Switching to a standard diet promotes complete restoration of blood biochemical parameters, but only partial restoration of liver histoarchitecture. Signs of active regeneration of the organ's parenchyma are found only with physical exercise combined with a change in diet.

Thus, the results obtained confirm that FH leads to the development of pronounced morphological and functional changes in the liver, similar to non-alcoholic fatty liver disease in humans. These changes are accompanied by hepatocyte dysfunction, MS and an inflammatory reaction, which underscores the significance of HFHCD in the pathogenesis of liver diseases. The FH model based on combined HFHCD leads, in all experimental rats, to the development of pronounced morphological and biochemical signs of this pathology.

The created models of experimental MS based on HFHCD revealed its main characteristic features: increased body weight of rats, hyperglycemia, hyperinsulinemia, insulin resistance, dyslipidemia, and a pronounced increase in blood triglyceride content in rats throughout the experiment.

The morphofunctional manifestation of FH is the accumulation of neutral fats in hepatocytes, accompanied by an increase in hepatocyte size, the appearance of numerous fat vacuoles in the cytoplasm, and displacement of nuclei to the periphery of the cells. In some areas, disturbances of the trabecular structure, dilation of sinusoids and signs of initial dystrophic processes are found. The results obtained confirm that excessive intake of HFHCD promotes pathological changes in the liver.

4. CONCLUSIONS

The findings demonstrate that prolonged exposure to a high-fat, high-carbohydrate diet (HFHCD) induces visceral obesity and pronounced structural and functional alterations in the liver of experimental rats, accompanied by significant disturbances in lipid and carbohydrate metabolism. The experimental group showed substantial increases in ALT, AST, total cholesterol, triglycerides, and blood glucose compared with the control group, indicating impaired hepatic function and the development of metabolic abnormalities. Morphological examination further revealed fatty degeneration, hepatocyte ballooning, inflammatory infiltration, disruption of hepatic

lobular architecture, increased liver weight, accumulation of lipid inclusions in hepatocytes, nuclear displacement, and reduced density of hepatic cords, confirming substantial diet-induced hepatic injury. These changes demonstrate that the HFHCD model reproduces key morphological and biochemical manifestations associated with fatty hepatitis and metabolic dysfunction. The study also indicates that switching from the hypercaloric diet to a standard diet can promote substantial normalization of serum cholesterol, triglycerides, total bilirubin, and hepatic enzyme activity, although restoration of liver histoarchitecture may remain incomplete. Overall, the findings support the use of this experimental model for further investigation of the mechanisms underlying diet-induced fatty liver disease and for evaluating preventive and corrective interventions aimed at reducing metabolic disturbances and hepatic structural damage.

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