Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 24 (1-2)
Sofia ● 2017

Morphology

Morphological Disorder Progression in Rat High-Fat, High-Carbohydrate Diet Induced Metabolic Syndrome

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High-fat-carbohydrate intake correlates with the epidemic rise in obesity and metabolic syndrome and related diseases. The aim of our work was to study the liver and mesenteric adipose tissue in rats with metabolic syndrome induced by high-fat-high-carbohydrate diet (HFHCD). Wistar rats (n = 10) were fed with HFHCD for 16 weeks. Control rats (n = 10) were on a normal diet. Metabolic control was determined by measuring BMI, adiposity, plasma parameter. Histopathological study was performed on the mesenteric adipose tissue and liver using PAS-reaction and Sudan-III-staining. Results: HFHCD increased body weight, BMI, adiposity, decreased HDL-cholesterol. Mesenteric adipose tissue was with larger adipocytes, liver steatosis and an increase in glycogen storage was also observed in the HFHCD group. In conclusion, nutritional stress caused by HFHCD promotes oxidative stress as evident by increased lipid peroxidation products and de novo lipogenesis which contributed to fat accumulation in the adipose tissue and the liver and to the development of non-alcoholic liver disorders.

Key words: metabolic syndrome, male rats, hepatic steatosis

Introduction

Obesity is a major health problem in the world. It is associated with the risk of cardiovascular disease, cancers, hyperlipidemia, and liver steatosis development [4]. Obesity constitutes a risk factor for contributing to the development of type 2 diabetes [11]. Sprague-Dawley rats were fed a high fat and high cholesterol diet demonstrated diet-induced non-alcoholic fatty liver disease [3]. High fat diet increases body and liver

weight, fasting blood glucose, serum aminotransferase (ALT) and lipid accumulation in the liver [3]. High fat diet administered to Prague hereditary hypertriglyceridemic (HTG) rats can induce signs of metabolic syndrome [5]. The increased consumption of high-fructose corn syrup may contribute to the worldwide epidemic of fatty liver [9]. High fats and sugars rich diets also induce obesity and other metabolic disorders. They lead the body to a pro-inflammatory pattern, which may affect the proper functioning of many tissues, including adipose tissue and liver. During the expansion of adipose tissue, a number of functions such as activation and release of cytokines and hormones may be affected [10]. After eight weeks high fat, high carbohydrates diet, body weights increased 2.4-fold, hepatic-triglyceride content increased progressively [1]. Most of the experimental animal models use male rats for studying these processes. Few of them use female rats [2]. Relatively little is known about gender-dependent susceptibility to obesity and hepatic injury induced by nutritional factors. Such is the work of Pektas et al. (2017) who investigated dietary fructose-induced hepatic degeneration in male and female rats [8]. Dietary fructose activates both insulin signaling and inflammatory pathway in the adipose tissues of male and female rats proposing no correlation between the tissue insulin signaling and inflammation [7].

The aim of our study was to detect morphological changes in the liver and adipose tissue of male and female rats with diet induced metabolic syndrome with high-fat, high-carbohydrate diet.

Materials and Methods

Male and female Wistar rats (n = 10) were fed with high-fat, high-carbohydrate diet (HFHCD) for 16 weeks. Control male and female rats (n = 10) were fed a normal diet for the same period of time. Metabolic control was determined by measuring body weight, BMI, adiposity, plasma biochemical parameter - total cholesterol, HDL and LDL cholesterol. Two-way ANOVA statistic analysis was used. Sections of the liver lobe and visceral mesenteric adipose tissue were fixed in 10% buffered formalin. Histopathological study was performed on the visceral mesenteric adipose tissue and liver (glycogen and lipid content) using routine staining with hematoxylin-eosin and histochemical PAS reaction and Sudan III staining.

Results

The obtained results show that dietary manipulated rats (male and female) began to increase their weight of the 11th week onwards, which was maintained until the end of the experiment (**Fig. 1**). Male and female dietary-manipulated rats had a higher weight at the end of the experiment (**Fig. 2**).

At the end of the experiment dietary-manipulated rats had a higher BMI as compared to the control rats (0.69 ± 0.02 g/cm^2 vs 0.63 ± 0.02 g/cm^2, $p < 0.01$). At the end of the experiment the diet had significant main effect on the total cholesterol plasma level, as it was significantly higher in dietary-manipulated rats compared to the controls (2.00 ± 0.09 mmol/l vs 1.67 ± 0.10 mmol/l, $p < 0.05$). The rats from the dietary-manipulated groups had lower concentrations of HDL-cholesterol compared to the control groups (1.11 ± 0.05 mmol/l vs 1.31 ± 0.05 mmol/l, $p = 0.01$).

No gender differences were noticed in the morphology of the liver of the dietary-manipulated rats. HFHCD leads to accumulation of a lot of lipid cytoplasmic inclusions in the hepatocytes of the experimental rats. These extensive lesions in the liver showed

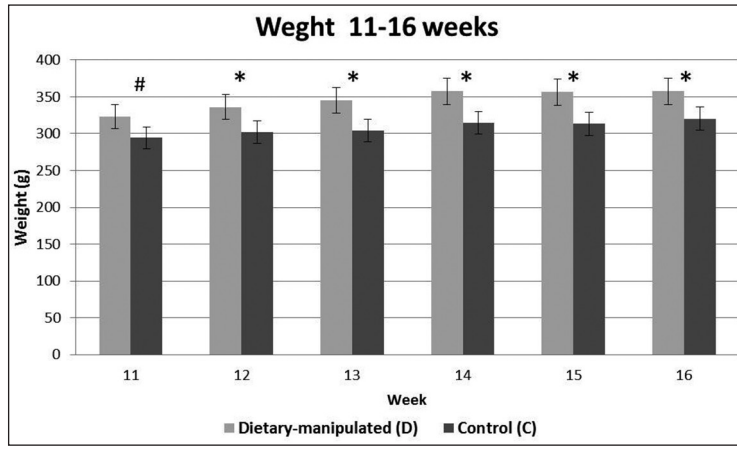


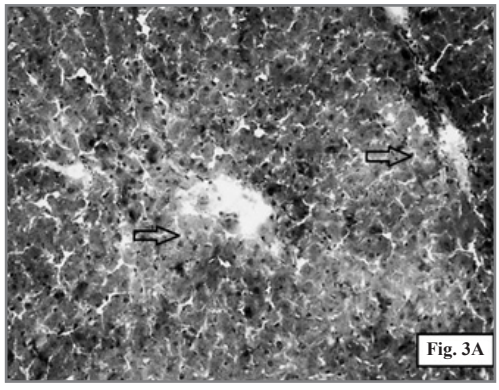
Fig. 1. Weight (g) of dietary-manipulated and control groups from the 11th week until the end of the experiment #P = 0.078, *p < 0.05 dietary-manipulated vs controls

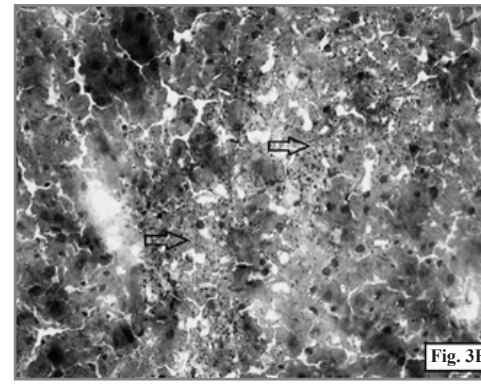
Group	Weight (g) 16th week
Male control group	371.50 ± 54.01
HFHCD male rats	394.50 ± 42.81
Female control group	269.33 ± 31.61
HFHCD female rats	320.17 ± 37.95

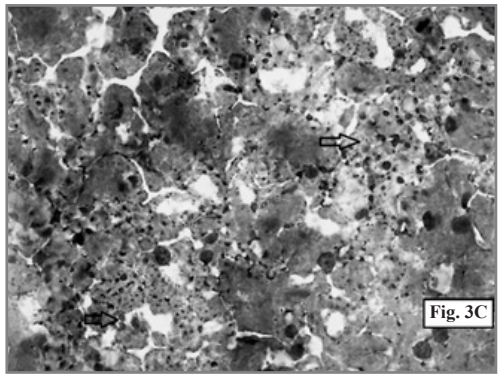
Fig. 2. Weight (g) of male and female rats: control and dietary-manipulated groups at the end of the experiment (16th week)

advanced lipodystrophy. Steatosis changes were observed in two areas - the periphery of the liver lobules and around the central vein (**Fig. 3A**). In the cytoplasm of the cells of these areas liposomes of various sizes were observed (**Fig. 3B**). Other cells had more severe dystrophic damage - microvesicles around the nucleus and large vacuoles in the periphery of the cytoplasm. There were also hepatocytes with irreversible lesions - presence of lipid cysts formed after rupture of the cell membrane and extracellular lipid droplets (**Fig. 3C**). Lipid inclusions stained with Sudan III were not observed in the hepatocytes of the control group (**Fig. 3D**).

Increased carbohydrate intake in the experimental group rats leads to the accumulation of glycogen inclusions in the liver hepatocytes. PAS-reaction was positive – a large number of cells equally scattered in the liver (**Fig. 4A**). Glycogen granules were accumulated to a greater degree in the hepatocytes, which do not have lipid inclusions (**Fig. 4B**). Cysts of extracted lipid droplets were seen (**Fig. 4C**). Hepatocytes of the control rats had a moderate amount of glycogen granules equally distributed in the cytoplasm of cells (**Fig. 4D**).







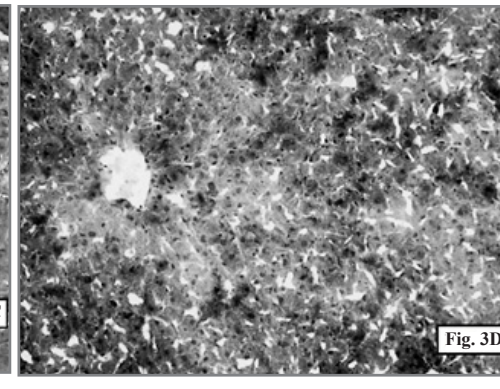


Fig. 3. A. HFHCD male rats' liver. Hepatic lobules with dystrophic processes around the central vein and periportal areas. Sudan III stainingp × 100; **B.** HFHCD male rats' liver. Wide area of hepatocytes with presence of large numbers of lipid droplets. Sudan III staining, × 200; **C.** HFHCD female rats' liver. Rounded hepatocytes with various degrees of lipodystrophy: lipid microvesicles, lipid vacuoles and lipid cysts. Presence of lipid droplets in extracellular space. Sudan III staining, × 400; **D.** Liver of the control group. Liver lobules with normal structure. Without lipid inclusions in the hepatocytes. Sudan III staining. × 100

No gender differences were noticed in the morphology of the adipose tissue of the dietary-manipulated rats. HFHCD leads to accumulation of extreme level of lipid inclusions in the cells of the adipose tissue of the experimental group rats compared to the controls. This was particularly expressed in the visceral mesenteric adipose tissue (**Fig. 5A**). Adipocytes were enlarged, as a result of the large lipid droplets, which occupied the whole cytoplasm. These drops pushed the nuclei into the periphery of the cells. The amount of the lipid drops in the adipose tissue of the controls is smaller compared to the dietary-manipulated rats (**Fig. 5B**).

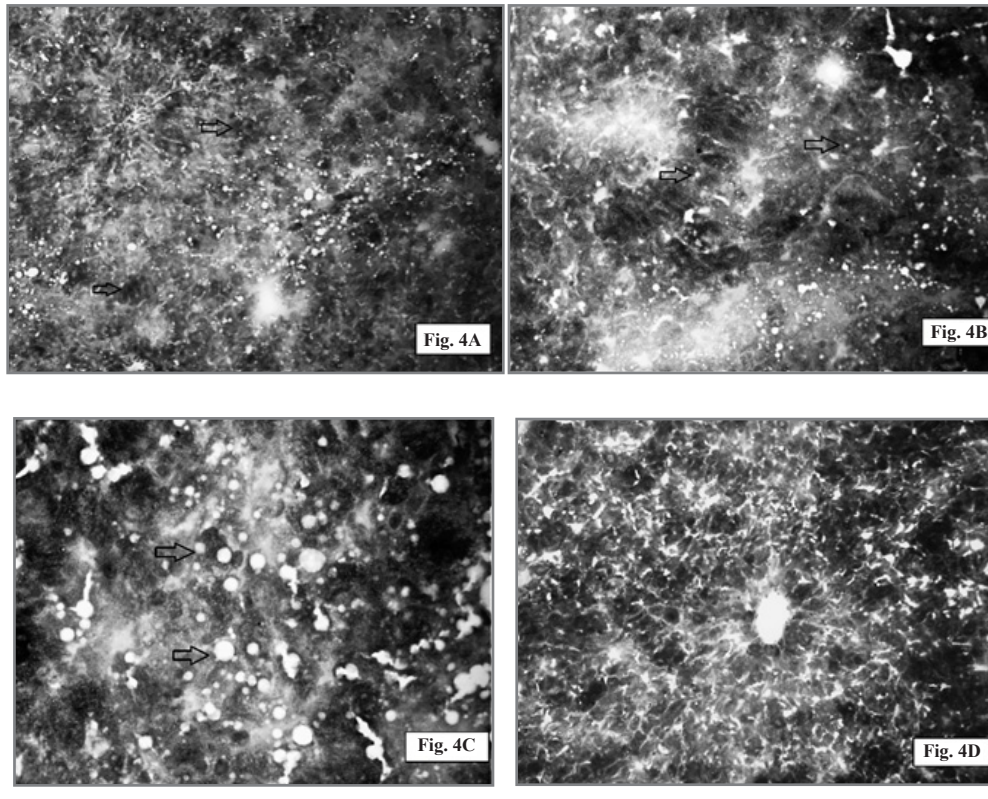


Fig. 4. A. Liver of HFHCD male rats. Drags of hepatocytes with raspberry-red glycogen granules in the cytoplasm. PAS–reaction, ×100; **B.** Liver of HFHCD male rats. Glycogen granules in hepatocytes without lipid inclusions. PAS–reaction, ×200; **C.** Liver of HFHCD female rats. Area a heavy dystrophy. Hepatocytes with glycogen and lipid inclusions, visualized by empty vesicles and vacuoles. PAS-reaction, × 400; **D.** Liver of control group. Hepatocytes with a uniform accumulation of glycogen granules in the cytoplasm. PAS-reaction, ×100

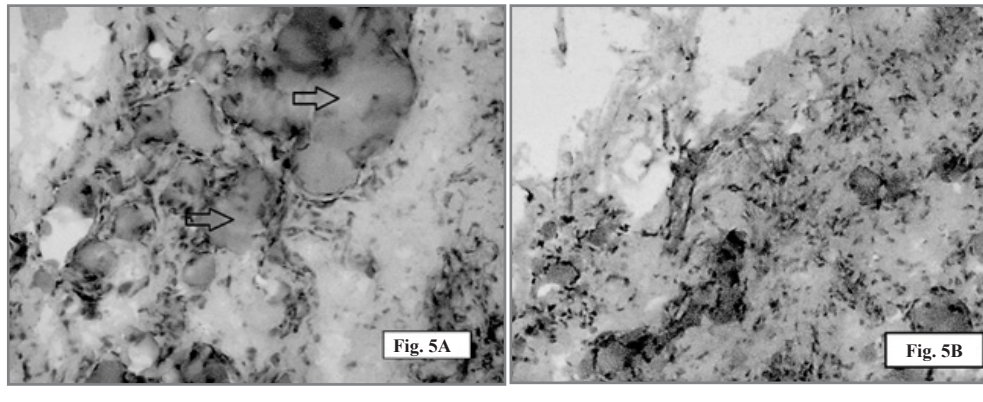


Fig. 5. A. HFHCD female rats' visceral mesenteric adipose tissue. Sudan III staining, × 400; **B.** Control group. Visceral mesenteric adipose tissue. Sudan III staining, × 100

Discussion

We found that HFHC diet consumption resulted in obesity development in both male and female rats. It is responsible for increased body weight, adiposity and liver steatosis. Increased body weight was accompanied by increased lipid plasma levels. We observed increased lipid deposition in the adipose tissue in both male and female HFHC diet fed rats. Lipid deposition was also seen in the liver of the same groups. Obesity increases the risk for non-alcoholic fatty liver disease through adipokine dysregulation and inflammation [2]. Our results were concomitant to a certain extent with other authors' data, regarding the effect of high-fructose diet [9], high-fat diet [5] or high-fat, high-carbohydrates diet [1] in rats. The increased consumption of high-fructose corn syrup increased triglyceride content and caused mild microvesicular steatosis in liver of male rats [9]. An increase in body and liver weight, fasting blood glucose, fasting insulin, serum aminotransferase (ALT) as well as lipid accumulation in the liver were documented in Sprague-Dawley rats fed with a high-fat and high-cholesterol diet [3]. Some of the works report that eight weeks of high fat, high carbohydrates diet caused 2.4-fold increase in body weights as well as hepatic-triglyceride content increased progressively and is enough for reaching obesity [1]. We think that a longer period of HFHC diet is more appropriate for this purpose, especially for the liver lipid dystrophy. Long-term fructose diet caused pronounced increase in body weight of males, but not of female rats [6]. Pektas et al. (2017) proved that long-term fructose diet caused parenchymal degeneration and hyperemia in liver of male and female rats and also led to increase in hepatic triglyceride content [8]. Some gender differences were found concerning body weight and plasma lipid levels. High-fructose diet increased plasma insulin, triglyceride and VLDL levels as well as omental weights in both genders [6]. But in another study, no gender-related differences, as a consequence of fructose consumption, were found by Pektas et al. (2017) [8]. In our study we also did not observed any gender-related differences in liver and adipose tissue disorders about the effects of HFHC diet in female and male rats.

Conclusion

We proved that diet rich in fats and carbohydrates is strongly associated with the development of obesity in male and female Wistar rats. It is also associated with liver steatosis. Intake of HFHCD establish the Wistar rats as an excellent experimental model for the study of metabolic and hepatic abnormalities resulting from obesity.

Acknowledgements: This study was financed by Medical University of Plovdiv - project SDP 09/2015.

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