Lipogenesis and lipid peroxidation in high-fructose and cafeteria diet rodent models

T. Mašek[1], P. Roškaric[2], M. Mauric[3], J. Barišic[4], M. Šperanda[5], K. Severin[6], K. Starcevic[2]

[1] Department of Animal Nutrition and Dietetics, Faculty of Veterinary Medicine, University of Zagreb, Croatia;
[2] Department of Chemistry and Biochemistry, Faculty of Veterinary Medicine, University of Zagreb, Croatia. e-mail: kristina.starcevic@vef.hr;
[3] Department of Animal Breading and Livestock Production, Faculty of Veterinary Medicine, University of Zagreb, Croatia
[4] Laboratory for Biotechnology in Aquaculture, Division of Materials Chemistry, Ruder Boškovic Institute, Croatia;
[5] Department of Animal Science, Faculty of Agriculture, University of Osijek, Croatia;
[6] Department of Forensic and State Veterinary Medicine, Faculty of Veterinary Medicine, University of Zagreb, Croatia

Objective

Our aim was to investigate the differences in lipogenesis of the different animal models of metabolic syndrome with the sex as additional variable.

Introduction

High fructose and cafeteria diet are extensively used rodent models in the investigations of metabolic syndrome. Although interchangeably used, these models have specific differences in lipogenesis. Therefore, we investigated the differences in the fatty acid profile and the extent of lipid peroxidation in these rodent models as well as the influence of sex.

Methods

Two month old Wistar rats (15 male and 15 female) were divided into control group (CON), high fructose group (HF, 15% of fructose in the drinking water) and cafeteria diet group (CAF) which was made by mixing basal diet and cafeteria diet in 1:1 ratio. The animals were kept in polycarbonate cages and fed with experimental diets over a period of 16 weeks. After 16 weeks liver and perirenal adipose tissue (fat) were harvested and frozen at -80°C.

Fatty acid composition: Analysis of fatty acid composition was performed using a Shimadzu GC-MS QP2010; Ultra Gas Chromatograph Mass Spectrometer equipped with capillary column BPX70.

Lipid peroxidation evaluation: Malondialdehyde (MDA) content of liver tissue homogenate was measured by the HPLC method onto a Shimadzu LC-2010CHT with an Inert Sustain C18 column.

Western blotting: The liver tissue was homogenised in lysis buffer with added protease inhibitors for protein preparation. The proteins were run on 10% SDS polyacrylamide gel electrophoresis and electrotransferred from gels to a nitrocellulose membrane. The nitrocellulose membranes were subsequently incubated at 4 °C with HNE primary antibody and with horse radish peroxidase-conjugated secondary antibodies. Reactive bands were detected by chemiluminescence. The ß-actin was used as loading control.

Routine histology was used to produce tissue sections for histological analyses of rat livers.

For statistical data analysis, GraphPad 8 was used. Data were compared by analysis of variance and Tukey post hoc test.

Results

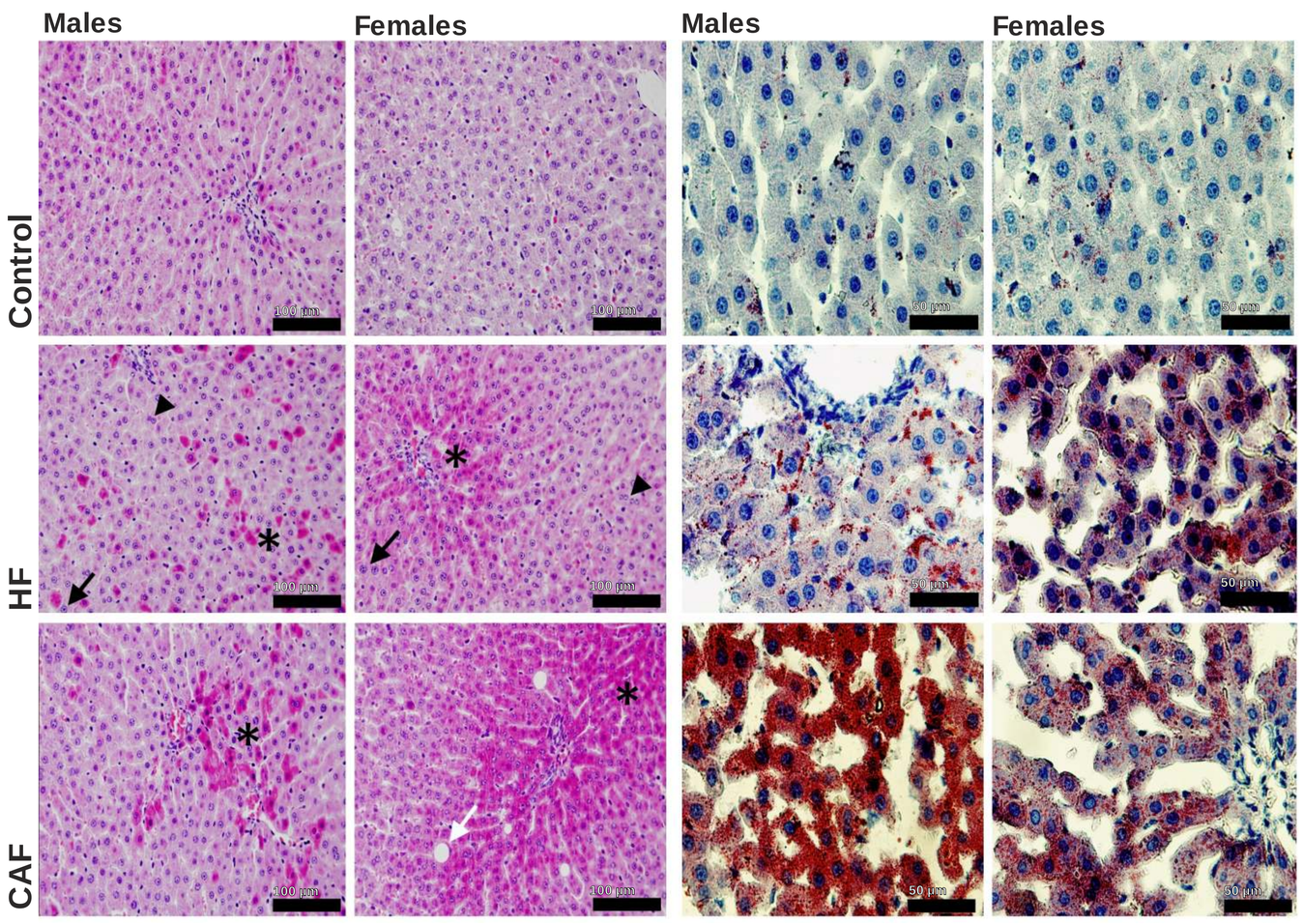

Figure 1. Hematoxylin and eosin (H&E) stained liver sections from male and female rats. Control group: normal histological appearance; high fructose diet (HF): incidence of binucleated hepatocytes (arrowhead); kariomegaly (black arrow); and areas with focal apoptosis in males and diffuse apoptosis in females (asterisks); Cafeteria diet (CAF): areas with diffuse apoptosis (asterisks), focal macrovesicular steatosis in females (white star). Magnification: 200×.

Figure 2. Oil Red stained liver sections from male and female rats revealing fat accumulation in hepatocytes. Control group: normal appearance of scattered microvesicular fatty droplets; High fructose diet (HF): Focal (males) and diffuse (females) microvesicular steatosis and Cafeteria diet (CAF): high accumulation of fatty droplets in hepatocytes, forming bigger vacuoles of fat. Magnification: 400×.

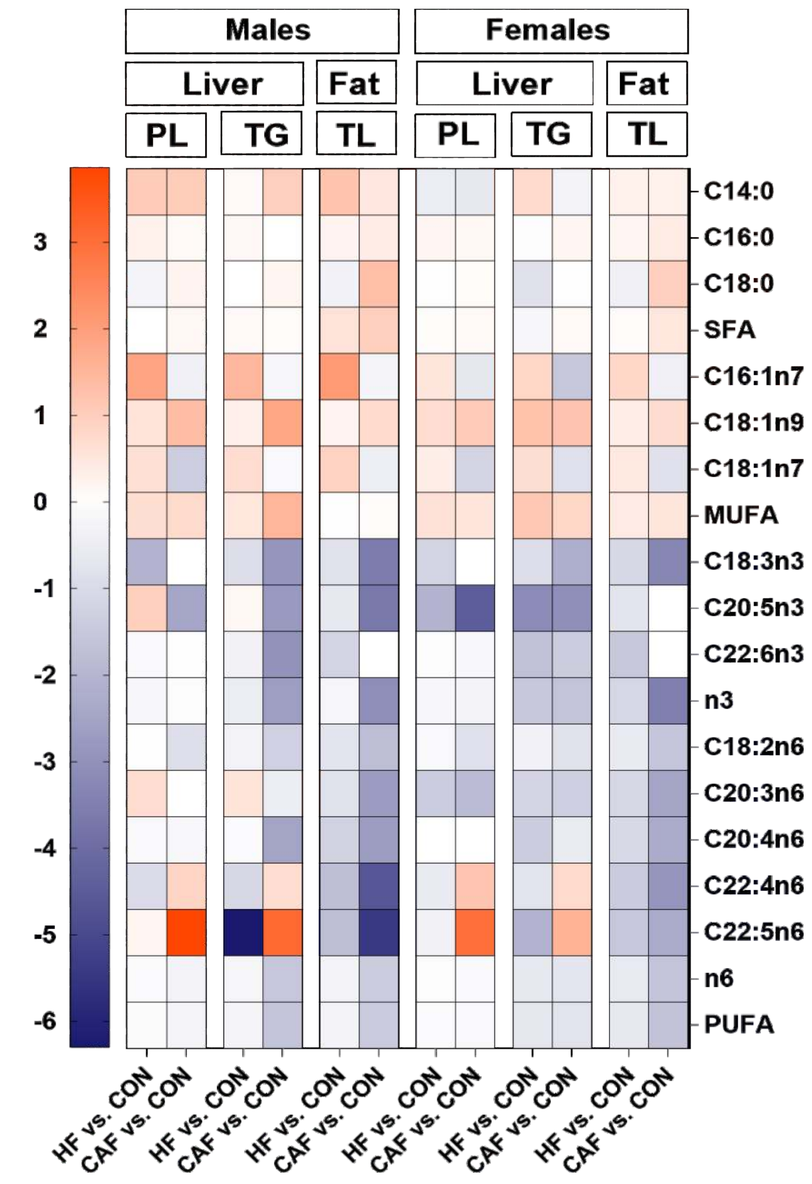

Figure 3. Heatmap showing log2 fold change of studied fatty acids in liver phospholipids (PL) and triglycerides (TG) and perirenal adipose tissue total lipids (TL). Experimental groups versus the Control group.

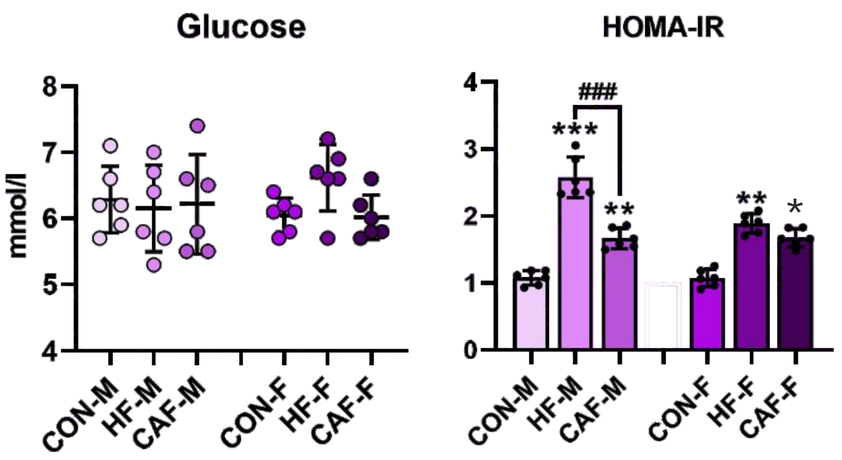

Figure 4. Non-fasting glucose values were not increased in the experimental groups, while HOMA-IR was increased in the HF and CAF diets in comparison to the CON. Male rats of the HF group showed significantly higher HOMA-IR than rats fed by CAF diet.

Figure 5. MDA as index of lipid peroxidation was increased in the CAF group in both male and female rats in comparison to the HF and CON rats.

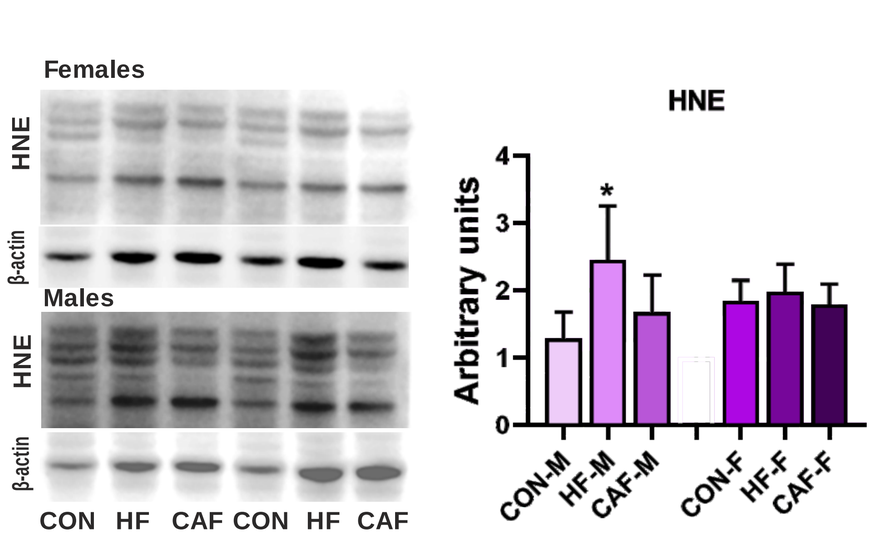

Figure 6. Western bloting analysis of the expression of the 4-hidroxynonenal-protein (HNE) in the liver. The expression of HNE protein was significantly increased in the HF male group.

Literature

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Conclusions

The results showed significant differences in lipogenesis and lipid peroxidation between examined models of metabolic syndrome.

The observed differences include fatty acids with important biological effects (e.g. EPA), which must be considered in investigations of metabolic syndrome.

Moreover, male and female rats respond differently to experimental diets.