Research Article

Metabolic syndrome: comparison of three diet-induced experimental models

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Abstract

The high-fat (HF) diets can be used to generate a valid rodent model for metabolic syndrome (METS). The aim of this study was to compare three different diets, namely a high-fat, high-carbohydrate diet (HF-HCD), a high-fat lard-based diet (HFD), and a cafeteria diet (CFD), in terms of the ability to induce METS. The next step was to characterize the syndrome according to the biochemical and histopathological changes in the liver and pancreas, and to determine the optimal animal model. As a result, all diets disturbed significantly the serum biochemical parameters. HF-HCD and CFD increased the uric acid levels and reduced the weight gain in comparison with the standard chow diet (SCD) and HFD. The HFD and CFD induced the highest fasting glycemia levels. Although the animals fed with HF-HCD had the lowest body weight, the most serious histopathological changes in the pancreas, hypertension, and oxidative stress were noted in them.

Keywords

Metabolic syndrome, high-fat diets, cafeteria diet, rat model

Introduction

Metabolic syndrome (METS) is a multifactorial pathological condition characterized by the simultaneous occurrence of at least three of the following five risk factors: obesity, especially visceral obesity, dyslipidemia with high triglycerides, low level of high-density lipoprotein (HDL) cholesterol, hypertension, and fasting hyperglycemia (Alberti et al. 2006). The risk factors characterizing METS are predictive for both cardiovascular diseases and type 2 diabetes, and affect between 20% and 30% of adult populations in most countries. Moreover, METS causes an increased risk of non-alcoholic fatty liver disease (Vanni et al. 2010), cancer (liver, pancreas, breast and bladder) (Johansen et al. 2010), kidney and pancreatic dysfunction (Panchal and Brown 2011).

The METS reasons are complex and resulted from an association between genetic, environmental and epigenetic factors. The main mechanisms that contribute to its development are insulin resistance and the abundance of circulating free fatty acids. These factors may have a genetic origin or could be caused by aging alterations but are often associated with obesity or at least an enlarged waist circumference, due to a sedentary lifestyle (Fuentes et al.

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2013). Adipose tissue produces pro-inflammatory cytokines responsible for the insulin resistance, lipolysis and liver production of pro-thrombotic factors. Thus, the inflammatory chronic state, elicited by obese adipose tissue through an abnormal adipokine production, results in endothelial dysfunction and a pro-thrombotic state (Fuentes et al. 2013). Lipolysis occurs in expanded subcutaneous adipose tissue as well as in hypertrophied visceral fat, in both cases affecting the hepatic metabolism with glucose production and synthesis of very low-density lipoproteins. The subsequent hypertriglyceridemia supports a change in the HDL composition, resulting in a decreased cholesterol content and increased clearance from the circulation. High levels of free fatty acids and reduced insulin activity may both lead to hyperglycemia, and contribute not only to increased glucose production by the liver but also to a reduced glucose uptake by muscle and adipose tissue (Eckel et al. 2005).

Recently, numerous reasons for the severe increase in overweight and obesity prevalence are presented. Genetic factors appear to be responsible for 40–70% of the variation of body mass index (BMI) (Pigeyre et al. 2016). However, the environmental factors play an important role for the weight gain and obesity. One of the most important factors is the lifestyle changes and diets that have occurred over the last three decades (Blüher 2019). Example of obesogenic diet is the excessive consumption of highly appetizing sugar and fat laden foods, often in the forms of "junk" or "fast" food, and it plays a key role in the development of obesity in humans (Mozaffarian et al. 2011; Basu et al. 2013).

The METS harmful effects attract research efforts in developing new interventions to reduce its weight on the healthcare system. Due to its multifactorial nature, selecting an adequate experimental model that best represents the pathophysiology of METS in humans can be rather challenging. Rats and mice are the most common animal models used for the METS investigating. Some of the various approaches used to induce METS in rodents include dietary manipulation, genetic modification and drugs (Wong et al. 2016). Numerous dietary approaches capable to induce METS in animals have been reported. They comprise the use of a single type of diet or a combination of diets, such as high fructose (HF), high-sucrose (HS), high-fat (HF), high fructose/-high-fat (HF-HF), or high-sucrose-high-fat (HS-HF) diets (Panchal and Brown 2016). A lot of studies have revealed that HF diets promote hyperglycemia and whole-body insulin resistance, and numerous researchers have examined their effects on muscle and liver physiology, and on insulin signal transduction. Based on this experience, it is generally accepted that HF diets can be used to generate a valid rodent model for the metabolic syndrome with insulin resistance and compromised β-cell function. However, these dietary interventions are not standardized, and the HF-induced phenotype varies distinctly among different studies. The question which HF diet type is best to model the meta-

bolic deterioration seen in human obesity remains unclear (Buettner et al. 2007).

Another used diet is the cafeteria diet (CFD). CFD model for animal experiments contains of the same tasty but unhealthy food products that people eat (e.g. hot dogs, muffins, cookies), and considers variety, novelty and secondary food features, such as smell and texture, and which can be easily obtained from supermarkets and fast food restaurant. For this reason, it is also called the "Junk Food Diet" restaurants, "Supermarket Diet" or "Western Diet". Therefore, the CFD model mimics a certain pattern of problematic human consumption. Thus, this model mimics human eating patterns better than other models. Using CFD, the metabolic and behavioral causes of eating junk food are the same in rodents and humans, as both share the same etiology.

The object of this study is to compare three different diets in terms of the ability to induce the METS and to characterize the syndrome according to the profile of the MS components and thus, to determine the optimal animal model. We aimed to compare biochemical and histopathological changes in the liver and pancreas obtained with different HF diet approaches.

Materials and methods

Animals, study design and diets

Animals

An interventional comparative study was designed. The experiments were conducted according to the Guidelines for Animal Care. The Animal Care Ethics Committee approved the study protocol and issued an ethical clearance (No. 346 of 28.02.2023) from the Bulgarian Food Safety Agency.

The rats were housed, maintained and euthanized in accordance with the relevant international rules and recommendations as outlined in the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS 123).

Sixteen male Wistar rats at three months of age (200–300 g) were used. Rats were housed in Plexiglas cages (4 per cage) in a 12/12 light/dark cycle under standard laboratory conditions (ambient temperature 20 ± 2 °C and humidity 72 ± 4%). All animals were purchased from the National Breeding Center, Sofia, Bulgaria and allowed a minimum of 7 days to acclimate to the new conditions before the start of the study. Food and fresh drinking water were available ad libitum throughout the experimental period of 10 weeks.

Experimental design

The rats were divided into four groups with four animals in each (n=4):

Group 1 (SC) - control group fed with a standard chow;

Group 2 (HF-HC) - rats fed with high fat - high carbohydrates diet (prepared in the Department of Pharmaceutical Technology at the Faculty of Pharmacy, Medical University - Sofia)

Group 3 (HFD) - rats fed with a lard-based high-fat diet (Gancheva et al. 2015).

Group 4 (CFD) - rats fed with fat- and sugar-rich supermarket foods, or cafeteria diet (Hristov et al. 2020).

Groups 2, 3, and 4 received an additionally 10% Fructose for drinking.

The body weight and the blood glucose level of the experimental animals were measured once a week for 10 weeks.

At the end of the experimental period, after overnight starvation, the animals were sacrificed with a laboratory guillotine, blood was collected, and serum biochemical parameters were measured. Afterwards, the livers were taken to assess the oxidative stress biomarkers malone dialdehyde (MDA) and glutathione (GSH), the activity of the antioxidant enzymes glutathione peroxidase (GPx), catalase (CAT) and superoxide-dismutase (SOD). Small pieces from the livers and pancreas were taken and fixed in 10% buffered formalin for histopathological investigation.

Diets

The rats in the control group were fed a standard diet appropriate to their species and age, and had access to clean drinking water in unlimited quantities. The diet of rats in the HFD group was prepared by mechanically mixing standard rodent chow with 17% lard. The rats of the third group (HF-HC) received a diet formulated by the Department of Pharmaceutical Technology. The fourth group of rats was fed a so-called cafeteria diet, which included a variety of packaged foods such as wafers, corn balls, fried peanuts, potato chips, and others purchased from the supermarket. The nutrient composition of CFD and HFD is presented in Table 1.

Preparation of fat and carbohydrates enhanced diet (HF-HCD) for rat feeding

The HF-HCD (Table 2) was prepared by moulding from a melt. Preparation of the base started with the melting of lard at 40 °C. Then cocoa butter was added, which should be melted at 35–36 °C because of the presence

of a significant amount of oleic acid that causes unstable crystalline modifications. Suppose that the temperature regime is not observed after cooling. In that case, the melting temperature of the cocoa butter drops to 23–24 °C, and it isn't easy to apply and store the resulting food at room temperature.

As sucrose, fructose, starch, protein, and standard rat chow are insoluble in the base (lard and cocoa butter), they are suspended in the melted base.

Pouring in the moulds is carried out in the so-called "cream" state when the processes of recrystallization in the base have already started. This method guarantees the absence of sedimentation of the dispersed materials in the base and their uniform distribution throughout the entire volume of the mould.

The displacement value (f) of solid substances in the base is initially determined to ensure the exact amounts of ingredients by using the formula:

$$f = \frac{100(N.E - G_n)}{G_n.X}$$

where,
N – number of pieces
E – the average mass of one piece without dispersed substances, g;
G_n – total mass of pieces with dispersed substances, g;
X – the content of dispersed substances in weight %.

The formula determines the calculation of the required amount of base using the displacement value:

$$M = F - (f_1.S_1 + f_2.S_2 + \ldots \ldots f_n.S_n) ,$$

where,
M - the amount of base for preparing the exact number of flat cylinders;
F - the capacity of the mould (g);
f - displacement coefficient of the substances that will be included in the base;
S - the amount of substance included in the base

Assessment of serum biochemical parameters

The weekly measurement of blood glucose levels was performed using a blood drop from the tail vein and mini glucometer Accu-Chek® (Roche diagnostics).

Table 1. Nutrient composition of standard rat show (SC), cafeteria (CF) and high fat (HF) diets.

Food item	Energy density (kcal)	Protein (g)	Total fat (g)	Total Carbohydrate (g)	Sugars (g)	Fibers (g)
SC	272	20.09	1.75	42.2	3.8	6.7
Roasted and salted peanuts	634	24	22	50	ND	4
Fried potato chips	530	5,60	35	48	ND	ND
Corn balls	452	7.40	20.70	59	ND	ND
Bruschette Maretti*	466	8,80	65,50	18,80	ND	2.30
Popcorn with butter	518	9	57.10	28.10	ND	ND
Waffle with chocolate filling	544	3.40	30.20	64.70	1.01	ND
HFD (SC + Lard)	902	20.09	18	42.2	3.8	6.7

ND – no data.

Table 2. Composition and caloric content of HF-HCD.

Content		g/piece (2 g)	Kilocalories (kcal) g/100 g	1 piece (2 g)	100 g
Fats		1.15	**57.5**	35.72	1786
	Cocoa butter	0.65	32.5	17.68	884
	Lard	0.50	25.0	18.04	902
Carbohydrates		0.65	**32.5**	22.12	1106
	Sucrose	0.20	10	7.74	387
	Fructose	0.20	10	7.36	368
	Wheat starch	0.25	12.5	7.02	351
Protein (milk)		0.10	**5.0**	7.34	367
Rat chow		0.10	**5.0**	5.44	272

Biochemical markers such as glucose, urea, creatinine, uric acid (UA), total protein, albumin, transaminases L-aspartate aminotransferase (ASAT) and L-alanine aminotransferase (ALAT), total bilirubin, direct bilirubin, amylase, total cholesterol, triglycerides, creatine kinase (CK), and creatine kinase-myocardial band (CK-MB) were assessed using commercial kits for biochemical analyser "Mindray BS-120" as described by the manufacturer.

Measurement of arterial blood pressure

The blood pressure and the heart rate were measured at the beginning and at the end of the experiment using CODA non-invasive blood pressure system from Kent Scientific. Changes in the tail volume are detected using volume-pressure recording technology. Those changes correspond to systolic and diastolic blood pressure. The method relies to two tail-cuffs to measure blood pressure: occlusion cuffs (O-cuff) and volume-pressure recording cuffs (VPR-cuff). At the beginning, the animals are restrained in specific cages to limit their ability to move. The placement of the animals in the restraining cages should be very gentle to minimize the stress of the animals. The animals are left for 5 to 10 minutes to acclimatize and calm down. During this time, the restraining cage, together with the animals are put on a heating plate in order to increase the temperature in the tail for more accurate measurement of the blood pressure. The heating plate is set at 35 degrees, and the testing animals are covered with a specific blanket to maintain this temperature. The measurement consists of 15 cycles, with 5 seconds between the cycles. Mean values of the blood pressure and heart rate are than calculated and used to compare between the groups (Daugherty et al. 2009; DiPetrillo 2009; Wang et al. 2017).

Measurement of lipid peroxidation (LPO) in liver homogenate

Lipid peroxidation was determined by measuring the formation rate of thiobarbituric acid (TBARS) (expressed as malondialdehyde (MDA) equivalents) as described by Polizio and Pena (2005). The amount of MDA was calculated and expressed in nmol/g wet tissue.

Measurement of reduced glutathione (GSH) in liver homogenate

GSH was estimated by measuring non-protein sulfhydryls after protein precipitation with trichloracetic acid (TCA) using the method described by Bump et al. (1983). Absorbance was determined, and results were expressed in nmol/g wet tissue.

Preparation of liver homogenates for antioxidant enzyme activity measurement

The excised livers were rinsed in ice-cold physiological saline and minced with scissors. 10% homogenates were prepared in 0.05 M phosphate buffer (pH=7.4), centrifuged at 7,000 × g, and the supernatant was used for antioxidant enzymes assay. The protein content of liver homogenate was measured by the method of Lowry (1951).

Catalase activity (CAT) (Aebi 1974). Briefly, 10 μL of homogenate was added to 1990 μL of H_2O_2 solution. CAT activity was determined by measuring the decrease in absorbance at 240 nm. The enzyme activity was expressed as nmol/mg/min.

Superoxide dismutase activity (SOD) was measured according to the method of Misra and Fridovich (1972), following spectrophotometrically the autoxidation of epinephrine at pH=10.4 at 30 °C, using the molar extinction coefficient of 4.02 $mM^{-1}cm^{-1}$.

Glutathione peroxidase activity (GPx) was measured by NADPH oxidation, using a coupled reaction system consisting of glutathione, glutathione reductase and cumene hydroperoxide (Tappel 1978). Results are expressed as nmol/mg/min.

Histopathological studies

For light microscopic evaluation, liver tissues and pancreas were fixed in 10% buffered formalin, and, then, thin sections (4 μm) were subsequently stained with hematoxylin/eosin to determine general histological features (Bancroft et al. 2008). Sections were studied under a light microscope Leica DM 500.

Results and discussion

Weekly blood glucose level monitoring

Weekly monitoring of blood glucose levels showed that at the end of the experimental period, blood glucose was elevated to the highest degree in animals fed HF-HCD and CFD by 32% and 53% respectively, compared to the control animals. In the past, peaks of postprandial hyperglycemia were attributed only to simple carbohydrates such as mono- or disaccharides like fructose or sucrose, but in recent years it has been found that some complex carbohydrates can exhibit the same hyperglycemic peaks when rapidly digested (Bessesen et al. 2001). The rapid catabolism of the highly refined corn starch used to make the corn balls, as well as the wafers administered in CFD, may explain the high fasting glucose levels seen in this group of animals.

Serum biochemical parameters

Serum biochemical parameters are presented in Table 3. Urea was lowered by 66%, 44%, and 70% in the HF-HC, HFD, and CFD-fed animals, respectively, relative to controls, but within the reference range. The uric acid level was higher in HF-HCD and in CFD by 44% and 102%, respectively, relative to control rats, probably due to high fructose intake which normally is metabolized to uric acid. Increased serum uric acid levels are commonly seen in patients with metabolic syndrome and are widely accepted as risk factors for hypertension, gout, non-alcoholic fatty liver disease, chronic kidney disease (CKD), and cardiovascular diseases. The high values of total protein and albumin are remarkable. Total protein was increased by 19, 21, and 24% relative to controls in HF-HCD, HFD, and CFD, respectively, and albumin was elevated by 61, 70, and 59%, respectively (Cho et al. 2012) found that the higher serum albumin levels are positively associated with

METS, probably through increased abdominal obesity, high fasting blood glucose, and triglycerides. Transaminases were also elevated relative to both controls and reference values. ALAT activity was increased by more than 200% in all diet groups, and ASAT activity was increased by more than 300% in all groups of experimental animals. Studies have demonstrated the association of METS with elevated ALAT and ASAT levels (Hanley et al. 2005; Liangpunsakul et al. 2005; Andre et al. 2007; Nikniaz et al. 2018).

Total bilirubin was elevated by 71% relative to control only in the group fed with the diet prepared by our Faculty. Amylase activity was many times lower, relative to control, by about 90% in all groups. Low serum amylase activity has been reported in certain common conditions such as obesity, diabetes (regardless of type), and metabolic syndrome, all of which appear to have a common etiology of insufficient insulin action due to insulin resistance and/or diminished insulin secretion (Nakajima et al. 2016). All these abnormalities in biochemical parameters indicate an extremely severe impairment of renal, hepatic, and pancreatic function, as well as damage to the musculoskeletal system and connective tissue, probably associated with myositis, rhabdomyolysis, and hence renal involvement. These results reveal a very severe nutritional and metabolic disorder, probably with endocrine dysfunction and hypothyroidism, which is also reflected in the weight change of the animals (Fig. 1).

It is worth noticed that cholesterol and triglycerides were in the normal reference range in all animal groups, which is most likely related to body weight reduction (Fig. 1).

The activity of CK and CK-MB fractions was extremely elevated in all groups of animals, which is probably related to the method of euthanasia and the abrupt release of these fractions from the muscle tissue of the animals before decapitation (O'Brien et al. 1997).

Table 3. Effect of the different diets on biochemical parameters, compared to reference values (n=6).

Parameters	SCD	HF-HCD	HFD	CFD	References
GLU mmol/L	5.23 ± 0,22	6.89 ± 0.33[ad]	5.81 ± 0.3[bd]	7.99 ± 0.28[abce]	4,2–7,5
UREA mmol/L	9.41 ± 0,32	3.18 ± 0.28[ac]	5.31±0.3[abd]	2.82 ± 0.18[ac]	3.8–9.5
CREAT μmol/L	48.83 ± 3,3	38.05 ± 4.1[ade]	45.35 ± 4.4	49.6 ± 3.8	30–48
UA mmol/L	45 ± 2,9	65 ± 2.5[acde]	40.5 ± 3.2[bd]	91 ± 2.1[abc]	12–54
TP g/L	58.8 ± 1.2	70.15 ± 1.5[ae]	70.9 ± 1.8[ae]	73.1 ± 1.2[ae]	53–63
ALB g/L	26.4 ± 1.8	42.6 ± 1.3[ae]	44.8 ± 1.8[ae]	42 ± 2.2[ae]	26–29
ASAT U/L	48.7 ± 4,5	231.6 ± 4.2[ae]	204.3± 4.3[ae]	231.4 ± 6.2 [ae]	49–98
ALAT U/L	28.3 ± 2,8	89.3 ± 2.2[ae]	87.9 ± 2.2[ae]	80.7 ± 1.8 [ae]	23–50
T-Bil μmol/L	7.2 ± 0,42	12.3 ± 0.38 [ae]	6.1 ± 0.51	10.1 ± 0.56 [a]	3.9–9.6
D-Bil μmol/L	2.4 ± 0,22	4.3 ± 0.41[a]	5.0 ± 0.35[ad]	3.3 ± 0.28	0–6.8
AMYL U/L	1390.8 ± 12,3	163.4 ± 8,4[acde]	295.15±9,5[abde]	51.05±3,5[acd]	1371–3207
CHOL mmol/L	1.01 ± 0.23	1.21 ± 0.31	1.19 ± 0.26	1.44 ± 0.42	1.1–2.1
TRIG mmol/L	0.4 ± 0.03	0.65 ± 0.02[ac]	1.05±0.04[abd]	0.72 ± 0.02 [ac]	0.3–2.1
CK U/L	3915.5 ± 16,3	2672.5 ± 123	4058.5±136	3928.5 ± 115	100–900
CK-MB pg/ mL	1164 ± 11.2	1193.5 ± 83.3	1053 ± 74.6	767.5 ± 36.6	30–50

[a]p< 0.05 vs control SCD; [b]p< 0.05 vs HF-HC diet; [c]p< 0.05 vs HFD; [d]p< 0.05 vs CFD; [e]p< 0.05 vs reference value (Boehm et al. 2007).
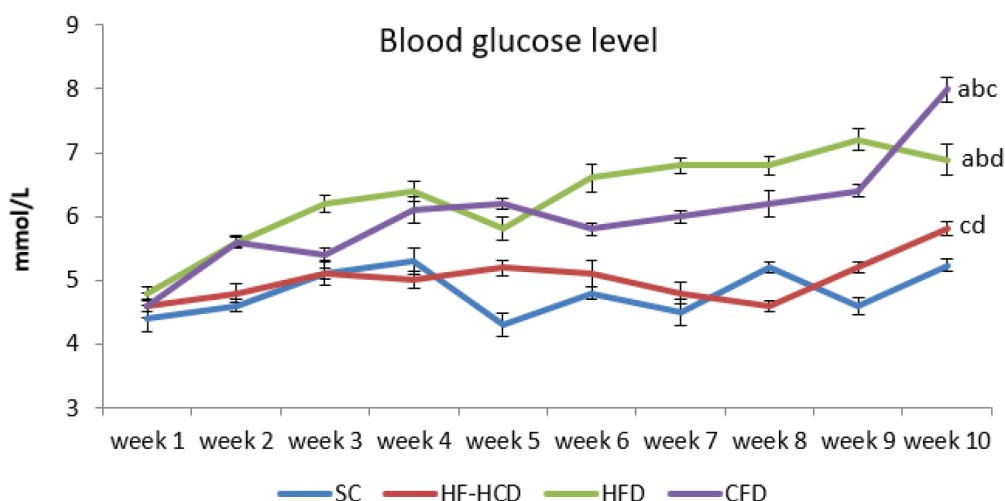
Figure 1. Weekly blood glucose (mmol/L) changes. Abbreviations: SCD (control group fed with a standard chow), HFD (lard-based high-fat diet), HF-HCD (high fat - high carbohydrates diet), and CFD (fat, salt, and sugar-rich supermarket foods (cafeteria diet). [a]p< 0.05 vs control SCD; [b]p< 0.05 vs HFD diet; [c]p< 0.05 vs HF-HCD; [d]p< 0.05 vs CFD.

Body weight changes

The change in body weight of the experimental animals is reflected in Fig. 2. At the beginning of the experiment, all the animals included in the four study groups had similar body weights. Body weight gain in the HFD group was progressively higher than in the other groups and showed weight gain, about 42%, respectively, from baseline weight. The animals fed the normal rodent chow without any other supplements gained by 29% (p< 0.05) compared to the beginning of the experiment. The HF-HCD and CFD groups showed a reduction of the weight with 18% and 16%, respectively compared with the first day of the experiment. We assumed that the weight loss in these groups is related to the intake of a small amount of food due to the unpleasant taste and lack of consistency of the food they are used to. Differences in the consistency of the food produced differences in food consumption when compared to standard chow (SC) and HFD, which in fact was a normal SC covered with lard.

Blood pressure measurement

The measurement of arterial blood pressure at the beginning and at the end of the experiment showed that systolic blood pressure (SBP) was increased to the highest degree in animals fed HF-CD and HFD, by 36 and by about 20%, respectively (Table 4). Hypertension is one of the main constituents of METS. Hypertension is strongly associated with metabolic syndrome through the pathophysiology, which involves obesity (Stanciu et al. 2023). Dietary fat contributes to the elevation of blood pressure and increases the risk of stroke and coronary artery disease. The increase of plasma fatty acids may contribute to the development of hypertension via a process involving the elevation of Ca^{2+} current density and an alteration of channel kinetics in the vascular smooth muscle membrane (Wilde et al. 2000).

Level of MDA and GSH

In the HF-HCD group, the amount of MDA (Fig. 3) was the highest by 188% compared to the controls and to a smaller extent to other groups, while the level of GSH (Fig. 4) was the lowest (75%), compared to the controls, indicating that this group was the most affected by oxidative stress. This pro-oxidant state was confirmed by the data obtained by measuring the activity of antioxidant enzymes (Fig. 5).

Antioxidant enzyme activity

The activities of the antioxidant enzymes CAT, SOD, and GPx were statistically significantly reduced in the group fed HF-HCD by 26%, 22%, and 28%, respectively, compared to control animals (Fig. 5). In the HFD group, we also observed an albeit smaller decrease in the activity of these enzymes, by 10, 15, and 12%, respectively. Many pieces of evidence suggest that increased oxidative stress may be considered the key to the generation of insulin resistance, diabetes, and cardiovascular disease. Data support the concept that free radical overgeneration may play an important role in METS and related manifestations (Ceriello et al. 2004; Mahjoub et al. 2012).

Oxidative stress could be an early event in the pathology of these chronic diseases rather than merely a consequence (Roberts et al. 2009). No change in the activity of antioxidant enzymes was observed in CFD-fed animals. This is probably related to the availability of antioxidant supplements to these foods available in supermarkets.

Table 4. Initial and terminal systolic blood pressure (SBP).

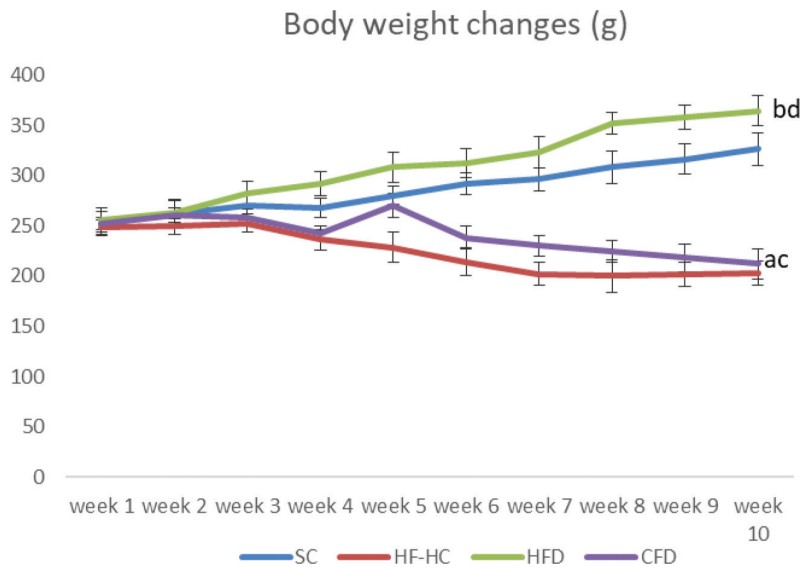| Group	Initial SBP	Terminal SBP	% change vs the initial SBP
SCD	122.5 ± 6.3	137.6 ± 8.2	12.3
HF-HCD	126.8 ± 9.1	172.5 ± 6.2	36*
HFD	118.7 ± 8.8	142.3 ± 4.6	19.9*
CFD	123.3 ± 6.6	127.4 ± 9.8	3.3

Body weight changes (g)

Figure 2. Weekly body weight changes (in grams). Abbreviations: SC (control group fed with a standard chow), HF-HC (high fat - high carbohydrates diet), HFD (lard-based high-fat diet), and CFD (fat, salt, and sugar-rich supermarket foods (cafeteria diet). [a]$p < 0.05$ vs control SCD; [b]$p < 0.05$ vs HF-HC diet; [c]$p < 0.05$ vs HFD; [d]$p < 0.05$ vs CFD.
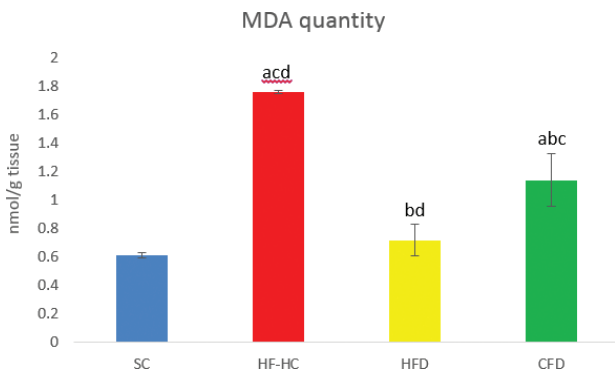
MDA quantity

Figure 3. Malondialdehyde quantity in the liver homogenate from rats fed with different diets; [a]$p < 0.05$ vs control SCD; [b]$p < 0.05$ vs HF-HC diet; [c]$p < 0.05$ vs HFD; [d]$p < 0.05$ vs CFD.
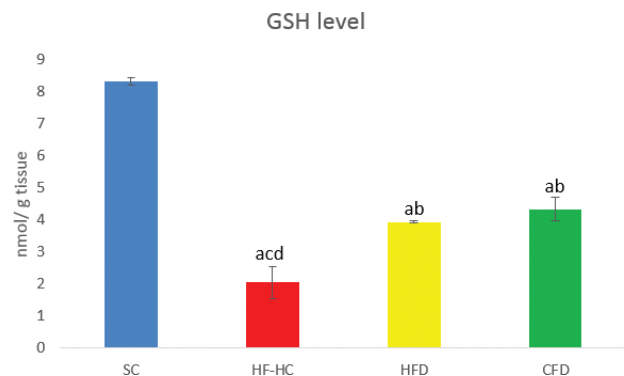
GSH level

Figure 4. Level of reduced glutathione (GSH) in the liver homogenate from rats fed with different diets; [a]$p < 0.05$ vs control SCD; [b]$p < 0.05$ vs HF-HC diet; [c]$p < 0.05$ vs HFD; [d]$p < 0.05$ vs CFD.

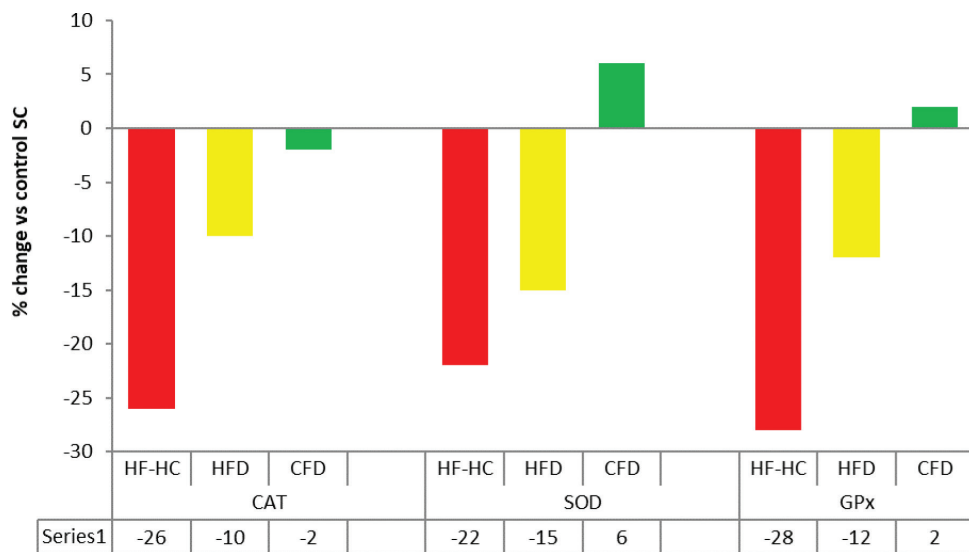|	HF-HC	HFD	CFD		HF-HC	HFD	CFD		HF-HC	HFD	CFD
		CAT				SOD				GPx	
Series1	-26	-10	-2		-22	-15	6		-28	-12	2

Figure 5. Changes (%) in the activity of antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) in the liver homogenate from rats fed with different diets, compared with control rats, fed with the normal rat chow. [a]$p < 0.05$ vs control SCD; [b]$p < 0.05$ vs HF-HC diet; [c]$p < 0.05$ vs HFD; [d]$p < 0.05$ vs CFD.

Histopathological studies

Morphological changes in the livers and pancreas are presented in Fig. 6. In the control animal group, histopathological changes in both investigated organs were not seen. The liver and Langerhans islets of the pancreas were without fatty deposits, and the structure of organs was normal. In the HF-HCD group fatty and glycogen degeneration was seen. Massive deposition of adipose tissue between the pancreatic lobules was visible. Non-alcoholic steatohepatitis (NASH) is considered the hepatic manifestation of METS. In the HFD group, weak morphological changes in the liver were shown. Slightly increased fatty tissue between the pancreatic lobules was also visible (Patton et al. 2010). The fatty pancreas also showed a strong correlation with metabolic syndrome. Animal studies reported that lipid deposition in pancreas islet cells due to a high fat/high glucose diet could damage pancreatic beta cells and make them hyperglycemic (Yin et al. 2004). Metabolic conditions give rise to pancreatitis (Kota et al. 2013). CFD group was presented with slight degenerative changes in hepatocytes, predominantly in the periphery of the lobules, and the pancreas was with preserved structure.
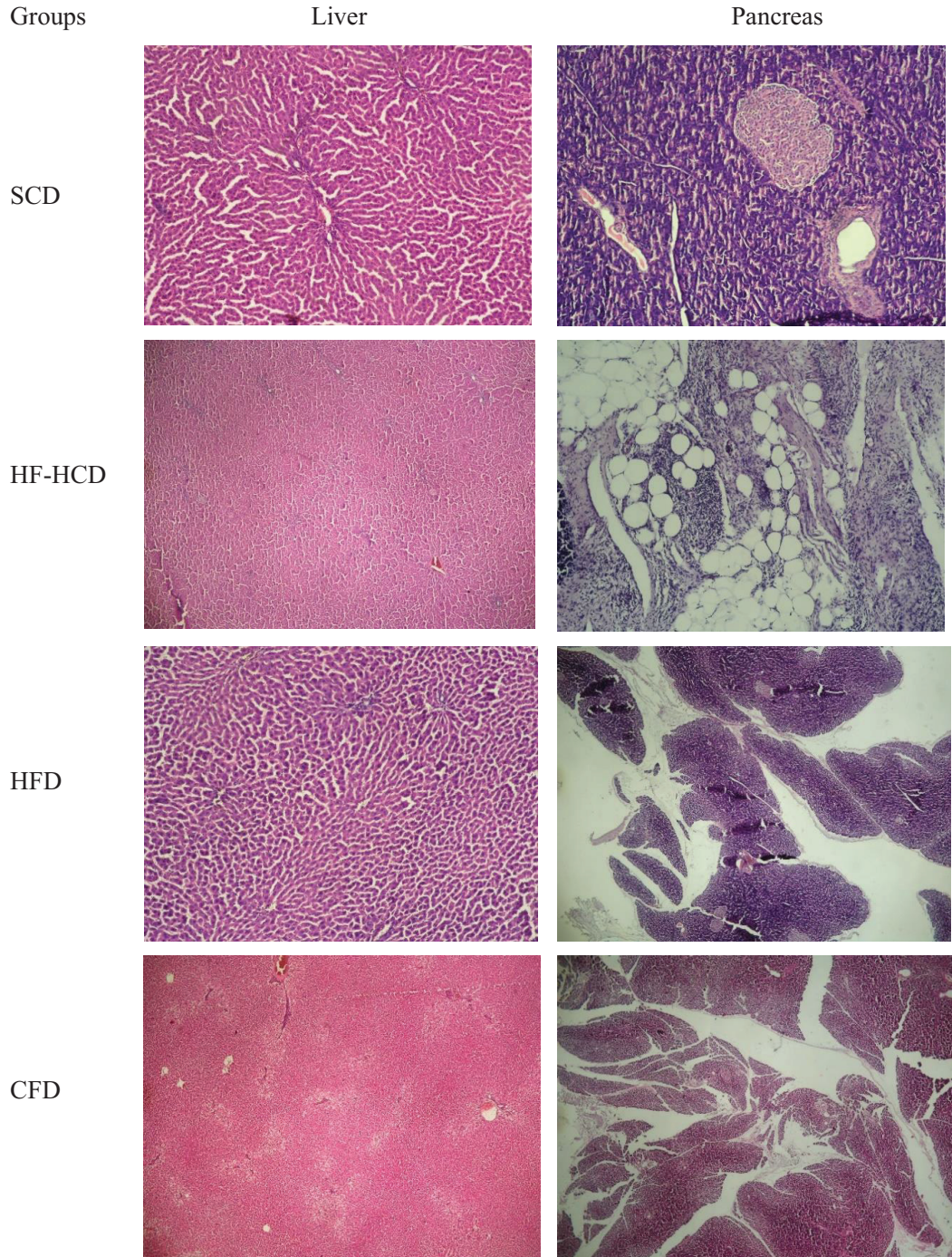
Groups	Liver	Pancreas
SCD		
HF-HCD		
HFD		
CFD		

Figure 6. Histological profile.

Conclusions

All three compared diets affected significantly the liver and serum biochemical parameters. The most serious histopathological damage, hypertension, and oxidative stress were caused by HF-HCD prepared in the Department of Pharmaceutical Technology, Faculty of Pharmacy, Medical University - Sofia. According to our study, this type of diet is most suitable for inducing metabolic syndrome and the comorbid complications that accompany it, in experimental animals.

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References

Aebi H (1974) Catalase. In: Bergmeyer HV (Eds) Methods in Enzymatic Analysis. Academic Press Inc., New York, 673–686. https://doi.org/10.1016/B978-0-12-091302-2.50032-3

Alberti KG, Zimmet P, Shaw J (2006) Metabolic syndrome - a new worldwide definition. A Consensus Statement from the International Diabetes Federation. Diabetic Medicine 23(5): 469–480. https://doi.org/10.1111/j.1464-5491.2006.01858.x

Andre P, Balkau B, Vol S, Charles MA, Eschwege E (2007) Gamma-glutamyltransferase activity and development of the metabolic syndrome (international diabetes federation definition) in middle-aged men and women: Data from the epidemiological study on the insulin resistance syndrome (desir) cohort. Diabetes Care 30: 2355–2361. https://doi.org/10.2337/dc07-0440

Bancroft JD, Gamble M (2008) Theory and practice of histological techniques. Churchill Livingstone Publications, Edinburgh.

Basu S, McKee M, Galea G, Stuckler D (2013) Relationship of soft drink consumption to global overweight, obesity, and diabetes: a cross-national analysis of 75 countries. American Journal of Public Health 103: 2071–2077. https://doi.org/10.2105/AJPH.2012.300974

Bessesen DH (2001) The role of carbohydrates in insulin resistance. Journal of Nutrition 131: S2782–S2786. https://doi.org/10.1093/jn/131.10.2782S

Blüher M (2019) Obesity: global epidemiology and pathogenesis. Nature Reviews Endocrinology 15: 288–298. https://doi.org/10.1038/s41574-019-0176-8

Boehm O, Zur B, Koch A, Tran N, Freyenhagen R, Hartmann M, Zacharowski K (2007) Clinical chemistry reference database for Wistar rats and C57/BL6 mice. Biological Chemistry 388(5): 547–554. https://doi.org/10.1515/BC.2007.061

Buettner R, Schölmerich J, Bollheimer LC (2007) High-fat diets: modeling the metabolic disorders of human obesity in rodents. Obesity 15: 798–808. https://doi.org/10.1038/oby.2007.608

Bump EA, Taylor YC, Brown JM (1983) Role of glutathione in the hypoxic cell cytotoxicity of misonidazole. Cancer Research 43: 997–1002.

Ceriello A, Motz E (2004) Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. Arteriosclerosis, Thrombosis, and Vascular Biology 24(5): 816–823. https://doi.org/10.1161/01.ATV.0000122852.22604.78

Cho HM, Kim HC, Lee JM, Oh SM, Choi DP, Suh I (2012) The association between serum albumin levels and metabolic syndrome in a rural population of Korea. Journal of Preventive Medicine and Public Health 45(2): 98–104. https://doi.org/10.3961/jpmph.2012.45.2.98

Daugherty A, Rateri D, Hong L, Balakrishnan A (2009) Measuring blood pressure in mice using volume pressure recording, a tail-cuff method. Journal of Visualized Experiments 15(27): 1291. https://doi.org/10.3791/1291-v

Eckel RH, Alberti KG, Grundy SM, Zimmet PZ (2010) The metabolic syndrome. Lancet 375: 181–183. https://doi.org/10.1016/S0140-6736(09)61794-3

Eckel RH, Grundy SM, Zimmet PZ (2005) The metabolic syndrome. Lancet 365: 1415–1428. https://doi.org/10.1016/S0140-6736(05)66378-7

Fuentes E, Fuentes F, Vilahur G, Badimon L, Palomo I (2013) Mechanisms of chronic state of inflammation as mediators that link obese adipose tissue and metabolic syndrome. Mediators of Inflammation 2013(2): 136584. https://doi.org/10.1155/2013/136584

Gancheva S, Zhelyazkova-Savova M, Galunska B, Chervenkov T (2015) Experimental models of metabolic syndrome in rats. Scripta Scientifica Medica 47(2): 14–21. https://doi.org/10.14748/ssm.v47i2.1145

Hanley AJ, Williams K, Festa A, Wagenknecht LE, D'Agostino Jr RB, Haffner SM (2005) Liver markers and development of the metabolic syndrome: The insulin resistance atherosclerosis study. Diabetes 54: 3140–3147. https://doi.org/10.2337/diabetes.54.11.3140

Hristov M, Landzhov B, Yakimova K (2020) Cafeteria diet-induced obesity reduces leptin-stimulated NADPH-diaphorase reactivity in the hypothalamic arcuate nucleus of rats. Acta Histochemica 122(7): 151616. https://doi.org/10.1016/j.acthis.2020.151616

Johansen D, Stocks T, Jonsson H, Lindkvist B, Bjorge T, Concin H, Almquist M, Häggström Ch, Engeland A, Ulmer H, Hallmans G, Selmer R, Nagel G, Tretli St, Stattin P, Manjer J (2010) Metabolic factors and the risk of pancreatic cancer: a prospective analysis of almost 580,000 men and women in the Metabolic Syndrome and Cancer Project. Cancer Epidemiology, Biomarkers & Prevention 19: 2307–2317. https://doi.org/10.1158/1055-9965.EPI-10-0234

Kota SK, Krishna SV, Lakhtakia S, Modi KD (2013) Metabolic pancreatitis: Etiopathogenesis and management. Indian Journal of Endocrinology and Metabolism 17(5): 799–805. https://doi.org/10.4103/2230-8210.117208

Liangpunsakul S, Chalasani N (2005) Unexplained elevations in alanine aminotransferase in individuals with the metabolic syndrome: Results from the third national health and nutrition survey (NHANES III). The American Journal of the Medical Sciences 329: 111–116. https://doi.org/10.1097/00000441-200503000-00001

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193(1): 265–275. https://doi.org/10.1016/S0021-9258(19)52451-6

Mahjoub S, Masrour-Roudsari J (2012) Role of oxidative stress in pathogenesis of metabolic syndrome. Caspian Journal of Internal Medicine 3(1): 386–396.

Misra HP, Fridovich I (1972) The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological Chemistry 247(10): 3170–3175. https://doi.org/10.1016/S0021-9258(19)45228-9

Mozaffarian D, Hao T, Rimm EB, Willett WC, Hu FB (2011) Changes in diet and lifestyle and long-term weight gain in women and men. New England Journal of Medicine 364: 2392–2404. https://doi.org/10.1056/NEJMoa1014296

Nakajima K (2016) Low serum amylase and obesity, diabetes and metabolic syndrome: A novel interpretation. World Journal of Diabetes 7(6): 112–121. https://doi.org/10.4239/wjd.v7.i6.112

Nikniaz L, Nikniaz Z, Tabrizi JS, Sadeghi-Bazargani H, Farahbakhsh M (2018) Is within-normal range liver enzymes associated with metabolic syndrome in adults? Clinics and Research in Hepatology and Gastroenterology 42: 92–98. https://doi.org/10.1016/j.clinre.2017.06.006

O'Brien PJ, Dameron GW, Beck ML, Kang YJ, Erickson BK, Di Battista TH, Miller KE, Jackson KN, Mittelstadt S (1997) Cardiac troponin T is a sensitive, specific biomarker of cardiac injury in laboratory animals. Laboratory Animal Science 47(5): 486–495.

Panchal SK, Brown L (2011) Rodent models for metabolic syndrome research. Journal of Biomedicine and Biotechnology 2011: 351982. https://doi.org/10.1155/2011/351982

Patton HM, Yates K, Unalp-Arida A, Behling CA, Huang TT, Rosenthal P, Sanyal AJ, Schwimmer JB, Lavine JE (2010) Association between metabolic syndrome and liver histology among children with nonalcoholic fatty liver disease. American Journal of Gastroenterology 105(9): 2093–2102. https://doi.org/10.1038/ajg.2010.152

Pigeyre M, Yazdi FT, Kaur Y, Meyre D (2016) Recent progress in genetics, epigenetics and metagenomics unveils the pathophysiology of human obesity. Clinical Science 130: 943–986. https://doi.org/10.1042/CS20160136

Polizio AH, Pena C (2005) Effects of angiotensin II type 1 receptor blockade on the oxidative stress in spontaneously hypertensive rat tissues. Regulatory Peptides 128: 1–5. https://doi.org/10.1016/j.regpep.2004.12.004

Roberts CK, Sindhu KK (2009) Oxidative stress and metabolic syndrome. Life Science 84(21–22): 705–712. https://doi.org/10.1016/j.lfs.2009.02.026

Stanciu S, Rusu E, Miricescu D, Radu AC, Axinia B, Vrabie AM, Ionescu R, Jinga M, Sirbu CA (2023) Links between metabolic syndrome and hypertension: The relationship with the current antidiabetic drugs. Metabolites 13(1): 87. https://doi.org/10.3390/metabo13010087

Tappel AL (1978) Glutathione Peroxidase and Hydroperoxides. Methods of Enzymology 52: 506–513. https://doi.org/10.1016/S0076-6879(78)52055-7

Vanni E, Bugianesi E, Kotronen A, De Minicis S, Yki-Jarvinen H, Svegliati-Baroni G (2010) From the metabolic syndrome to NAFLD or vice versa? Digestive and Liver Disease 42: 320–330. https://doi.org/10.1016/j.dld.2010.01.016

Wang Y, Thatcher SE, Cassis LA (2017) Measuring blood pressure using a noninvasive tail cuff method in mice. Methods in Molecular Biology 1614: 69–73. https://doi.org/10.1007/978-1-4939-7030-8_6

Wilde DW, Massey KD, Walker GK, Vollmer A, Grekin RJ (2000) High-fat diet elevates blood pressure and cerebrovascular muscle Ca(2+) current. Hypertension 35(3): 832–837. https://doi.org/10.1161/01.HYP.35.3.832

Wong SK, Chin K-Y, Suhaimi FH, Fairus A, Ima-Nirwana S (2016) Animal models of metabolic syndrome: a review. Nutrition & Metabolism 13: 65. https://doi.org/10.1186/s12986-016-0123-9

Yin W, Liao D, Kusunoki M, Xi S, Tsutsumi K, Wang Z, Lian X, Koike T, Fan J, Yang Y, Tang C (2004) NO-1886 decreases ectopic lipid deposition and protects pancreatic beta cells in diet-induced diabetic swine. Journal of Endocrinology 180: 399–408. https://doi.org/10.1677/joe.0.1800399