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Comparative analysis of the impact of a high-fat, high-carbohydrate diet on two generations of naïve mice

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ABSTRACT

Background: A maternal high-fat diet influences offspring and may elevate their susceptibility to obesity and type 2 diabetes mellitus. This study aims to examine the impact of a high-fat, high-carbohydrate diet on the development of obesity, type 2 diabetes mellitus, non-alcoholic fatty liver disease, and cardiovascular disease in mice and their progeny.

Methods: Twenty one-month-old BALB/c mice (10 males and 10 females) were divided into control and first-generation groups of five males and five females. The control mice ate a butter-based diet with 10%–11% fat calories, while the first-generation mice ate 45% fat and 30% carbohydrates. The weights of both groups were measured for 4 months. In the first generation, females and males cohabited and mated. Mothers ate a high-fat, high-carbohydrate diet throughout pregnancy and nursing. After weaning, 10 second-generation pups (five males and five females) were randomly picked and isolated from their mothers in other cages. Isolated weaned offspring ate a high-fat, high-carbohydrate diet for 4 months. Several biochemical parameters were estimated for each mouse in the three groups.

Results: Higher levels of random blood glucose, triglycerides, total cholesterol, and LDL-C were seen in the second generation than in the first generation, whereas lower HDL-C was observed in the second generation. The second generation had considerably greater HbA1c levels than the first generation. Significantly greater liver enzyme activity was seen in the second generation. Proteinuria was observed in the second generation. Second-generation mice had greater total CK and CK-MB activity. The second-generation group showed considerably lower nitric oxide and adropin levels than the first-generation group. The second-generation mice had greater serum endothelin-1 and vascular endothelial growth factor levels than the first-generation mice.

Conclusions: The study concludes that a high-fat, high-carbohydrate diet induces obesity and T2DM in mice. The offspring of mothers that consume a high-fat, high-carbohydrate diet during both prenatal and postnatal periods exhibit a greater risk of developing these diseases than their progenitors.

Keywords: high-fat high-carbohydrate diet, obesity, non-alcoholic fatty liver disease, type 2 diabetes mellitus

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INTRODUCTION

Around 2.3 billion people worldwide are overweight or obese, and the prevalence of adult obesity has risen to 12% [1]. A major risk factor for chronic diseases, such as cancer, heart disease, type 2 diabetes mellitus, and high blood pressure, obesity has a huge negative impact on society's health and finances [2]. Researchers have long assumed that diets contribute to the development of obesity and other metabolic illnesses; nonetheless, in recent decades, diets have become the subject of extensive investigation. Thus far, the majority of research demonstrating an intricate relationship between dietary patterns and metabolism has been conducted on a restricted number of samples or tissues in animal models [3,4]. A high-fat, high-carbohydrate diet causes ectopic lipid accumulation, notably in the liver, inflammation, oxidative stress, and fibrosis, causing organ damage and loss of function [5]. Since the 20th century, a high-fat, high-calorie diet has been linked to obesity and glucose and lipid metabolic diseases [6]. Lipid-induced insulin resistance can be studied using a high-fat, high-calorie diet, which reduces insulin sensitivity in healthy young men [7]. These diets also reduce bile salt synthesis and turnover, decreasing cholesterol clearance [8]. Animal models of atherosclerosis, type 2 diabetes mellitus (T2DM), and non-alcoholic fatty liver disease (NAFLD) have been created using a high-fat, high-carbohydrate diet [9]. A previous study found that mice that were fed a high-fat, high-carbohydrate diet developed liver fibrosis and nonalcoholic steatohepatitis (NASH) [10]. Another

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study observed that severe dysbiosis could result from a low-fiber, high-fat, and high-carbohydrate diet [11]. Maintaining a healthy lifestyle for an extended period of time, especially dietary practices, has always been regarded as the first line of treatment for obesity and other metabolic diseases before the use of medications. The nongenetic inheritance of newly acquired phenotypes is a novel idea in biology, which posits that alterations prompted by specific environmental stimuli in parents (mothers and/or fathers) can be passed to subsequent generations [12]. The ability of environmental signals to alter the molecular hereditary information in spermatozoa illustrates the phenomenon of environmentally induced epigenetic alterations [13].

The present study aimed to investigate the effect of a high-fat, high-carbohydrate diet on two generations of naïve BALB/c mice.

METHODS

The present trial was conducted in the Applied Medical Sciences Department, Taif University, between September 2024 and January 2025.

The animal study protocol was accredited by the National Committee for Bioethics at Taif University (protocol code HAO-02-T-105) and the Committee considered that the proposal fulfills the requirements.

Study design

Twenty weaning (1-month-old) BALB/c mice (10 males and 10 females) were acquired from the animal facility at Umm Al-Qura University. The mice were housed in an ordinary rodent enclosure with woodchip bedding in a wide, well-ventilated room kept at 25 °C and exposed to a 12-h light/dark cycle. The animal study protocol was accredited by the National Committee for Bioethics at Taif University (protocol code HAPO-02-T-105), and the Committee concluded that the proposal fulfilled the requirements.

The mice were categorized into two groups, each comprising five males and five females.

1. The first group was the control group. In this group, the mice were fed a butter-based diet containing 10%–11% fat calories and drank tap water.

2. The second group was designated as the first-generation group. In this cohort, the mice were administered a high-fat diet comprising 45% fat, along with a high-carbohydrate diet of 30% (purchased from Research Diets Inc., New Brunswick, NJ, USA), with unrestricted access to tap water during the study [14]. After 4 months, the final body weight of this group was measured, and then the females and males lived with each other and mated; mating was verified through the identification of a vaginal plug. During the gestation period (21 days) and the lactation period (21 days from birth of offspring to weaning), the mice in this group (dams or first generation) were still fed a high-fat, high-carbohydrate diet. After weaning, 10 offspring (five males and five females) were randomly chosen and isolated from their mothers in other cages and designated as the second-generation group.

3. Second-generation group (offspring): For 4 months, the isolated weaned offspring were fed the same high-fat, high-carbohydrate diet as their parents. Subsequently, after the 4-month-long experiments with the control and first-generation groups, followed by the experiment with the second-generation group, blood samples were drawn from the retro-orbital venous plexus of each mouse in the three groups. The samples were collected in plain tubes and immediately centrifuged, and the sera were kept at -20 °C until analysis. In addition, for each mouse, a urine sample was collected in a Petri dish by applying gentle trans-abdominal pressure on the mice, and then the sample was immediately transferred to a sterile tube to prevent evaporation [15]. Finally, all the mice from the three groups were euthanized, and the liver and heart were harvested and preserved in 10% formaldehyde for a minimum of 1 day for fixation.

Body weight measurement

The body weight of each mouse in the three groups was recorded for 4 months (the control and first-generation groups, followed by the second-generation group) of the experiment using a digital scale (OHAUS, Scout Pro SPU601, China).

Random blood glucose and HbA1c levels

After 4 months of the experiment, random blood glucose levels were assessed using a colorimetric assay (Dimension EXL 200, SIEMENS). Furthermore, HbA1c was measured for all mice using a glycohemoglobin kit (POINTE Scientific Inc., USA).

Lipid profile measurement

At the end of the trial, a colorimetric assay (Dimension EXL 200, SIEMENS) was used to measure triglycerides, total cholesterol, HDL-C, and LDL-C for each mouse in the study.

Liver and kidney function tests

After 4 months, the serum sodium (Na+), potassium (K+), chloride (Cl-), urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, albumin, total bilirubin, and direct bilirubin levels were estimated using a colorimetric assay (Dimension EXL 200, SIEMENS). Urinary protein levels (proteinuria) were estimated using a colorimetric assay (Elabscience, E-BC-K252-M).

Measurement of cardiac markers

After 4 months, serum creatine kinase (CK), CK-MB isoenzyme, and cTn-I were measured for mice in the three groups using a colorimetric assay (Dimension EXL 200, SIEMENS).

Adropin, NO, ET-1, VEGF, leptin, and adiponectin

At the end of the experiment, serum adropin, NO, ET-1, VEGF, leptin, and adiponectin were estimated using the quantitative competitive ELISA kits from Thermo Fisher. The serum levels of these parameters were measured using the Thermo 5111340 Microplate Reader (Fisher Scientific).

Estimation of cardiac homogenate

Cardiac homogenate was prepared with a 2-mL bead kit from OMNI International Inc. Small testicular tissue pieces, radio-immunoprecipitation assay (RIPA) buffer, and a protease inhibitor were introduced into a 2-mL microtube containing beads. The Bead Ruptor 12 (OMNI International Inc.) was employed to homogenize the microtube for a duration of 2 min. The sample was subsequently centrifuged for 30 min at 8 °C and 15,000 revolutions per minute (SIGMA 1-14 k). The supernatant, obtained by aspirating into a microtube and centrifuging for 15 min at 8 °C and 15,000 rpm, was stored at-20 °C until analysis [16].

Oxidants and antioxidants in cardiac tissue

After 4 months, lipid peroxidation products (LPO) (Elabscience, Cat. No. E-BC-K176-M), reduced glutathione (GSH) (Elabscience, Cat. No. E-BC-K030-S), glutathione peroxidase (GSH-Px) (Elabscience, Cat. No. E-EL-R2491), superoxide dismutase (SOD) (Elabscience, Cat. No. E-EL-R1424), and catalase (Elabscience, Cat. No. E-BC-F006) were estimated in cardiac homogenate using an ELISA reader.

Histopathological study

The liver tissue was fixed in formaldehyde and then dehydrated and cleaned in ethanol. After the tissue was attached to glass slides, the paraffin was removed before the tissue was stained by heating to 56 °C for at least one hour; after that, it was immersed in xyline for 3–5 min. Finally, the tissue was stained with hematoxylin and eosin (H&E) and then observed by a light microscope at 10X [17].

Statistical analysis

Analysis was done using Prism software version 10 (GraphPad). All data were expressed as mean ± SD. The comparisons of body weight, HbA1c, random blood glucose, lipid profile, kidney, liver, and cardiac function tests, adropin, NO, ET-1, VEGF, leptin, adiponectin, oxidant and antioxidant parameters between all groups were made with a one-way analysis of variance (ANOVA), followed by Post-Hoc test (Tukey`s HSD test) for the determination which groups have significant statistical differences. The level of significance was set at $p < 0.05$.

RESULTS

Body weight of mice

The body weight of mice in all groups is displayed in Figure 1A. The body weight of the first and second generations was markedly increased compared to the control group. Furthermore, the second generation had higher body weight compared to the first generation.

Random blood glucose, HbA1c, and lipid profile

Figure 1B displays random blood glucose, triglyceride, total cholesterol, LDL-C, and HDL-C levels of mice in the three groups. Random blood glucose levels and lipid profiles were significantly worse in the first generation compared with controls, and in the second generation compared with the first: higher random glucose, triglycerides, total cholesterol, LDL-C, and lower HDL-C. Glycated hemoglobin in the three groups is displayed in Figure 1C. The study observed that HbA1c is increased in the first- and second-generation groups compared to the control group. Moreover, the second-generation group had significantly higher HbA1c than the first-generation group.

Kidney function tests

Table 1 displays the kidney function tests for mice in the three groups. The serum albumin level was significantly decreased in the first- and second-generation groups compared to the control group, and in the second-generation group, it was lower than in the first-generation group. Regarding proteinuria, both first-generation and second-generation groups have proteinuria; however, the second-generation group has the greatest level.

Liver function tests

Figure 1D represents the liver enzymes of mice in the three groups. The study observed that first- and second-generation mice had considerably higher ALT, AST, and ALP activities than the control group, and the second-generation group had the highest liver enzyme activities.

Cardiac markers

Cardiac markers of mice in the three groups are displayed in Table 1. CK and CK-MB activities were higher in first- and second-generation groups compared to the control group, and the second-generation mice had the highest activities. The first- and second-generation groups showed greater cardiac troponin-I levels than the control group, and higher levels in the second-generation than in the first-generation.

Serum NO, adropin, ET-1, and VEGF levels

NO, adropin, ET-1, and VEGF levels are displayed in Figure 1E. NO and adropin levels decreased in first- and second-generation groups compared to the control

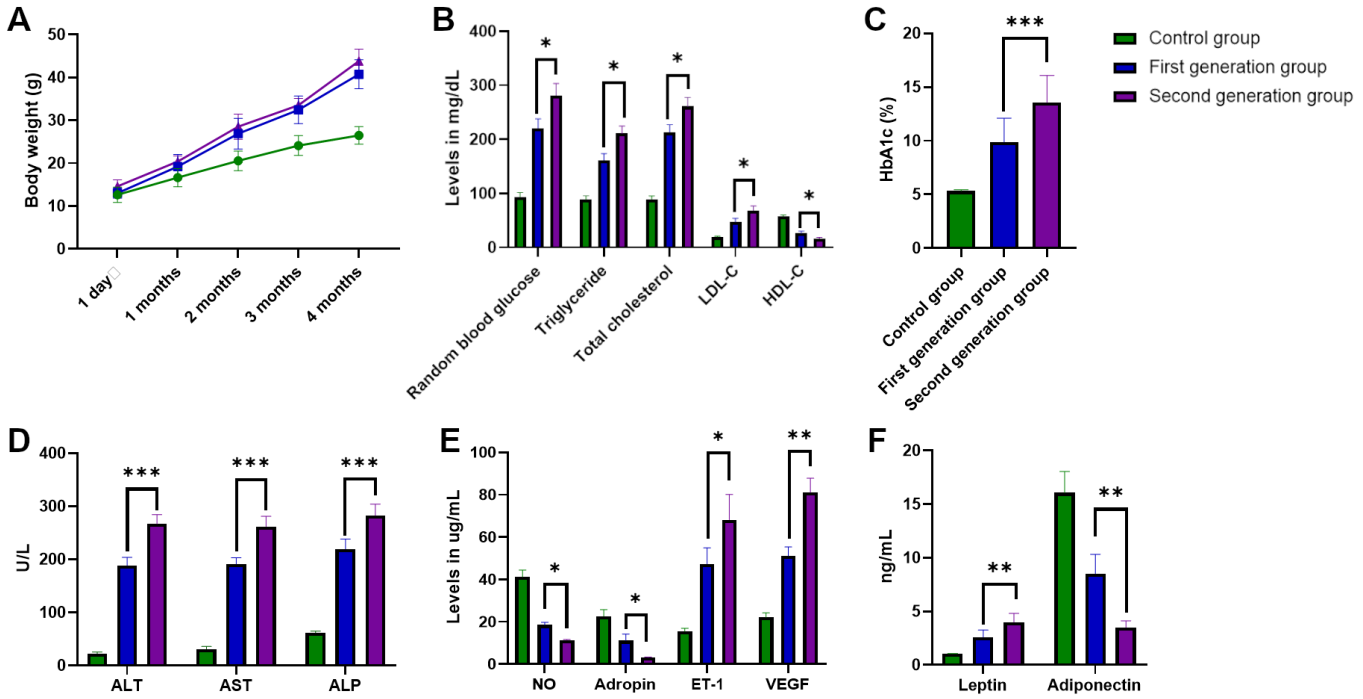

Figure 1. Comparison of body weight and blood parameters between mice groups

Abbreviations: ALP – alkaline phosphatase, ALT – alanine aminotransferase, AST – aspartate aminotransferase, ET-1 – endothelin-1, HbA1c – glycated hemoglobin, HDL-C – high-density lipoprotein cholesterol, LDL-C – low-density lipoprotein cholesterol, NO – nitric oxide, VEGF – vascular endothelial growth factor. Statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 1. Kidney function tests, cardiovascular biomarkers, and oxidative stress parameters of mice in the three groups

Parameter	Control (n=10)	First generation (n=10)	Second generation (n=10)
Na+ (mmol/L)	147.21 ± 3.26	146.71 ± 2.55	146.70 ± 2.71
K+ (mmol/L)	5.11 ± 1.02	6.14 ± 1.51	6.11 ± 1.11
Cl- (mmol/L)	116.87 ± 3.60	114.22 ± 2.87	112.91 ± 3.70
Urea (mg/dL)	20.57 ± 3.11	28.29 ± 5.20	31.44 ± 3.91
Creatinine (mg/dL)	1.72 ± 0.31	2.10 ± 0.93	2.31 ± 0.67
Albumin (g/dL)	4.99 ± 0.50	1.71 ± 0.24*	0.61 ± 0.08*
Proteinuria (mg/dL)	6.41 ± 0.40	58.39 ± 6.24**	172.28 ± 10.24**
Total CK (IU/mL)	19.46 ± 2.66	98.38 ± 11.72**	162.22 ± 17.34**
CK-MB (%)	2.81 ± 0.65	28.16 ± 4.29**	51.58 ± 6.64**
c-troponin-I (ng/mL)	80.87 ± 3.60	154.22 ± 7.87*	193.91 ± 11.70*
LPO (nmol/g)	31.38 ± 4.11	67.80 ± 8.03*	93.39 ± 11.80*
GSH (nmol/g)	8.66 ± 1.04	1.26 ± 0.16	1.41 ± 0.49
Catalase (U/g)	50.48 ± 6.32	20.57 ± 1.60*	4.82 ± 0.71*
GSH-Px (U/g protein)	6.44 ± 0.13	1.92 ± 0.03*	0.37 ± 0.02*
SOD (ug/g protein)	17.20 ± 2.99	7.35 ± 1.29*	2.64 ± 0.50*

Abbreviations: CK – creatine kinase, GSH – glutathione (reduced), GSH-Px – glutathione peroxidase, LPO – lipid peroxidation products, SOD – superoxide dismutase. Statistical significance: * $p < 0.05$, ** $p < 0.01$.

group, and the second-generation had significantly the lowest levels. First- and second-generation groups had higher endothelin-1 and VEGF levels than the control group, and the second-generation group had considerably the highest levels.

Serum leptin and adiponectin levels

Serum leptin levels were considerably increased in first- and second-generation groups, and the second-generation had the worst levels (Figure 1F). Serum adiponectin levels were significantly reduced in the first- and second-generation groups compared to the control group, with the lowest levels in the second-generation.

Oxidants and antioxidants in cardiac homogenate

Table 1 presents the level of oxidative stress parameters in the cardiac homogenate of mice. LPO levels were considerably higher in first- and second-generation groups, with the highest levels in the second generation. Antioxidant enzymes (catalase, GSH-Px, and SOD) were considerably lower in first- and second-generation compared to the control group, and the second-generation had the lowest value.

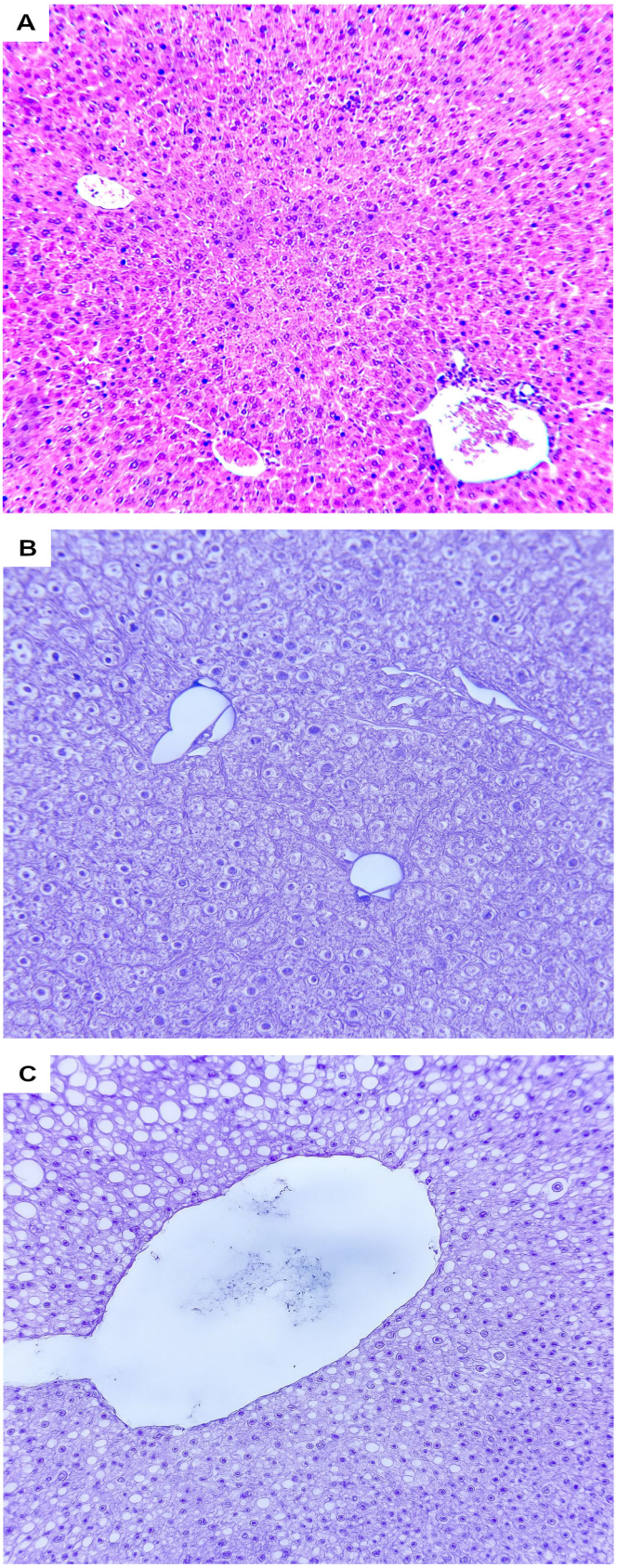

Figure 2. Liver histology: HE stain, 10x magnification

(A) Liver of the control group appears with normal architecture; (B) Liver of the first-generation group appears to have steatosis (NAFLD); (C) Liver of the second-generation group appears to have steatosis and infiltration of acute inflammatory cells (polymorphs) (NASH).

DISCUSSION

The incidence of obesity has risen globally. This rise has been attributed to lifestyle modifications, notably the intake of high-fat and high-carbohydrate diets. Various experimental methods have been used to explore diet-induced obesity. These methods have compared the effect of a diet inducing obesity with a control diet in a single rodent strain, the response of two rodent strains with different susceptibility to diet-induced obesity, or the range of diet-induced obesity responses in a single rodent strain. Although most rodents often exhibit obesity when subjected to high-fat diets, responses in weight gain, glucose tolerance, insulin resistance, triglycerides, and other parameters may vary according to the strain. Certain inbred strains exhibit greater susceptibility to obesity induction, whereas others demonstrate increased resistance. This varied susceptibility to high-fat diets was similarly noted in human populations [18].

Most diet-induced obesity investigations have used adult animals, but few studies have examined young and early adult animals. Previous studies found that pups born from a high-fat, high-carbohydrate diet group had elevated body weight and obesity at weaning, whereas at 16 weeks, there was no significant difference in body weight between control-fed pups and high-energy-fed pups [14,15]. In the present study, the second generation exhibited the highest body weight at weaning and throughout the experiment. Over 6 months, the first-generation group experienced an approximate monthly gain of 4 g, whereas the second-generation group saw a monthly increase of 6 g.

Adiponectin and leptin are two regulating hormones synthesized by adipose tissue. Leptin induces hunger and satiety. The concentration of circulating leptin is directly proportional to the quantity of energy reserves (triglycerides) accumulated in adipose tissue [19]. The current study found that the levels of leptin increased in the first and second generations, but the levels were higher in the second generation. Adiponectin plays a role in the regulation of glucose levels and the catabolism of fatty acids. Elevated adiponectin levels are associated with a reduced risk of T2DM. Adiponectin plasma concentrations are reduced in obese individuals compared with their lean counterparts [20]. Adiponectin levels appear to be inversely correlated with a high-fat, high-carbohydrate diet and the body weight of mice.

The present study observed that mice in the first-generation and second-generation cohorts developed T2DM after 6 months. The random blood glucose levels and HbA1c were higher in the second-generation group than in the first-generation group. These results are in line with two other studies that identified a significant increase in glucose levels in obese mice subjected to

a high-fat, high-carbohydrate diet [14,21]. The current study revealed that a high-fat, high-carbohydrate diet elevated triglycerides, total cholesterol, and LDL-C, while it decreased HDL-C. Furthermore, these parameters were higher in the second-generation mice than in the first-generation mice, except for HDL-C.

NAFLD has emerged as the predominant liver disease worldwide. It is induced by multiple factors, including nutrition. The defining characteristic of NAFLD is the non-harmful buildup of triacylglycerols; nonetheless, this illness can progress to non-alcoholic steatohepatitis, a more severe variant linked to inflammation and fibrosis. No therapies are now available; dietary adjustments constitute the sole option [22]. The results of the current study showed that a high-fat, high-carbohydrate diet induced liver steatosis. Liver enzyme levels were elevated in the first- and second-generation mice, with the highest levels observed in the second-generation mice. The histopathological study revealed a significant accumulation of triglycerides in the hepatocytes of both generations (NAFLD) (Figure 2B). Moreover, the second generation exhibited infiltration of hepatic tissue by chronic inflammatory cells, a phenomenon that did not appear in the first generation. This means that NASH developed only in the second-generation group (Figure 2C).

Concerning renal function, both groups had hypoalbuminemia and albuminuria. Hypoalbuminemia and albuminuria were higher in the second-generation group. Serum urea and creatinine levels remained within the normal range for both groups; however, the potential for future deterioration of kidney function should not be overlooked.

Regarding cardiac integrity, the present study showed that serum CK, CK-MB, and cardiac troponin-I were higher in the first- and second-generation groups. These parameters in the second-generation group exceeded those in the first-generation group. Besides these markers, the current study measured other parameters associated with cardiac function.

NO is a free radical with vasodilatory properties and is necessary for maintaining and regulating blood flow in healthy tissues, especially microcirculation [23]. Besides NO, adropin is a peptide hormone that plays a crucial role in protecting vascular endothelial cells from injuries [24]. The present study found that NO and adropin levels were decreased in the first- and second-generation groups, with the second-generation group exhibiting the lowest values. Endothelin-1 is a peptide generated by endothelial cells in blood vessels and various other cells throughout the body. Endothelin-1 exerts its effects in a paracrine manner on the adjacent vascular smooth muscle cells (VSMCs), leading to a significant vasoconstrictive response [25]. VEGFs are a collection of glycoproteins that regulate angiogenesis by promoting endothelial cell differentiation. Increased VEGF activity has been associated with inflammation, elevated blood pressure, and the formation of atherosclerotic plaques, hence facilitating the onset of coronary heart disease [26]. The present study observed that a high-fat, high-carbohydrate diet induced endothelin-1 and VEGFs in the first- and second-generation groups. These proteins were higher in the second-generation group than in the first-generation group.

Oxidative stress, resulting from the excessive intracellular buildup of reactive oxygen species (ROS) and other free radicals, plays a significant role in the initiation and advancement of numerous diseases, including obesity, diabetes mellitus, cardiovascular damage, and hepatic injuries. Oxidative stress occurs when there is a reduction in antioxidant molecules, including glutathione, catalase, SOD, and GSH-Px [27]. The current study demonstrated that a high-fat, high-carbohydrate diet increased lipid peroxidation and reduced the activities of catalase, SOD, and GSH-Px. This may give insight into the mechanism by which a high-fat, high-carbohydrate diet induced obesity, T2DM, cardiovascular injuries, and NAFLD and NASH in mice in the current study.

A question that warrants attention is why the second-generation mice exhibited greater susceptibility to the development of these disorders than their progenitors. Rodent studies are extensively used to investigate the impact on offspring of maternal consumption of a high-fat diet, which serves as a prevalent model for overnutrition. Research indicates that maternal high-fat diets result in numerous adverse outcomes in mice progeny, including heightened susceptibility to NAFLD, skeletal deformities, glucose intolerance, and insulin resistance. Some studies suggest an increase in rat offspring obesity, while other studies indicate no effect. Intrauterine programming has been suggested, indicating that the mother's diet may influence the offspring's metabolism throughout their lifetime. The prenatal and early postnatal phases are crucial periods for adult life. Furthermore, maternal nutritional conditions and health status cause epigenetic alterations that influence the prevalence of T2DM in progeny. Some studies have elucidated the role of DNA methylation alterations in fetal programming related to metabolic disease in both human and animal models. In human studies, neonates with fat parents exhibited modified methylation in several imprinted genes within their cord blood. In an animal study, exposure to a maternal high-fat diet resulted in DNA hypermethylation in the livers of offspring mice [28]. This may explain why offspring are at a greater risk than their dams of developing injuries resulting from an unhealthy diet during prenatal and postnatal life.

The current study is limited by its small sample size and brief duration, indicating the need for extension. Furthermore, future studies in humans are recommended.

CONCLUSIONS

The study concludes that a high-fat, high-carbohydrate diet induces obesity and T2DM in mice. Furthermore, this diet contributes to NAFLD, NASHD, cardiovascular injuries, and proteinuria. The offspring of mothers that consume a high-fat, high-carbohydrate diet during both prenatal and postnatal periods exhibit a greater risk of developing these diseases than their progenitors.

ABBREVIATIONS

ALP – alkaline phosphatase

ALT – alanine aminotransferase

AST – aspartate aminotransferase

CK – creatine kinase

ET-1 – endothelin-1

GSH – glutathione (reduced)

GSH-Px – glutathione peroxidase

LPO – lipid peroxidation products

NO – nitric oxide

NAFLD – non-alcoholic fatty liver disease

NASH – non-alcoholic steatohepatitis

ROS – reactive oxygen species

SOD – superoxide dismutase

T2DM – type 2 diabetes mellitus

VEGF – vascular endothelial growth factor

VSMC – vascular smooth muscle cell

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AUTHORS' CONTRIBUTION

AA: conceptualization, methodology, investigation, writing – original draft.

MA: formal analysis, writing – review & editing.

All authors have read and approved the final version of the manuscript.

CONFLICT OF INTEREST

None to declare.

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