Original Article

PORTO
BIOMEDICAL
JOURNAL
WHERE SCIENCE MEETS KNOWLEDGE

OPEN

An albino mouse model of nonalcoholic fatty liver disease induced using high-fat liquid "Lieber-DeCarli" diet: a preliminary investigation

Ayokanmi Ore, MSc[a,b,*], Regina Ngozi Ugbaja, PhD[a], Abideen Idowu Adeogun, PhD[c], Oluseyi Adeboye Akinloye, PhD[a]

Abstract

Background: Experimental diet models have proven to be vital to understanding the pathophysiology and management of nonalcoholic fatty liver disease (NAFLD). Lieber-DeCarli high-fat, liquid diet have been used to produce NAFLD in rat models. There is, however, currently no information on the effects of this diet in the mouse model.

Methods: Ten (n = 10) male albino mice (27.7 ± 2.0 g) were divided into 2 diet groups (n = 5/group). Animals from group 1 were fed with standard chow diet (CD group) and those from group 2 were fed with Lieber-DeCarli high-fat, liquid diet (high-fat diet or HFD group) *ad libitum* for a period of 4 weeks.

Results: Data obtained show insulin resistance in the HFD group with a significant increase in plasma lipid profile. Level of cholesterol and triglycerides in the liver and plasma increased significantly ($P < .05$) in the HFD group compared with the CD group. Plasma level of tumor necrosis factor alpha increased significantly in the HFD group compared to control. Also, indicators of oxidative stress (malondialdehyde and protein carbonyls) increased significantly coupled with a significant reduction in reduced glutathione (GSH) level and activity of glutathione peroxidase in the liver of mice in the HFD group compared to CD group. Histopathological evaluation of liver sections reveals steatosis with ballooned hepatocytes.

Conclusions: Data from the present study suggest that the Lieber-DeCarli high-fat, liquid diet may be vital in the study of fatty liver disease in albino mouse. This model may also produce the features of NAFLD in a shorter time in albino mice.

Keywords: hepatocellular ballooning, inflammation, Lieber-DeCarli high-fat liquid diet, non-alcoholic fatty liver disease, oxidative stress, steatosis

Introduction

Non-Alcoholic Fatty Liver Disease (NAFLD) refers to a hepatic condition associated with fat deposits in the liver which occur independently of alcohol consumption.[1] As described by the "multiple-hit" hypothesis,[2] NAFLD begins with an initial stage of fatty liver (hepatic steatosis) where metabolic syndrome plays a vital role, due to insulin resistance. Hepatic steatosis progresses into nonalcoholic steatohepatitis (NASH) due to inflammation and fibrosis and ultimately to liver cirrhosis and hepatocellular carcinoma (Fig. 1).[3] The term "nonalcoholic steatohepatitis" was first introduced by Ludwig et al.[4] to describe steatohepatitis in patients with no history of alcohol consumption or abuse.

Several experimental models have been used to better understand the pathogenesis, clinical management, and treatment of NAFLD. Most of these models have been extensively reviewed[5,6] with various advantages and disadvantages against each model. Efforts are, however, still in place to create a definite animal model of NAFLD/NASH.[7] An ideal model should be able to produce the human symptoms of NAFLD/NASH in the shortest possible time and be reproducible. One of the most commonly used diet models is the high-fat diet model with several variants and advantages. These diets are available in solid (pelletized) or liquid forms. Although the solid diet models are commonly used, few articles have reported the use of liquid diet models. Lieber et al[8,9] and Zou et al[10] reported the use of high-fat liquid diet to induce NASH in 3 and 6 weeks, respectively in Sprague–Dawley rats. The former involved feeding rats *ad libitum*, whereas in the latter, rats were orally treated with the high-fat emulsion (10 mL/kg) once daily, in addition to normal rat pellets.

Although a report showed an inability to reproduce the NASH condition in Sprague–Dawley rats in 3 weeks using Lieber-DeCarli high-fat liquid diet (LDHFLD),[11] another reproduced various degrees of NAFLD in albino rats at 4, 8, and 12 weeks.[12] At the time of this study, there was no information on the effects of LDHFLD in mouse model. The current study was therefore

Ore *et al.* Porto Biomed. J. (2020) 5:4

Porto Biomedical Journal

Figure 1. The multihit hypothesis and pathophysiology of nonalcoholic fatty liver disease (NAFLD)[3]. NASH = nonalcoholic steatohepatitis.

designed to explore the effect of LDHFLD *ad libitum* for 4 weeks in a mouse model.

Materials and Methods

Chemicals and reagents

Chemicals used in this research including ethanol, *p*-nitrophenyl phosphate, 2,4-dinitrophenylhydrazine, sodium carbonate, and glutathione (GSH), were purchased from Merck (Darmstadt, Germany). Guanidine hydrochloride was purchased from AK Scientific (Union City, CA). All other chemicals used were of analytical grade.

Assay kits

ELISA kit for Mouse tumor necrosis factor alpha (TNF-α), and Mouse Insulin (INS) were procured from Elabscience Biotechnology Co. Limited (Houston, TX). Assay kits for alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TC), triglycerides (TGs), HDL-cholesterol are products of Fortress Diagnostics Limited (Antrim, UK). Assay kit for glucose was purchased from Randox Diagnostics Limited.

Experimental animals

Albino mice used in this study were acquired from a private animal breeding facility in Iwo, Osun State, Nigeria. They were contained in wire-meshed cages and supplied with commercial chow diet (CD) and water *ad libitum*. Animal procedures were carried out in accordance with the International Guidelines on laboratory animal handling. Ethical approval for this study (FNS/ERC/2018005) was provided by the Faculty of Natural Sciences Ethical Review Committee, Ajayi Crowther University, Oyo.

Diets

Lieber-DeCarli 71% Fat Derived Calories Liquid Rodent Diet used in this study was purchased from DYETS Inc (Bethlehem,

PA). Commercial CD used was a product of Ladokun Feeds (Ibadan, Oyo State Nigeria).

Experimental design

After 1 week of acclimatization, 10 (n = 10) mice (27.7 ± 2 g) were divided into 2 diet groups (5 mice/group). Animals from group 1 were fed with CD and group 2 with LDHFLD (as high-fat diet, HFD; Table 1) *ad libitum* for a period of 4 weeks. Body weight was recorded weekly.

Blood and tissue collection

After 28 days, mice were weighed and sacrificed in the fed state. The blood sample was collected via the retro-orbital vein into fluoride oxalate coated sample tubes for preparation of plasma. The liver was excised, washed in ice-cold phosphate buffered saline (pH 7.4) to remove residual blood. The liver was blotted to dry and weighed. Sections from liver samples were fixed in 10%

Table 1	
Composition of Lieber-DeCarli high-fat liquid diet	
Ingredient	**g/L**
Casein (100 mesh)	41.4
L-cystine	0.5
D,L-methionine	0.3
Corn oil	48.5
Olive oil	28.4
Safflower oil	2.7
Maltose dextrin	25.6
Cellulose	10
Mineral mix 210011	8.75
Vitamin mix 310011	2.5
Choline bitartrate	0.53
Xanthan gum	3

Ore *et al.* Porto Biomed. J. (2020) 5:4

www.portobiomedicaljournal.com

neutral buffered formalin for histopathology and the remainder used for the preparation of liver homogenate.

Preparation of plasma and liver homogenate

The blood sample was subjected to centrifugation at 4000 rpm for 5 minutes to obtain plasma. Approximately 0.5 g of the liver was minced and homogenized in phosphate buffered saline (10% w/v). The homogenate was centrifuged at 10,000 g for 10 minutes at 4°C. The resulting supernatant was collected and stored frozen until used for biochemical analyses. Protein contents of samples (serum and liver homogenate) were determined using the biuret method.[13]

Biochemical analyses

Activities of ALT, AST, and levels of TC, TGs, HDL-cholesterol, and glucose were determined using assay kits according to the manufacturer's protocol. Plasma alkaline phosphatase activity was determined according to Wright et al.[14] Hepatic level of reduced GSH and glutathione peroxidase (GSH-Px) activity was determined according to Jollow et al[15] and Gross et al,[16] respectively. Hepatic levels of oxidative damage products, malondialdehyde (MDA) and protein carbonyls were measured according to Varshney and Kale[17] and Reznick and Packer,[18] respectively.

The index of insulin resistance was calculated by the homeostasis model assessment [HOMA][19] using the following formula:

$$HOMA - IR = \frac{Insulin(mlU/L) \times Blood\ Glucose(mmol/L)}{22.4}$$

The concentration of insulin and TNF-α in plasma was quantified by Sandwich-ELISA principle using ELISA Kits following the manufacturer's procedure.

Liver histopathology

Histological evaluation was performed on liver sections fixed in 10% neutral formalin for 24 hours. Sections were dehydrated in ethanol embedded in paraffin wax. Sections were cut at 4 μm in thickness, stained with hematoxylin and eosin for histological examination.

Statistical analysis

Data were expressed as mean ± standard error of mean (SEM) of 5 mice in each group. The significant difference between the diet groups was analyzed by *t*-test using Graphpad Prism software (v6.01). *P* value <.05 were considered to be significant.

Results

Weight changes in mice

Data presented in Table 2 show weight changes in mice. There was a significant decrease in relative liver weight in HFD groups (*P* <.05) compared to normal control.

Plasma glucose, insulin, and insulin resistance

Table 3 shows the plasma level of glucose, insulin, and index of insulin resistance. Plasma glucose level was high in the HFD

Table 2

Weight changes in mice fed normal chow diet or high fat diet for 4 weeks

Parameters	CD	HFD
Initial body weight (g)	22.1 ± 2.8	27.7 ± 1.6
Final body weight (g)	29.3 ± 3.2	35.1 ± 3.7
Liver weight (g)	1.45 ± 0.07	1.48 ± 0.07
Relative liver weight	0.05 ± 0.004	0.04 ± 0.004*

Data are expressed as mean ± SEM of 5 mice in each group. Significant difference between groups was analyzed by *t* student test.
CD = chow diet; HFD = high fat diet.
* *P* <.05 compared to control.

Table 3

Plasma level of glucose, insulin, and Index of insulin resistance in mice fed normal chow diet or high fat diet for 4 weeks

Parameters	CD	HFD
Glucose (mmol/L)	7.5 ± 0.2	8.6 ± 0.7[ns]
Insulin (mlU/L)	6.8 ± 0.1	8.4 ± 0.3*
HOMA-IR	2.2 ± 0.1	3.2 ± 0.3*

Data are expressed as mean ± SEM of 5 mice in each group. Significant difference between groups was analyzed by *t* student test.
CD = chow diet; HFD = high fat diet; HOMA = homeostasis model assessment; ns = not significant.
* *P* <.05 compared to control.

group compared to control, although the increase was not significant. Plasma insulin and HOMA-IR index were significantly higher in the HFD group (*P* <.05) compared to the CD group (Table 3).

Inflammation

Figure 2 shows the plasma concentrations TNF-α in mice after 4 weeks. There was a significant increase (*P* <.05) in the level of TNF-α in HFD group compared to the CD group.

Liver function biomarkers

Figure 3 shows the biomarkers of liver function. Plasma activities of ALT, AST, and alkaline phosphatase increased significantly in the HFD group compared with the control group.

Lipid profile

Figure 4 shows the lipid profile of mice fed CD and HFD for 4 weeks. TC (Fig. 4A and B) and TGs (Fig. 4C and D) levels increased significantly (*P* <.05) in the plasma and liver of mice in the HFD group compared to the CD group. Plasma level of HDL-cholesterol (Fig. 4E) decreased significantly in the HFD group compared to control. LDL-cholesterol level (Fig. 4F) increased significantly (*P* <.05) in the HFD group compared to control.

Oxidative stress

Figure 5 shows the status of hepatic antioxidant and oxidative stress markers in mice fed CD and HFD for 4 weeks. There is a significant reduction in the level of GSH and GSH-Px in the liver of mice in the HFD group compared to CD group. Hepatic MDA content and protein carbonyls increased significantly in the HFD group compared with control.

Ore *et al.* Porto Biomed. J. (2020) 5:4

Porto Biomedical Journal

Histopathology

Figure 6 shows the representative images of liver sections from the CD group and the HFD group. A normal histoarchitecture is shown in the CD group (Fig. 6A). In the HFD group, however, microvesicular steatosis can be seen, with hepatocellular ballooning in the zone 3 hepatocytes (Fig. 6B).

Discussion

Several experimental models have been created to better understand the pathophysiology and treatment of NAFLD. Lack of a definite experimental model that, however, reproduces the major features of the disease has been a challenge. This gap has been largely filled by some recent models. In this preliminary study, we aim to provide biochemical and histological information on the role of LDHFLD in the induction of hepatic features of NAFLD (insulin resistance, steatosis, inflammation, and oxidative stress) in the mouse.

Accumulating evidence shows close association between NAFLD and metabolic syndrome. Data from this study show hyperinsulinemia and insulin resistance in mice fed with HFD. HFD is known to induce hyperglycemia, hyperinsulinemia, and insulin resistance (or impaired glucose tolerance).[20] Insulin is involved in mediating the metabolic actions required for maintaining the balance between nutrients intake and storage. Insulin also promotes energy storage in adipose tissue, liver, and muscle. NAFLD is characterized by insulin resistance, where higher levels of insulin are required to reduce hyperglycemia and maintain blood glucose level.[21] Data obtained in the present study on insulin resistance is similar to that reported previously from feeding LDHFLD to rats.[12]

NAFLD has been reported to be associated with an increase in levels of proinflammatory factors such as TNF-α and IL-6. In this study, HFD caused a significant increase in the plasma level of TNF-α in mice. The cytokines, including the TNF-α, are recognized as vital components in metabolic inflammation as observed previously in NAFLD associated with adipose tissue inflammation.[22] Adipose tissue inflammation is characterized by an increased expression of various proinflammatory cytokines such as TNF-α, IL-1, and IL-6, and cellular infiltrates including monocytes/macrophages, neutrophils, etc. Increase in TNF-α level observed in this study is similar to those reported earlier.[8,9]
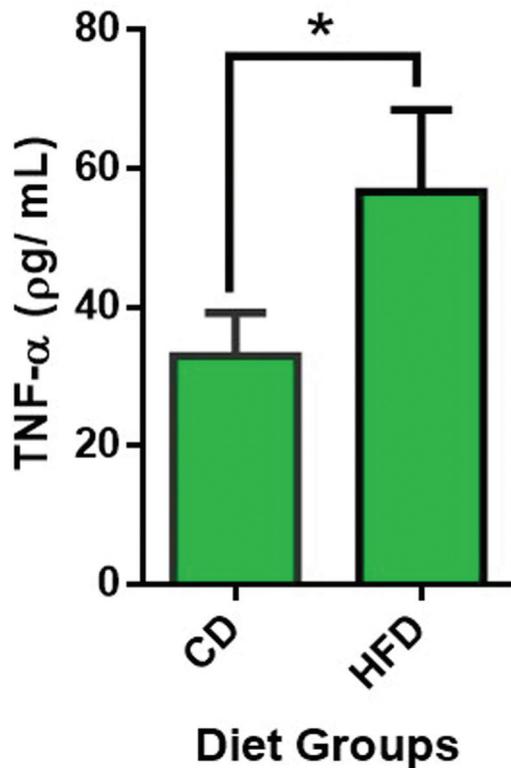
Figure 2. Plasma level of tumor necrosis factor alpha (TNF-α) in mice fed normal chow diet or high fat diet for 4 weeks. Bars represent the mean ± SEM of 5 mice per group. *Significantly different ($P < .05$). CD = chow diet; HFD = high fat diet.
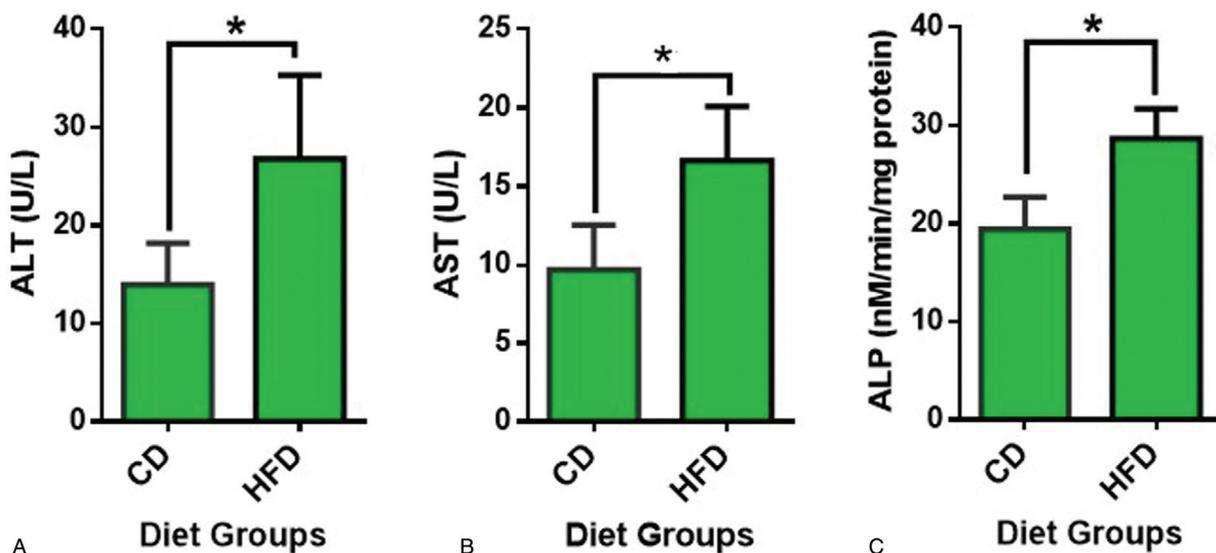
Figure 3. Liver function biomarkers measured in the plasma of in mice fed normal chow diet or high fat diet for 4 weeks: (A) ALT – alanine aminotransferase, (B) AST – aspartate aminotransferase, (C) ALP – alkaline phosphatase. Bars represent the mean ± SEM of 5 mice per group. *Significantly different ($P < .05$). CD = chow diet; HFD = high fat diet.
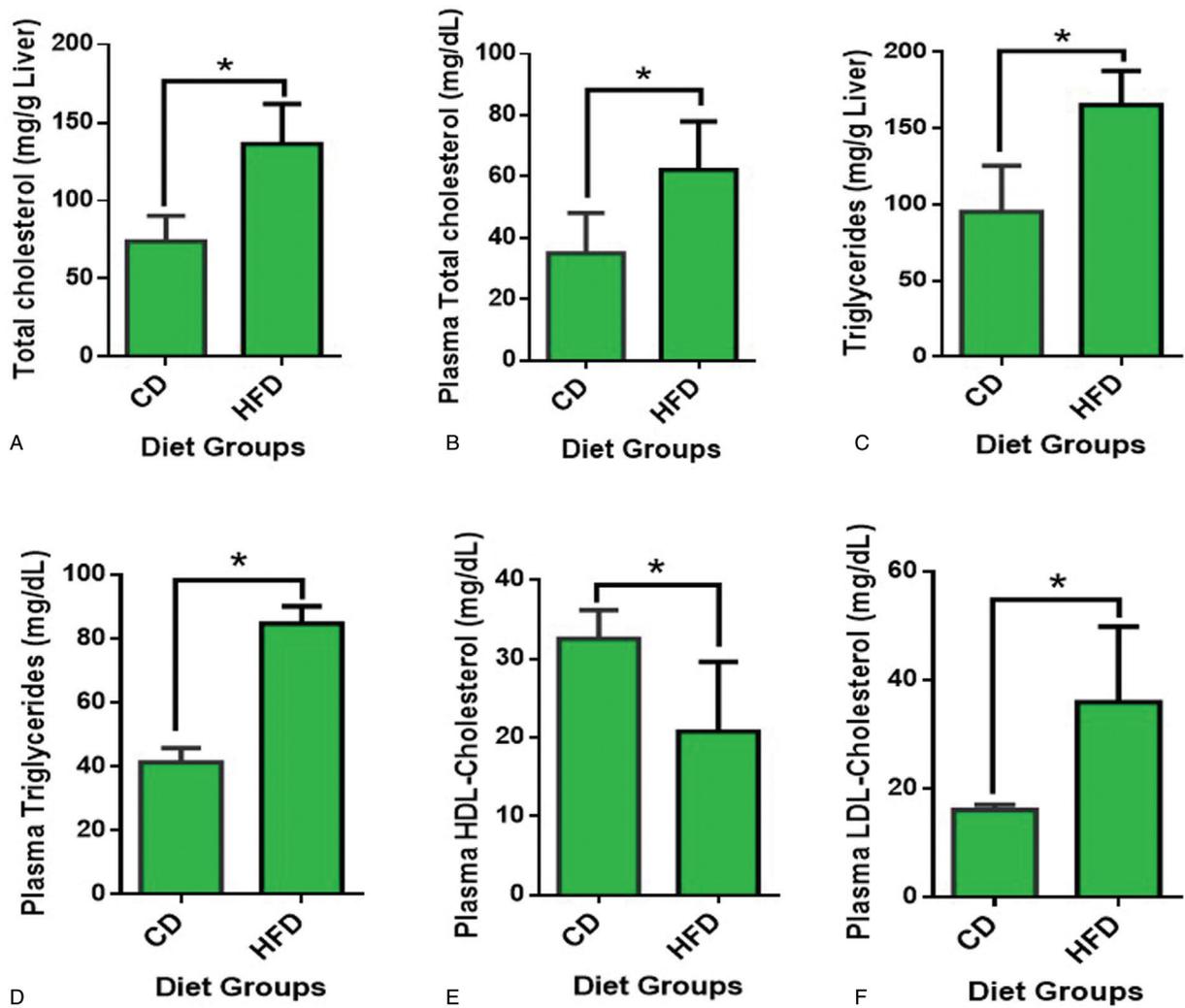
Figure 4. Hepatic and plasma lipid profile in mice fed normal chow diet or high fat diet for 4 weeks: (A) hepatic total cholesterol, (B) plasma total cholesterol, (C) hepatic triglycerides, (D) plasma triglycerides, (E) plasma HDL-cholesterol, and (F) plasma LDL-cholesterol. Bars represent the mean ± SEM of 5 mice per group. * Significantly different ($P < .05$). CD = chow diet; HFD = high fat diet.
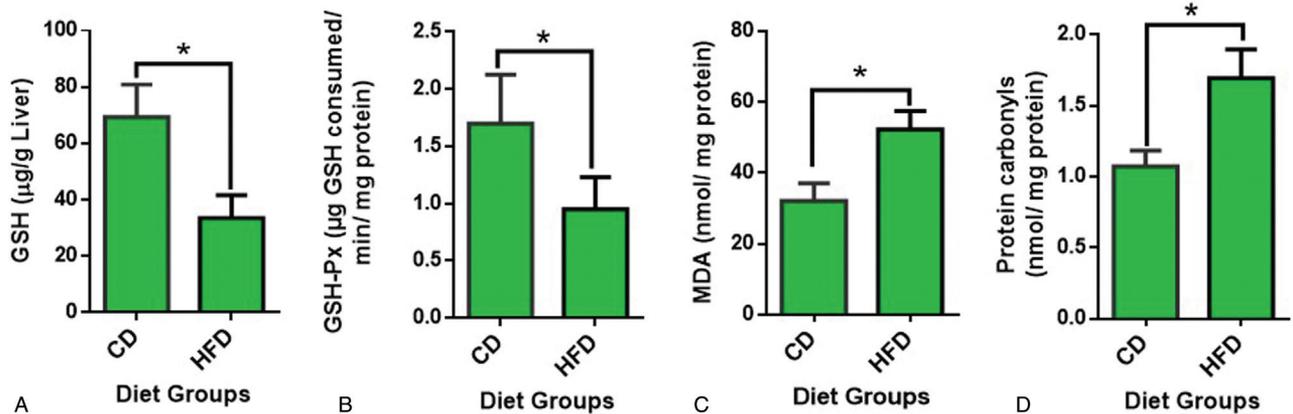
Figure 5. Hepatic antioxidants and biomarkers of oxidative stress in mice fed normal chow diet or high fat diet for 4 weeks: (A) reduced glutathione level, (B) glutathione peroxidase activity, (C) malondialdehyde level, and (D) level of protein carbonyls. Bars represent the mean ± SEM of 5 mice per group. * Significantly different ($P < .05$). CD = chow diet; HFD = high fat diet.

Ore *et al.* Porto Biomed. J. (2020) 5:4

Porto Biomedical Journal
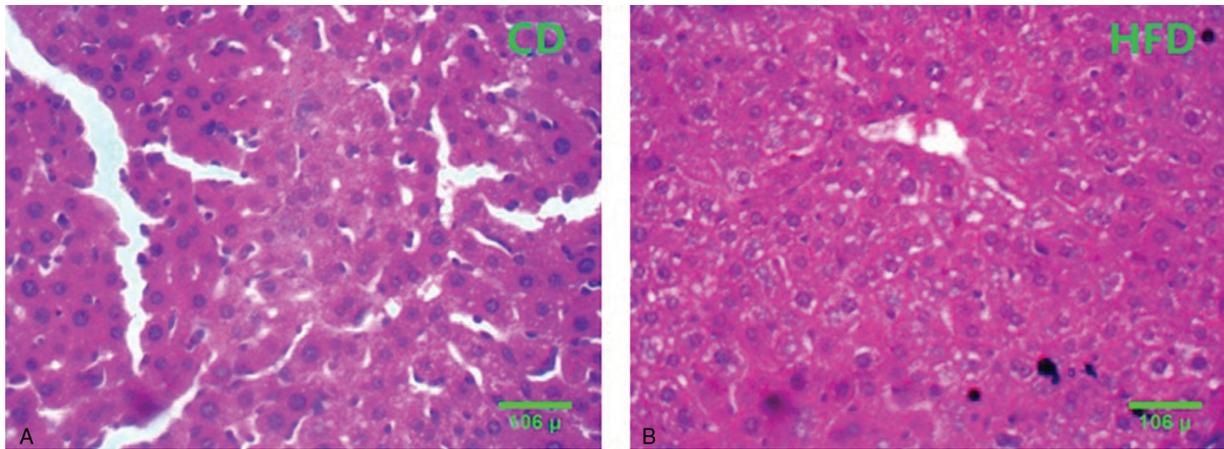
Figure 6. Representative images of liver sections from mice fed normal chow diet or high fat diet for 4 weeks: (A) chow diet shows normal hepatic structure. (B) High fat diet shows steatosis (mainly microvesicular), with hepatocellular ballooning in the zone 3 hepatocytes (centrilobular hepatocellular degeneration).

Previous studies have shown that oxidative stress and antioxidants play some roles in the pathogenesis of NAFLD.[23,24] Metabolic activities of the mitochondria are important contributors to the level of oxidative stress in NAFLD.[25] The mitochondria generate reactive oxygen species (ROS) during hepatic β-oxidation reactions. Increase in ROS production is related to increases in free fatty acid delivery in NAFLD.[3,25] An increase in fatty acid induces oxidative metabolism, causing an increase in the level of oxidative stress and inflammation. ROS produced can damage the hepatocytes contributing to inflammation and oxidative damage products such as MDA (from lipid oxidation) and protein carbonyls (from protein oxidation).[3] In the present study, we observed a significant decrease in antioxidant levels in the HFD group. Hepatic concentration/ activity of 2 antioxidants involved in the clearance of ROS: GSH (a nonenzymic antioxidant) and GSH-Px (an enzymic antioxidant) were decreased in HFD group. These antioxidants have been identified as key players in the pathogenesis of NAFLD.[3] Their reduction may be related to the presence of reactive species as also evident by an increase in the levels of oxidative damage products (MDA and protein carbonyls) in this study. Findings from the present study on antioxidant and oxidative stress are similar to those reported earlier in both human and experimental NAFLD.[10,26]

Histological evidence in mice revealed that ingestion of LDHFLD during a period of 4 weeks produced most of the prominent characteristics of NAFLD. These features including steatosis, hepatocellular degeneration, and ballooning of hepatocytes are consistent with previous reports regarding NAFLD induced with LDHFLD.[8,12]

Conclusions

In conclusion, data from this preliminary investigation show that feeding LDHFLD to mice for 4 weeks produces the various characteristics of NAFLD including the associated metabolic and histologic characteristics. It is hoped that this mouse model will serve as a relevant model in the understanding of the disease progression and also in identifying drug targets against the disease. Further studies with a wider selection of biomarkers are required to explain the mechanism underlying the development of NAFLD using this model.

Author contributions

Conflicts of interest

The author (s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

[1] Puri P, Sanyal AJ. Nonalcoholic fatty liver disease: definitions, risk factors, and workup. Clin Liver Dis (Hoboken). 2012;1:99–103.

[2] Buzzetti E, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of nonalcoholic fatty liver disease (NAFLD). Metabolism. 2016;65:1038–1048.

[3] Ore A, Akinloye OA. Oxidative stress and antioxidant biomarkers in clinical and experimental models of non-alcoholic fatty liver disease. Medicina. 2019;55:1–13.

[4] Ludwig J, Viggiano TR, McGill DB, et al. Non-alcoholic steatohepatitis: Mayo clinic experiences with a hitherto unnamed disease. Mayo Clin Proc. 1980;55:434–438.

[5] Bertola A. Rodent models of fatty liver diseases. Liver Res. 2018;2:3–13.

[6] Anstee QM, Goldin RD. Mouse models in non-alcoholic fatty liver disease and steatohepatitis research. Int J Exp Pathol. 2006;87:1–16.

[7] Castro RE, Diehl AM. Towards a definite mouse model of NAFLD. J Hepatol. 2018;69:272–274.

[8] Lieber CS, Leo MA, Mak KM, et al. Model of non-alcoholic steatohepatitis. Am J Clin Nutr. 2004;79:502–509.

[9] Lieber CS, Leo MA, Mak KM, et al. Acarbose attenuates experimental non-alcoholic steatohepatitis. Biochem Biophys Res Comm. 2004;315:699–703.

[10] Zou Y, Li J, Lu C, et al. High-fat emulsion-induced rat model of non-alcoholic steatohepatitis. Life Sci. 2006;79:1100–1107.

[11] Akin H, Deniz M, Tahan V, et al. High-fat liquid "Lieber-DeCarli" diet for an animal model of non-alcoholic steatohepatitis: does it really work? Hepatol Int. 2007;1:449–450.

[12] Nazmy MH, Abdel-Ghany MI. Serum markers versus histopathological scoring for discrimination between experimental fatty liver and non-alcoholic steatohepatitis. Int Res J Med Med Sci. 2015;3:51–59.

[13] Gornall AG, Bardawill CJ, David MM. Determination of serum proteins by means of the Biuret reaction. J Biol Chem. 1949;177:751–766.

[14] Wright PJ, Leathwood PD, Plummer DT. Enzymes in rat urine: alkaline phosphatase. Enzymologia. 1972;42:317–327.

[15] Jollow DJ, Mitchell JR, Zampaghone N, et al. Bromobenzene induced liver necrosis, protective role of glutathione and evidence for 3,4 bromobenzene oxide as the hepatotoxic metabolite. Pharmacology. 1974;11:151–169.

[16] Gross RT, Bracci R, Rudolph N, Schroeder E, Kochen JA. Hydrogen peroxide toxicity and detoxification in the erythrocytes of new born infants. Blood. 1967;29:481–493.

[17] Varshney R, Kale RK. Effect of calmodulin antagonist on radiation induced lipid peroxidation in microsomes. Int J Radic Biol. 1990;58:733–743.

[18] Reznick AZ, Packer L. Oxidative damage to proteins: spectrophotometric method for carbonyl assay. Methods Enzymol. 1994;233:357–363.

[19] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28:412–419.

[20] Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. Nature. 2001;414:799–806.

[21] Dongiovanni P, Rametta R, Meroni M, Valenti L. The role of insulin resistance in nonalcoholic steatohepatitis and liver disease development – a potential therapeutic target? Expert Rev Gastroenterol Hepatol. 2016;10:229–242.

[22] Rabelo F, Oliveira CPMS, Faintuch J, et al. Pro- and anti-inflammatory cytokines in steatosis and steatohepatitis. Obes Surg. 2010;20:906–912.

[23] Matsunami T, Sato Y, Ariga S, et al. Regulation of oxidative stress and inflammation by hepatic adiponectin receptor 2 in an animal model of nonalcoholic steatohepatitis. Int J Clin Exp Pathol. 2010;22:472–481.

[24] Leclercq A, Farrell GC, Field J, Bell DR, Gonzalez FJ, Robertson GR. CYP2E1 and CYP4A as microsomal catalysts of lipid peroxides in murine non-alcoholic steatohepatitis. J Clin Investig. 2000;105:1067–1075.

[25] Satapati S, Kucejova B, Duarte JA, et al. Mitochondrial metabolism mediates oxidative stress and inflammation in fatty liver. J Clin Investig. 2015;125:4447–4462.

[26] Videla LA, Rodrigo R, Orellana M, et al. Oxidative stress-related parameters in the liver of non-alcoholic fatty liver disease patients. Clin Sci (Lond). 2004;106:261–268.