Histol Histopathol (2022) 37: 813-824

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

REVIEW **Open Access**

Characterization of diet based nonalcoholic fatty liver disease/nonalcoholic steatohepatitis in rodent models: Histological and biochemical outcomes

Ghaidafeh Akbari[1], Seyyed Ali Mard[2], Feryal Savari[3], Barat Barati[4] and Maryam Jafar Sameri[5,6]

[1]Medical Plants Research Center, Yasuj University of Medical Sciences, Yasuj, [2]Clinical Sciences Research Institute, Alimentary Tract Research Center, Department of Physiology, The school of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, [3]Department of Basic Sciences, [4]Department of Radiologic Technology, Shoushtar Faculty of Medical Sciences, Shoushtar, [5]Department of Physiology, The School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz [6]Department of Physiology, The School of Medicine, Abadan University of Medical Science, Abadan,Iran

Summary. Nonalcoholic fatty liver disease (NAFLD), as the most common chronic liver disease, is rapidly increasing worldwide. This complex disorder can include simple liver steatosis to more serious stages of nonalcoholic fibrosis and steatohepatitis (NASH). One of the critical concerns in NASH research is selecting and confiding in relying on preclinical animal models and experimental methods that can accurately reflect the situation in human NASH. Recently, creating nutritional models of NASH with a closer dietary pattern in human has been providing reliable, simple, and reproducible tools that hope to create a better landscape for showing the recapitulation of disease pathophysiology. This review focuses on recent research on rodent models (mice, rats, and hamsters) in the induction of the dietary model of NAFLD /NASH. This research tries to compile the different dietary compositions of NASH, time frames required for disease development, and their impact on liver histological features as well as metabolic parameters.

Key words: NAFLD /NASH, Dietary models, Metabolic syndrome, Mice, Rats, Hamsters

Introduction

The most frequent liver disease worldwide that is unceasingly rising in parallel with the growing obesity epidemic is nonalcoholic fatty liver disease (NAFLD) (Hansen et al., 2017; Younossi et al., 2018). Indeed, NAFLD is quickly becoming a worldwide public health problem, and it is predicted that it will have an exponential growth during 10 next years due to high caloric intake combined with a sedentary lifestyle (Younossi, 2019). The NAFLD includes two histological forms: nonalcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH) (Sberna et al., 2018). When hepatic steatosis without hepatocellular necrosis and no or minimal inflammation is observed, the NAFL occurs. On the other hand, NASH characteristics include steatosis, hepatocellular ballooning, and liver inflammation, with or without fibrosis (Soret et al., 2020). When inflammation and fibrosis in NASH are developed, it underlies cirrhosis and hepatocellular carcinoma (HCC) (Sberna et al., 2018). Currently, NASH is known as the second main cause of orthotopic liver transplantation, and it will become the most common reason for liver transplantation within developing countries (Agopian et al., 2012). The molecular and cellular mechanisms of how the progression from NAFL to NASH is not completely elucidated. The "two-hit hypothesis" is one of the leading theories in the issue (Dowman et al., 2010; Stephenson et al., 2018). The first hit is the accumulation of fat in the liver which results in the liver being at increased susceptibility to further damages (Donnelly et al., 2005). The effect of oxidative stress on the liver is the second hit, as it results in liver cell damage and inflammation, which signifies the progression of NAFL to NASH (Caldwell and Crespo, 2004). From a pathogenesis point of view, many mechanisms have been proposed for inducing fatty liver infiltration and NASH appearing (Schuppan and Schattenberg, 2013). Some different metabolic diseases such as central obesity, type 2 diabetes, and hyperlipidemia are well-known risk factors and been strongly associated with NASH (Kořínková et al., 2020). Since the NAFLD and metabolic syndromes that lead to NASH are affected by an unhealthy diet, adopting healthy dietary habits can be considered as a suitable approach for all patients with

Corresponding Author: Feryal Savari, Department of Basic Sciences, Shoushtar Faculty of Medical Sciences, Shoushtar, Iran. e-mail: savari-f@shoushtarums.ac.ir or feryal.savari@gmail.com

DOI: 10.14670/HH-18-462

NASH (Finelli and Tarantino, 2012; Torres Silva et al., 2020). Thus, to investigate the relationship between diets and the development of NASH and to clarify the involved pathophysiological mechanisms of NASH, appropriate experimental models need to be used. Since the morphological alteration in NAFLD/NASH animal models is considered as the endpoint of common pathogenic pathways despite diversities of the primary etiology, the pathologist tends to focus on morphological alterations of models (Denk et al., 2019). Currently, physiology scientists will prefer models that based on the administration of toxins, special diets, or genetic alterations resembling the metabolic background of human disease with less emphasis on morphology. As such, the experimental models that mimic the human condition as causative mechanisms responsible for studies in humans are many times more difficult or impossible to understand how this disease develops and progresses. The animal model may serve as an excellent preclinical platform to study novel therapeutic strategies for inhibiting NAFLD/NASH progression. This review provides a more comprehensive exploration of the known dietary animal models of NASH, with a focus on comparing the effects of various dietary components on the metabolic parameters of the disease, biochemical alterations, and the histological features to help in selecting an effective dietary intervention during the study design phase.

Research method

All published data until December 1, 2021, including articles and research relevant to the *in vitro* and *in vivo* studies that assayed the known dietary animal models of NAFLD/NASH were considered. The information was collected by searching in Google Scholar, Scopus, and PubMed. The keywords including "Nonalcoholic fibrosis and steatohepatitis", "Nonalcoholic fatty liver disease", "dietary model of NAFLD /NASH", "Metabolic syndrome" were combined with "Rodent animal", "Rat", "Hamster", OR "Mice" to be used as search terms. The inclusion criteria were studies evaluating different dietary models of NAFLD /NASH in animal models. The studies that did not meet the inclusion criteria, involved human research or clinical studies, and duplicate studies were removed. There were no search restrictions in the type of studies. The English language studies were used for reviewing.

Characteristics of NASH

The newest hypothesis that explains the pathogenesis of NASH is the "multi-parallel hit" hypothesis. According to this hypothesis, endoplasmic reticulum stress and cytokine-mediated stress can induce steatosis as well as necroinflammation. Thus, multiple hits act together in the development of NASH (Dowman et al., 2010). As such, the individual markers for NASH, such as inflammatory or fibrogenic factors and biochemical markers can improve the understanding of disease pathogenesis and allow therapies to be developed. Nonspecific hepatocellular damage is reflected by biochemical markers measured in plasma or the liver, such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Kořínková et al., 2020). Other primary markers of NASH include inflammation markers (such as C-reactive protein (CRP)), inflammatory cytokines and chemokines such as interleukin (IL-6), tumor necrosis factor-α (TNFα), and chemokine CC ligand-2 (CCL2) (Neuman et al., 2014; Schuppan et al., 2018). For verifying or diagnosing the stage of NASH to evaluate histopathological changes in NASH, liver biopsy can be used. Several histological scoring systems such as the NAFLD activity score (NAS) and the fibrosis scoring system are applied to monitor these histopathological changes (Kim et al., 2011). Currently, noninvasive approaches methods such as magnetic resonance spectroscopy, other inflammatory, and fibrotic markers discovered by hepatic mRNA sequencing provide sensitive and fast methods to detect NASH (Szczepaniak et al., 2005; Hansen et al., 2017; Tølbøl et al., 2018).

Animal models of NASH

Over the last decade, various animal models with various degrees of fidelity to the metabolic profile and the histological patterns of human NASH have been developed. The NASH models can be divided into two groups which involve environmental induction (diets, drugs, or toxins) and genetic modifications that naturally occur or target overexpression or deletions of genes (Diehl 2005; London and George, 2007). The outcomes of these models are decreased hepatic fatty acid oxidation or export, as well as increased hepatic lipogenesis and uptake (Diehl 2005; Larter and Yeh, 2008; Ying-Rong et al., 2021). Among all models, the over-nutrition-based models have shown substantial metabolic similarity to humans with NASH (Ibrahim et al., 2016). These models have reproducibility of the histological features of NASH, such as hepatocellular ballooning (Charlton et al., 2011; Verbeek et al., 2015) and progressive hepatic fibrosis (Kohli et al., 2010; Charlton et al., 2011). This review provides a comprehensive description of dietary strategies in the three common animal models (mouse, rat, and hamster) for selecting the best diet choice during the study design phase.

Diet-induced NASH models in animal models

High-Fat Diet

The High-Fat Diet (HFD) includes a wide range of diet formulas with different fat types (30-60 kcal% fat) with other compositional differences such as different forms (i.e., pellet or liquid) and low or high sucrose

amounts (Radhakrishnan et al., 2020). The high-fat diet in experimental animals is widely applied to induce hepatic steatosis and NASH (Table 1). Nevertheless, the obtained results are variable concerning the degree of steatosis, inflammation, and fibrosis (Kanuri and Bergheim, 2013; Nakamura and Terauchi, 2013). These different results are due to differences rodent models strain, physical activity (mainly sedentary behavior), the sex and genetic background of the models, the fat content of the diet, the composition of the dietary fat, and the duration of treatment (Kanuri and Bergheim, 2013; Kořínková et al., 2020). Depending on the fat source, the HFD typically increases body weight and body fat as well as insulin resistance in rodent models (Imajo et al., 2013).

Sprague-Dawley rats are the first NASH animal models that showed panlobular steatosis and inflammatory infiltrate with predominantly mononuclear cells developed after 3 weeks of consuming HFD (71 % of energy from fat, 11 % from carbohydrates, 18 % from protein). Rats increased hepatic molecular markers of inflammation, oxidative stress and developed insulin resistance and induced fibrogenesis, which was indicated by elevation of TNF-α, 4-hydroxynonenal (4-HNE), plasma insulin, and collagen I, respectively. However, this diet could not increase body weight or plasma ALT levels after 3 weeks (Lieber et al., 2004). Another study fed Sprague-Dawley rats HFD (60% fat-derived calories) for 7 weeks and reported hypertriglyceridemia, hyperinsulinemia, and hyperglycemia, which are

Table 1. High fat diet- induced rodent models of NAFLD/NASH.

Rodent model	Dietary model	Composition/definition	Treatment length	outcome	Phenotypes/outcomes
SD rat (Liu et al., 2015)	HFD (D12492)	60% kcal-fat	12 weeks	NAFLD	Steatosis; ↑BW; ALT, AST (no change); ↑TG (plasma) TC (plasma; no change); ↑FBS, ↑Insulin, ↑HOMA-IR
SD rat (Liu et al., 2015)	HFD + STZ (D12492)	60% kcal-fat + single dose of STZ (25 mg/kg)	12 weeks	NAFLD +T2DM	Steatosis; ↑BW ALT, AST (no change); ↑TG (plasma) TC (plasma; no change); ↑FBS, ↑Insulin, ↑HOMA-IR
SD rat (Hsu et al., 2013)	HFD + STZ (D12492)	60% kcal-fat + single dose of STZ (200 mg/kg)	8 weeks	NASH	Steatosis (macrovesicular); Ballooning Inflammation; severe fibrosis; ↑BW, ↑ALT, ↑FBS
C57BL/6N mice (Kubota et al., 2013)	HFD + CCl4	64% kcal-fat, 21% kcal-carbohydrate, 14% protein + 8 times CCl4 administration	12 weeks	NASH	Steatosis; Inflammation; Ballooning Fibrosis; ↑ALT, ↑TNF-α, ↑TGF-β
C57BL/6N mice (Kubota et al., 2013)	HFD	64% kcal-fat, 21% kcal-carbohydrate, 14% protein	12 weeks	NAFLD	Steatosis; ↑BW, ↑ALT, ↑AST
BALB/c mice (Farrell et al., 2014)	HFD	(23% fat, 45% carbohydrate, 20% protein, 0.19% cholesterol)	24 weeks	*failed to induce NAFLD*	↑BW, ↑Insulin, ↑FBS
Obese (foz/foz) BALB/c mice (Farrell et al., 2014)	HFD	(23% fat, 45% carbohydrate, 20% protein, 0.19% cholesterol)	24 weeks	NAFLD	Steatosis; Necroinflammation; Mild fibrosis ↑BW, ↑TC, ↑Insulin, ↑FBS, ↑TNFα, ↑α-SMA
C57BL6/J mice (Farrell et al., 2014)	HFD	(23% fat, 45% carbohydrate, 20% protein, 0.19% cholesterol)	24 weeks	NASH	Steatosis; Ballooning; Necroinflammation fibrosis ↑BW, ↑ALT, ↑TC, ↑FBS, ↑TNFα, ↑α-SMA
Obese (foz/foz); 57BL6/J mice (Farrell et al., 2014)	HFD	(23% fat, 45% carbohydrate, 20% protein, 0.19% cholesterol)	24 weeks	Severe NASH	Steatosis; Ballooning; Necroinflammation fibrosis ↑BW, ↑ALT, ↑TC, ↑Insulin, ↑FBS, ↑TNFα, ↑α-SMA
C57BL6/J mice (Kirpich et al., 2011)	HFD	60 kcal%-fat, mainly lard	8 weeks	NAFLD	Macrovesicular steatosis without necrosis Inflammation; ↑BW, ↑ALT, ↑Insulin FBS (no change); ↑TC, ↑TG, ↑TG (liver)
C57BL6/J mice (Flores-Costa et al., 2018)	HFD (D12492)	60% kcal from fat, 20% kcal-carbohydrate, 20% kcal-protein	16 weeks	NASH	Steatosis; Mild inflammation; Mild fibrosis ↑BW, ↑AST, ↑TG (liver), ↑SREBP-1c (mRNA)
C57BL6/J mice (van der Heijden et al., 2015)	HFD (D12492)	60% kcal from fat (60% kcal-fat)	52 weeks	NASH	Steatosis (micro- & macrovesicular); Ballooning Multifocal inflammation fibrosis; ↑BW, ↑Insulin, HOMA-IR (no change); ↑TNFα (mRNA)
C57BL6/J mice (Lieber et al., 2004; Eccleston, et al., 2011)	HFD	71% kcal-fat, 11% kcal-carbohydrate, 18% kcal-protein	16 weeks	NAFLD	Steatosis; Ballooning; BW (no change), ↑TG (liver), plasma TG (no change); ALT (no change) ↑FBS, Insulin (no change); TNFα (no change)
Golden Syrian hamster (Tzeng et al., 2015)	HFD (D12451)	1825 kcal fat, 1420 kcal carbohydrate, 811 kcal protein	10 weeks	NASH	Steatosis (micro- & macrovesicular); Multifocal inflammation; ↑BW, ↑FBS, ↑Insulin, ↑TG, ↑TC, ↑LDL (plasma), ↑TG, ↑TC (liver), ↑HOMA-IR, ↑TNFα (mRNA), ↑SREBP-1c, FAS (mRNA)
Golden Syrian hamster (Ou et al., 2019)	HFD	742 kcal-fat	8 weeks	NAFLD	Steatosis (microvesicular); Mild inflammatory infiltrate; ↑BW, ↑FBS; Insulin (no change); ↑TG, ↑TC, ↑LDL (plasma), ↑TG, ↑TC (liver), FAS (mRNA, no change); ↓SREBP-1c (mRNA)

HFD, high fat diet; STZ, streptozocin; α-SMA, α-smooth muscle actin; COL1A1, collagen type I; FBS, fast blood sugar, SREBP-1c, sterol regulating element binding protein-1c; FAS; fatty acid synthase, TNF-α; tumor necrosis factor-α; TGF, transforming growth factor; BW, body weight; TG, triglyceride; TC, total cholesterol; SD, sprague dawley; LDL, low density lipoprotein; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance.

associated with the development of NASH histologically revealed by high stage of steatosis, ballooning, and inflammation (Emamat et al., 2018). Some HFD in Sprague-Dawley rats could increase liver fat concentrations very rapidly (within days) and hepatic insulin resistance, before significant increases occur in peripheral fat deposition (Samuel et al., 2004). Increasing the duration of HFD in Wistar rats, when they fed with 54% lipids (50% lard, 4% soya-bean oil w/w) for 8 weeks, could inhibit fatty acid oxidation plus mitochondrial oxidative phosphorylation and induce NAFLD. In this model, hepatic fat content increased, but liver parenchyma remained unchanged (Vial et al., 2011). In rat models, a combination of chemicals such as streptozotocin or carbon tetrachloride (CCl4) with HFD feeding could induce NASH associated with fibrosis (Hsu et al., 2013; Kubota et al., 2013; Liu et al., 2015).

The most common mouse strain applied in experimental NASH after obesogenic diets such as HFD is C57BL/6 mice, as this mouse strain exhibits high sensitivity to obesogenic diets (Hansen et al., 2017). In addition, the C57BL/6 mice model compared with BALB/c and C3H/HeN mice model is significantly more susceptible to developing diet-based NASH model exhibited by hepatic necroinflammation and fibrosis (Yamazaki et al., 2008; Farrell et al., 2014). In the experimental NASH researches, use of D12492 (60 kcal% fat, mainly lard) as widely used HFD could induce hepatic steatosis characterized by significantly elevated liver and plasma alanine aminotransferase, free fatty acid (FFA), and triglyceride concentrations, as well as inflammation in C57BL/6 mice fed for 8 weeks (Kirpich et al., 2011). In another study, C57BL/6 mice fed diet D12492 for 16 weeks showed widespread hepatic steatosis and increased hepatic TGs, adipose tissue inflammation, and mild fibrosis (Flores-Costa et al., 2018). The C57BL/6 mice were fed D12492 diet for 16 weeks had ~2- NAS scores (Liu et al., 2015). When C57BL/6 mice were fed D12492 diet for chronic time (52 weeks), the NASH was induced with inflammation along with excess body weight, hypercholesterolemia, and hyperinsulinemia (Ito et al., 2007; van der Heijden et al., 2015). The liver fibrosis degree in the mice model was worsened after the addition of sucrose in HFD (36 kcal% fat, 30 kcal% sucrose) as shown with increased expression of the α-smooth muscle actin (α-SMA), as a fibrotic marker (Ishimoto et al., 2013). Thus, the addition of sucrose and type of fat in the HFD formulation plays a critical role in these studies (de Wit et al., 2012). In rodent NASH models, increasing the fat content of HFD is also practical in inducing hepatic steatosis, and lipid concentrations related to increase in the content of dietary TGs (Lieber et al., 2004; Eccleston et al., 2011).

The HFD without other ingredients in rat models could induce mild steatosis (Imajo et al., 2013). This demonstrated that the genetic difference in all rodent models caused them not to have the same response to HFD. It seems that rats are the most susceptible to HFD among animal models of NASH. Rats have shown to experience severe steatosis with hyperlipidemia and hyperinsulinemia in a shorter time than other rodent models following the same HFD (Ito et al., 2007; Emamat et al., 2018). In addition, prolonged HFD feeding in rats could induce weak inflammation and ballooning, while such a diet was not sufficient to develop ballooning degeneration or inflammation in mice (Charlton et al., 2011).

Development of NASH was observed in golden Syrian hamsters due to HFD (45 kcal% fat diet, mainly lard, D12451) fed for 10 weeks. This diet caused an increase in histological grade of steatosis, liver and plasma lipid (total cholesterol and TGs) contents, and expression of inflammatory cytokine genes such as monocyte chemoattractant protein 1 (MCP-1), IL-1β, IL-6, and TNF-α which led to mild inflammatory foci development (Tzeng et al., 2015). Another study on the hamster model fed an HFD composed of 30% fat mainly derived from lard with 0.2% cholesterol after 8 weeks. It showed a predominantly microvesicular pattern of steatosis and elevation of liver lipids, plasma total cholesterol, LDL cholesterol, and TGs (Ou et al., 2019). Although the HFD fed in rodent models can replicate the altered metabolic parameters observed in human NASH, the hepatic pathological outcome is not as severe.

High-fructose diet

Fructose-rich diet forms can quickly lead to metabolic syndrome diseases (Hannou et al., 2018). The result of metabolic disorders in the body and liver mediated by fructose-rich food consumption can induce and contribute to development of NAFLD and then NASH (Roeb and Weiskirchen, 2021). Various techniques have been used in animal models to assess the influence of a fructose-rich diet on NASH development (Table 2). One of them is adding high-fructose corn syrup to water, combined with HFD feeding (Table 5) (Kohli et al., 2010; Savari et al., 2019a,b). This diet in 57BL/6 mice fed 8 kcal% fat diet and high fructose corn syrup in water for 16 weeks could increase lipogenesis and VLDL, hepatic oxidative stress, inflammatory markers (IL-1β, intercellular adhesion molecule-1), and metabolic disorders such as increased plasma lipids and glucose intolerance. Then, the result of these changes was a progression of liver fat deposition and occurrence of NASH scored according to the micro- and macrovesicular steatosis, lobular inflammation, and portal fibrosis (Kohli et al., 2010). Another technique is adding fructose directly in the context of a pelleted diet with 60-70% calories derived from fructose (Nigro et al., 2017). C57BL/6 mice (60 kcal% as fructose and 10 kcal% fat) could increase steatosis, liver TG and inflammation as compared to a low-fat matched control diet group (Nigro et al., 2017).

The high fructose diet (70 kcal%) in Sprague-Dawley rats induced NAFLD/NASH those pathological symptoms including high score steatosis, hepatic

ballooning, fibrosis, and inflammation (Svendsen et al., 2017). In Wistar rats fed high fructose diet (73% kcal fructose), increases in hepatic TGs were observed along with promoted liver histophathological changes such as a mixed micro/ macrovesicular pattern of steatosis and lobular inflammation. In this model, liver fibrotic scores were also significantly higher as compared to the control group (Kawasaki et al., 2009).

Golden Syrian hamsters fed high fat and high fructose diet (5.1% fat, 19.3% protein, 55.5% carbohydrates) for 10 to 20 weeks showed development of NASH, including hepatocellular ballooning and bridging fibrosis (Briand et al., 2021). A high fructose diet (60 kcal%) in golden Syrian hamsters could elevate plasma and liver TG, plus insulin (Kasim-Karakas et al., 1996).

Western diet (WD) or Fast-Food Diet

High-Fat, High-Fructose, and High-Cholesterol Combination Diets

A modern dietary pattern that exists in industrialized countries which includes high intakes of fat and sugar is the "Western diet or fast food" diet (Kopp, 2019). This dietary model, which is based on a combination diet rich in fat, sugar, and cholesterol can induce NAFLD and then NASH in animal models (Table 3) (Soret et al., 2020).

It has been demonstrated that high dietary fat together with high dietary cholesterol and high fructose can have synergistic interaction to induce steatohepatitis as well as the metabolic disturbances associated with human NASH (Savard et al., 2013, Jensen et al., 2018; Eng and Estall, 2021). C57BL/6 mice fed a combination diet containing high fat and fructose with added a modest amount of cholesterol (0.2%) for 14 weeks experienced increased hepatic steatosis and inflammation as well as plasma alanine aminotransferase level, in addition to exacerbation of insulin resistance and blood glucose (Kim et al., 2017). In NMRI mice, the addition of 0.4% cholesterol to a high-fat diet supplemented with fructose in drinking water could increase hepatocellular ballooning, hepatic TNF-α levels, and NAFLD activity score (NAS). In this NASH model, exposing animals to smoke led to more severe steatohepatitis (Savari et al., 2019b). A high-cholesterol diet on a low-fat diet background (added 1% cholesterol to a low-fat diet) (1% cholesterol, 11 kcal% fat) in C57BL/6 mice after 30 weeks caused increased hepatic cholesterol esters, where both the high-cholesterol diet and high-fat diet (33 kcal% fat, added cocoa butter) increased hepatic cholesterol esters and steatosis. While combined, synergistic interaction of the high-fat content and cholesterol amount caused NASH via moderate steatohepatitis and mild fibrosis. In addition, this diet could induce more significant elevations in weight gain, serum ALT concentrations, hepatic fat accumulation, and fibrosis, compared with high-fat diet. It also was associated with obesity and IR (Savard et al., 2013). The addition of cholic acid in the context of a high fat high cholesterol diet which has been commonly used in atherosclerosis related studies in rodent models (Lichtman et al., 1999) could be progressed steatosis and fibrosis after 12 weeks in male C57BL/6 mice and induced NASH (Montandon et al., 2019).

In rats and mice model, combination diets containing high-fat, high-fructose, and high-cholesterol, widely known as the AMLN diet (D09100301) and similar to the ALIOS (American Lifestyle-Induced Obesity

Table 2. High-Fructose diets- induced rodent models of NAFLD/NASH.

Rodent model	Dietary model	Composition/definition	Treatment length	Outcome	Phenotypes/outcomes
NMRI mice (Savari et al., 2019b)	HFrD	30% fructose in drinking water	8 weeks	Failed to induce NAFLD	Mild inflammation; Mild ballooning ↑FBS, ↑Insulin, ↑HOMA-IR plasma TC & TG (no change); ↑ALT, ↑AST
C57BL6/J mice (Charlton et al., 2011)	HFrD	23.1 g/l fructose in drinking water	25 weeks		
C57BL6/J mice (Nigro et al., 2017)	HFrD D02022704	60% kcal-fructose diet	12 weeks	NASH	Steatosis (macrovesicular); Inflammation; BW (no change); ↑FBS, Insulin (no change), ↑TG, ↑TC (plasma), ↑TG, TC; no change (liver), ↑SREBP-1c (mRNA)
SD rat (Jensen, et al., 2018)	HFrD	70% fructose, 10% fat, 20% protein	12 weeks	NASH	Steatosis; Inflammation; Ballooning; Fibrosis ↑BW, ↑FBS; ALT, AST (no change), ↓ALP ↑TG, ↑TC (plasma), ↑TNFα (mRNA)
Wst rat (Kawasaki et al., 2009)	HFrD	74.2% kcal-fructose	5 weeks	NASH	Steatosis (micro- and macrovesicular); Lobular inflammation; Fibrosis; BW, FBS (no change) ↑TG (liver); Plasma TG (no change)
Golden Syrian hamster (Kasim-Karakas et al., 1996)	HFrD	60% kcal-fructose			↑BW; FBS (no change), ↑Insulin, ↑TG (liver), ↑TG (plasma)

HFD, high fat diet; STZ, streptozocin; α-SMA, α-smooth muscle actin; COL1A1, collagen type I; FBS, fast blood sugar, SREBP-1c, sterol regulating element binding protein-1c; FAS; fatty acid synthase, TNF-α; tumor necrosis factor-α; TGF, transforming growth factor; BW, body weight; TG, triglyceride; TC, total cholesterol; SD, sprague dawley; LDL, low density lipoprotein; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance.

Syndrome), are a preferred diet by much research of NASH animal models (Tetri et al., 2008; Jensen et al., 2018). The AMLN diet caused liver damage and NASH development (steatosis, inflammation, ALT), similar to the mechanism that has been reported to occur in human (Jensen et al., 2018). The AMLN diet would use trans-fat source (~25% of fat), Primex, as a fat source formulated with a mixture of partially hydrogenated soybean/palm oils and the FDA banned its use in foods in 2018 (Radhakrishnan et al., 2020). Currently, in the AMLN diet, an alternative approach to inducing this phenotype of NASH model in C57BL/6 mice has been successful by replacing Primex with palm oil (D09100310) (Hansen et al., 2020). Use of modified AMLN diets in rodent NASH models can provide a more human-like model of this disease development, and help find a model for effective drug discovery. Hepatocyte fat deposition was mainly distributed as macrovesicular steatosis (grade 3) accompanied with lobular inflammation, ballooning (grade 1), and perisinusoidal fibrosis scored as 4-7 in mice which were comparable to 5-6 NAS score in NASH patients. In golden Syrian hamsters, a high fat-high fructose diet (30% fat, 40% fructose) with 0.05-0.25% cholesterol for 6 weeks could elevate liver TG and cholesterol concentrations. In addition, glucose tolerance and insulin sensitivity in this diet were worsened in a dose-dependent way in the hamster model (Basciano et al., 2009).

Methionine and choline deficient (MCD) diet

The methionine and choline deficient (MCD) diet is a frequently used dietary model among the different nutritional methods for diet-induced NAFLD/NASH in animal models. This diet can induce the most severe phenotype of NASH within the shortest possible time, which is highly desirable for those finding ways to reverse this phenotype or slow it down. This diet during 1-4 weeks in rodent animal model can induce measurable hepatic steatosis (predominantly macrovesicular pattern), while a longer feed (1-2 months) can induce the development of fibrosis and inflammation (Table 4) (Weltman et al., 1996; Sahai et al., 2004). This diet contains high sucrose (40%) and moderate fat (10%) (Ibrahim et al., 2016). In rodent model, the deficiency in choline and methionine in this diet which are essential nutrients, caused impairment of β-oxidation and production of very-low-density lipoprotein particles (Ibrahim et al., 2016). In addition, choline deficiency can be impaired in hepatic low-density lipoprotein secretion and hepatic fat accumulation, oxidative stress, liver cell death, and changes in cytokines and adipokines, but only minor inflammation and fibrosis have been observed (Lau et al., 2017). The added methionine deficiency leads to further early development of fibrosis and distinct inflammation (Itagaki et al., 2013; Lau et al., 2017).

Table 3. Western diet- induced rodent models of NAFLD/NASH.

Rodent model	Dietary model	Composition/definition	Treatment length	Outcome	Phenotypes/outcomes
C57BL6/J mice (Charlton et al., 2011)	WD (FFD)	40% kcal-fat, 2% kcal-cholesterol, 23.1 g/l fructose in drinking water	25 weeks	NASH	Steatosis; Ballooning (severe); Inflammation; Fibrosis ↑BW, ↑TC (plasma), ↑AST, ↑FBS, ↑Insulin, ↑αSMA, ↑COL (immunostaining)
SD rat (Jensen et al., 2018)	WD	40% kcal-fat, 2% kcal-cholesterol, 40% kcal-carbohydrate	16 weeks	NASH	Steatosis (micro- and macrovesicular); Inflammation Fibrosis; ↑BW, ↑ALT, ↑AST, ↑TG, ↑TC (liver), ↑FBS, Insulin (no change)
C57BL6/J mice (Kim et al., 2017)	WD	41% kcal-fat, 0.2% kcal-cholesterol	14 weeks	NASH	Steatosis; Lobular Inflammation; Fibrosis (no evidence) ↑BW, ↑ALT, ↑FBS, ↑Insulin, ↑TC, ↑HOMA-IR, ↑TG (liver), ↑TNFα, ↑COL1a1 (mRNA)
NMRI mice (Savari et al., 2019b)	WD	30% kcal-fat, 0.75% kcal-cholesterol, 30% fructose in drinking water	8 weeks	NASH	Steatosis (micro- and macrovesicular); Inflammation ballooning; ↑TG, ↑TC (plasma), ↑FBS, ↑Insulin, ↑HOMA-IR, ↑ALT, ↑AST, ↑TNFα
NMRI mice (Savari et al., 2019b)	WD + CS	30% kcal-fat, 0.4% kcal-cholesterol, 30% fructose in drinking water	8 weeks	NASH	Steatosis (micro- and macrovesicular); Severe Inflammation; Severe ballooning; ↑TG, ↑TC (plasma) ↑FBS, ↑Insulin, ↑HOMA-IR, ↑ALT, ↑AST, ↑TNFα
SD rat (Jensen et al., 2018)	WD	40% kcal-fat, 40% kcal-fructose, 2% kcal-cholesterol	16 weeks	NASH	Steatosis (micro- and macrovesicular); Inflammation Fibrosis; Plasma TG & TC (no change); ↑BW, ↑FBS Insulin; ↑TG, ↑TC (liver), ↑ALT, ↑AST
C57BL6/J mice (Hansen et al., 2020)	WD	40% kcal-fat, 22% kcal-fructose, 2% kcal-cholesterol	38-44 weeks	NASH	Steatosis (micro- and macrovesicular); Inflammation Ballooning; Fibrosis (perisinusoidal); ↑BW, ↑TG, ↑TC (liver), ↑Insulin, ↑HOMA-IR, ↑TC (plasma); plasma TG (no change), ↑ALT, ↑AST, ↑TNFα
Golden Syrian hamster (Basciano et al., 2009)	WD	30% kcal-fat, 40% kcal-fructose, 0.25% kcal-cholesterol	22 weeks	NASH	Steatosis; ↑BW, ↑TG, ↑TC (liver), ↑Insulin, ↑HOMA-IR FBS (no change), ↑TC, ↑TG (plasma)

WD, western diet; CS, cigarette smoke; STZ, streptozocin; α-SMA, α-smooth muscle actin; COL1A1, collagen type I; FBS, fast blood sugar, SREBP-1c, sterol regulating element binding protein-1c; FAS; fatty acid synthase, TNF-α; tumor necrosis factor-α; TGF, transforming growth factor; BW, body weight; TG, triglyceride; TC, total cholesterol; SD, sprague dawley; LDL, low density lipoprotein; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance.

Notably, NASH histopathological features increased along with biochemical markers from 2 to 16 weeks, but revealed reversing of NASH by 30 weeks with 8 to 6 NAS score reduction (Itagaki et al., 2013). Since reduced methionine intake caused by mitochondrial S-adenosyl-Lmethionine depletion can be mitochondrial dysfunction and mitochondrial glutathione depletion, this dietary model leads to mitochondrial glutathione depletion and mitochondrial dysfunction (Caballero et al., 2010). The result of mitochondrial dysfunction is hepatocyte injury via oxidative stress and inflammation. The methionine-deficient diet also developed most of the NASH features derived by the MCD diet including macrovesicular steatosis, ballooning, inflammation, and fibrosis accompanied with loss of body weight (Caballero et al., 2010).

In male C57BL/6J mice fed with a methionine- and choline-deficient diet (MCD) for 5 weeks, obvious hepatic steatosis and inflammation were observed compared with those who were fed a chow diet (CD) (Matthews et al., 2021). After feeding MCD diet to Wistar rats for 7 weeks, higher scores of liver inflammation and steatosis, a rapid rise in ALT, and evidence of fibrosis induction in the liver were reported (Veteläinen et al., 2007). In this diet, the source of dietary fat and sucrose and the addition of 1% cholesterol can alter the phenotype and liver injury, especially fibrosis progression (Hussein et al., 2007; Lee et al., 2007; Pickens et al., 2010; Radhakrishnan et al., 2020). In C3H/HeOuJ mice, the fructose -MCD diet could worsen hepatocellular injury as compared to the mice fed MCD-glucose and caused a significant increase in inflammation and hepatic lipid peroxidation (Table 5) (Pickens et al., 2010). Although mice and rats fed MCD diets showed a rapid onset of the NASH phenotype with ballooning and lobular inflammatory infiltrate in a short time (2-4 weeks) (Van Herck et al., 2017), in this NAFLD/NASH diet versus humans with NAFLD/NASH, weight loss owing to restricting calorie intake, low insulin resistance, glucose, leptin, and TG concentrations were observed (Kirsch et al., 2003; Tolosa et al., 2011; Liu et al., 2015). Thus, the extrapolation of data from this NASH model is limited and MCD diets are generally considered sufficient to

Table 4. Methionine- and choline-deficient diet- induced rodent models of NAFLD/NASH.

Rodent model	Dietary model	Composition/definition	Treatment length	outcome	Phenotypes/outcomes
C57BL6/J mice (Yamazaki et al., 2008)	MCDD	40% kcal-sucrose, 10%kcal- fat and is deficient in methionine and choline	8 weeks	NASH	Macrovesicular and panlobular steatosis ballooning; Necroinflammation fibrosis ↓BW, ↑ALT, ↑TG (liver), ↑COL1a1 (mRNA)
C57BL6/J (a/j strain) mice (Sahai et al., 2004)	MCDD	40% kcal-sucrose, 10%kcal- fat and is deficient in methionine and choline	12 weeks	NASH	Steatosis (macrovesicular); Inflammation (lobular & portal); Fibrosis; ↑TG (liver), ↑ALT, ↑TNFα, ↑Col- α I (mRNA)
Wst rat (Weltman et al., 1996)	MCDD	40% kcal-sucrose, 10%kcal- fat and is deficient in methionine and choline	13 weeks	NASH	Severe Steatosis (macrovesicular); Inflammation Ballooning; Fibrosis; ↓BW, ↑ALT
Wst rat (Weltman et al., 1996)	MCDD	40% kcal-sucrose, 10%kcal- fat and is deficient in methionine and choline	4 weeks	NAFLD	Steatosis (macrovesicular); ↓BW, ↑ALT, ↓TG, ↓TC (plasma)
C57BL6/J mice (Itagaki et al., 2013)	MCDD	40% kcal-sucrose, 10%kcal- fat and is deficient in methionine and choline	4-16 weeks	NASH (more severe)	Steatosis; Ballooning; Inflammation; Fibrosis ↓BW, ↑ALT, ↑AST
C57BL6/J mice (Itagaki et al., 2013)	MCDD	40% kcal-sucrose, 10%kcal- fat and is deficient in methionine and choline	30 weeks	NASH	Steatosis; Ballooning; Inflammation; Fibrosis ↓BW, ↓ALT, ↓AST
C57BL6/J mice (Caballero et al., 2010)	MCDD	40% kcal-sucrose, 10%kcal- fat and is deficient in methionine and choline	15 days	NASH	Steatosis (macrovesicular); Ballooning; Inflammation; Fibrosis; ↓BW, ↑ALT, ↑AST, ↑TG (liver); TC (liver; no change)
C57BL6/J mice (Matthews et al., 2021)	MCDD	40% kcal-sucrose, 10%kcal- fat and is deficient in methionine and choline	5 weeks	NASH	Steatosis; Inflammation; ↓BW, ↓FBS, ↑TNFα (mRNA)
Wst rat (Radhakrishnan et al., 2020)	MCDD	40% kcal-sucrose, 10%kcal- fat and is deficient in methionine and choline	7 weeks	NASH	Steatosis (micro- and macrovesicular); Ballooning Inflammation; ↓BW, ↑ALT, ↑bilirubin, ↓TG, ↓TC (plasma), HOMA-IR (no change), ↑TG, ↑TC (liver), ↑TNFα (liver)
SD rat (Liu et al., 2015)	MCDD	40% kcal-sucrose, 10%kcal- fat and is deficient in methionine and choline	8 weeks	NASH	Steatosis; Ballooning; Lobular inflammation; Mild fibrosis; ↓BW, ↑ALT, ↑AST
Wst rat (Kirsch et al., 2003)	MCDD	40% kcal-sucrose, 10%kcal- fat and is deficient in methionine and choline	4 weeks		Severe Steatosis (macrovesicular); ↓BW, ↑ALT
F1B Golden Syrian hamster (Tashiro et al., 2010)	MCDD	40% kcal-sucrose, 10%kcal- fat and is deficient in methionine and choline	8 weeks	NASH	Steatosis; Inflammation; Fibrosis; BW (no change) ↑TG (plasma)

MCDD, methionine-choline-deficient diet; STZ, streptozocin; α-SMA, α-smooth muscle actin; COL1A1, collagen type I; FBS, fast blood sugar, SREBP-1c, sterol regulating element binding protein-1c; FAS; fatty acid synthase, TNF-α; tumor necrosis factor-α; TGF, transforming growth factor; BW, body weight; TG, triglyceride; TC, total cholesterol; Wst, wistar; SD, sprague dawley; LDL, low density lipoprotein; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance.

evaluate the NASH-related intrahepatic events, genetic modifications, and the pharmacological therapy of NASH (Byrne and Targher, 2015; Radhakrishnan et al., 2020).

However, to counteract the effect of traditional MCD diets on weight loss in mice and rats' model, some modifications have been made that allow the research plans to fine-tune the phenotype to meet their needs (Matsumoto et al., 2013).

Choline-deficient L-amino acid-defined (CDAA) diet, due to choline deficiency, is similar to the MCD diet. However, in this diet, proteins are substituted with an equivalent and corresponding mixture of L-amino acids (Nakae et al., 1995). Feeding the animal model by a CDAA diet can induce the same or somewhat more severe degree of NASH and elevate ALT levels, though in a marginally longer period of time (Miura et al., 2010). C57BL/6 mice fed a CDAA diet for 22 weeks showed an increase in body weight, plasma triglyceride, total cholesterol levels, and insulin resistance (Ishioka et al., 2017). The combination of a CDAA diet with a high-fat diet in C57BL/6J mice after 6-9 weeks could rapidly induce NASH with fibrosis, without any weight loss (Matsumoto et al., 2013).

In male F1B hamsters, similar to the rats and mice, can drive significant steatosis, liver fibrosis, and elevated plasma TG, within 8 weeks. However, hamsters fed an MCD diet did not show any changes in the body weight (Tashiro et al., 2010). Another study on F1B hamsters reported that a diet containing 0.13% methionine with 21 kcal% fat and no added choline could induce severe hepatocellular ballooning and macrovesicular after 4 weeks (Bhathena et al., 2011).

Conclusions

Dietary animal models for NASH should be reproducible and efficient which can faithfully replicate

Table 5. Other dietary models.

Rodent model	Dietary model	Composition/definition	Treatment length	Outcome	Phenotypes/outcomes
C3H/HeOuJ mice (Pickens et al., 2010)	Fructose-MCDD (90% of diet's carbohydrate composed of fructose)	40% kcal-fructose, 10%kcal- fat and is deficient in methionine and choline	21 days	NASH	Steatosis (micro- and macrovesicular) Ballooning; Inflammation; ↓BW, ↓FBS, ↑ALT ↑TG (liver), ↓TG, ↓TC (plasma), ↑TNFα (mRNA)
C57BL6/J mice (Tetri et al., 2008)	WD (ALIOS; American Lifestyle-Induced Obesity Syndrome)	45% kcal-fat, 55% kcal-fructose, 30% kcal-MUFA and PUFA	16 weeks	NASH	Steatosis (macrovesicular); Inflammation Ballooning; ↑BW, ↑TG (liver), ↑ALT, ↑AST ↑TC (plasma); plasma TG (no change)
C57BL6/J mice (Savard et al., 2013)	HFHCD	(High fat high cholesterol diet) 15% kcal-fat, 1% kcal-cholesterol	30 weeks	NASH	Severe steatosis (macrovesicular) Inflammation; Fibrosis; ↑BW, ↑ALT, ↑TG, ↑TC, ↑cholesterol ester (liver), ↑TC (plasma) plasma TG, FBS (no change), ↑Insulin, , HOMA-IR, ↑TNFα, ↑COL1a1 (mRNA)
Wst rat (Kawasaki et al., 2009)	HFHFrD	43.3% kcal-carbohydrate, 39.3% kcal-fat, 17.4% kcal-protein	5 weeks	NAFLD	Steatosis (macrovesicular), ↑BW, FBS (no change); Plasma TG (no change)
C57BL6/J mice (Charlton et al., 2011)	HFHFrD	60% kcal-fat, 23.1 g/l fructose in drinking water	25 weeks	NAFLD	Steatosis (microvesicular); Mild inflammation ↑BW, ↑TC (plasma), AST (no change), ↑FBS, ↑Insulin
C57BL6/J mice (Kohli et al., 2010)	HFHCrD	(High fat high carbohydrate diet) 58 kcal-%fat & 55%-fructose, 45% sucrose in drinking water (42g/l carbohydrate)	16 weeks		Steatosis (micro- and macrovesicular); Inflammation Fibrosis; ↑TG (liver), ↑ALT, ↑BW, ↑FBS, ↑Insulin, ↑HOMA-IR, ↑COL1a1 (mRNA)
NMRI mice (Savari et al., 2019b)	HFHFrD	30% kcal-fat, 30% fructose in drinking water	8 weeks	NASH	Steatosis; Ballooning; Mild inflammaion ↑TG, ↑TC, ↑LDL (plasma), ↑FBS, ↑Insulin ↑HOMA-IR, ↑ALT, ↑AST, TNF
C57BL6/J mice (Ishimoto et al., 2013)	HFHSD	(High fat high sucrose diet) 36% kcal-fat, 43,2% carbohydrate-kcal without sugar, 30% kcal-sucrose	15 weeks	NASH	Steatosis (micro- and macrovesicular) Mild inflammation; fibrosis; ↑BW, ↑TG (liver) ↑ALT, ↑AST, ↑TNFα (mRNA), ↑COL1a1 (mRNA), ↑αSMA (mRNA)
C57BL6/J mice (Flores-Costa et al., 2018)	CDAAHFD (A06071302)	(Choline-deficient, L-amino acid-defined, high-fat; 60% kcal-fat)	9 weeks	NASH	Steatosis; Inflammation; Fibrosis; ↑BW, ↑FBS, ↑TC, ↑ALT, ↑AST, ↑PPARα (mRNA), ↑TNFα (mRNA)
C57BL6/J mice (Ishioka et al., 2017)	CDAAD	Choline-deficient L-amino acid-defined diet	8 weeks	NASH	Steatosis; Ballooning; Inflammation Fibrosis (not found histologically); ↑BW, ↑ALT ↑TG (liver), ↑COL1a1 (mRNA), ↑TNFα (mRNA)

HFHFrD, high fat high fructose diet; HFHCD, High fat high cholesterol diet; HFHCrD, High fat high carbohydrate diet; HFHSD, High fat high sucrose diet; CDAAHFD, Choline-deficient, L-amino acid-defined, high-fat; CDAAD, choline-deficient L-amino acid-defined diet; STZ, streptozocin; α-SMA, α-smooth muscle actin; COL1A1, collagen type I; FBS, fast blood sugar, SREBP-1c, sterol regulating element binding protein-1c; FAS; fatty acid synthase, TNF-α; tumor necrosis factor-α; TGF, transforming growth factor; BW, body weight; TG, triglyceride; TC, total cholesterol; Wst, wistar; SD, sprague dawley; DL, low density lipoprotein; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance.

the multifactorial disease mechanisms. Choosing the best available dietary animal models for preclinical research of NAFLD/NASH can be time-consuming and difficult. To achieve the best phenotype of varying degrees of NAFLD/NASH spectrum, the individual components of experiment diet can be selectively manipulated. Although each of these selective diets have limitations in representing human's NAFLD/NASH conditions, use of these diets in different animal models can be a powerful means for clarifying and studying the pathogenesis and progression of the NASH as well as uncovering potential treatment targets for it. This study encourages researchers to consider a combination of proper diet choice in the different animal models and work on modeling the pathophysiology of human's NAFLD/NASH, while taking important points into account such as appropriate rodent model, age, and sex.

Acknowledgements. We wish to thank Dr. Anahita Rezaie; Department of Pathobiology, School of Veterinary Medicine, Shahid Chamran University of Ahvaz, Iran, for notable suggestions that improved the quality of the manuscript.

Author Contributions. All authors contributed to the study conception and design, and all authors have read and agreed to the published version of the manuscript. FS was responsible for the design, draft and writing of the manuscript, GHA, SAM, BB and MJS provided Conceptualization, critical review and revision of the manuscript.

Conflicts of Interest. The authors declare no conflict of interest.

Funding. No funding was received to assist with the preparation of this manuscript.

References

Agopian V.G., Kaldas F.M., Hong J.C., Whittaker M., Holt C., Rana A., Zarrinpar A., Petrowsky H., Farmer D. and Yersiz H. (2012). Liver transplantation for nonalcoholic steatohepatitis: the new epidemic. Ann. Surg. 256, 624-633.

Basciano H., Miller A.E., Naples M., Baker C., Kohen R., Xu E., Su Q., Allister E.M., Wheeler M.B. and Adeli K. (2009). Metabolic effects of dietary cholesterol in an animal model of insulin resistance and hepatic steatosis. Am. J. Physiol. Endocrinol. Metab. 297, E462-473.

Bhathena J., Kulamarva A., Martoni C., Urbanska A.M., Malhotra M., Paul A. and Prakash S. (2011). Diet-induced metabolic hamster model of nonalcoholic fatty liver disease. Diabetes Metab. Syndr. Obes. 4, 195-203.

Briand F., Maupoint J., Brousseau E., Breyner N., Bouchet M., Costard C., Leste-Lasserre T., Petitjean M., Chen L., Chabrat A., Richard V., Burcelin R., Dubroca C. and Sulpice T. (2021). Elafibranor improves diet-induced nonalcoholic steatohepatitis associated with heart failure with preserved ejection fraction in Golden Syrian hamsters. Metabolism 117, 154707.

Byrne C.D. and Targher G. (2015). NAFLD: a multisystem disease. J. Hepatol. 62 (Suppl 1), S47-64.

Caballero F., Fernández A., Matías N., Martínez L., Fucho R., Elena M., Caballeria J., Morales A., Fernández-Checa J.C. and García-Ruiz C. (2010). Specific contribution of methionine and choline in nutritional nonalcoholic steatohepatitis: impact on mitochondrial S-adenosyl-L-methionine and glutathione. J. Biol. Chem. 285, 18528-18536.

Caldwell S.H. and Crespo D.M. (2004). The spectrum expanded: cryptogenic cirrhosis and the natural history of non-alcoholic fatty liver disease. J. Hepatol. 40, 578-584.

Charlton M., Krishnan A., Viker K., Sanderson S., Cazanave S., McConico A., Masuoko H. and Gores G. (2011). Fast food diet mouse: novel small animal model of NASH with ballooning, progressive fibrosis, and high physiological fidelity to the human condition. Am. J. Physiol. Gastrointest. Liver Physiol. 301, G825-834.

de Wit N., Derrien M., Bosch-Vermeulen H., Oosterink E., Keshtkar S., Duval C., de Vogel-van den Bosch J., Kleerebezem M., Müller M. and van der Meer R. (2012). Saturated fat stimulates obesity and hepatic steatosis and affects gut microbiota composition by an enhanced overflow of dietary fat to the distal intestine. Am. J. Physiol. Gastrointest. Liver Physiol. 303, G589-599.

Denk H., Abuja P.M. and Zatloukal K. (2019). Animal models of NAFLD from the pathologist's point of view. Biochim. Biophys Acta Mol. Basis Dis. 1865, 929-942.

Diehl A.M. (2005). Lessons from animal models of NASH. Hepatol. Res. 33, 138-144.

Donnelly K.L., Smith C.I., Schwarzenberg S.J., Jessurun J., Boldt M.D. and Parks E.J. (2005). Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. J. Clin. Invest. 115, 1343-1351.

Dowman J.K., Tomlinson J. and Newsome P. (2010). Pathogenesis of non-alcoholic fatty liver disease. QJM 103, 71-83.

Eccleston H.B., Andringa K.K., Betancourt A.M., King A.L., Mantena S.K., Swain T.M., Tinsley H.N., Nolte R.N., Nagy T.R., Abrams G.A. and Bailey S.M. (2011). Chronic exposure to a high-fat diet induces hepatic steatosis, impairs nitric oxide bioavailability, and modifies the mitochondrial proteome in mice. Antioxid. Redox Signal. 15, 447-459.

Emamat H., Foroughi F., Eini-Zinab H. and Hekmatdoost A. (2018). The Effects of onion consumption on prevention of nonalcoholic fatty liver disease. Indian J. Clin. Biochem. 33, 75-80.

Eng J.M. and Estall J.L. (2021). Diet-induced models of non-alcoholic fatty liver disease: Food for thought on sugar, fat, and cholesterol. Cells 10, 1805-1821.

Farrell G.C., Mridha A.R., Yeh M.M., Arsov T., Van Rooyen D.M., Brooling J., Nguyen T., Heydet D., Delghingaro-Augusto V. and Nolan C.J. (2014). Strain dependence of diet-induced NASH and liver fibrosis in obese mice is linked to diabetes and inflammatory phenotype. Liver Int. 34, 1084-1093.

Finelli C. and Tarantino G. (2012). Is there any consensus as to what diet or lifestyle approach is the right one for NAFLD patients? J. Gastrointestin. Liver Dis. 21, 293-302.

Flores-Costa R., Alcaraz-Quiles J., Titos E., López-Vicario C., Casulleras M., Duran-Güell M., Rius B., Diaz A., Hall K., Shea C., Sarno R., Currie M., Masferrer J.L. and Clària J. (2018). The soluble guanylate cyclase stimulator IW-1973 prevents inflammation and fibrosis in experimental non-alcoholic steatohepatitis. Br. J. Pharmacol. 175, 953-967.

Hannou S.A., Haslam D.E., McKeown N.M. and Herman M.A. (2018). Fructose metabolism and metabolic disease. J. Clin. Invest 128, 545-555.

Hansen H.H., Ægidius H.M., Oró D., Evers S.S., Heebøll S., Eriksen P.L., Thomsen K.L., Bengtsson A., Veidal S.S., Feigh M., Suppli M.P., Knop F.K., Grønbæk H., Miranda D., Trevaskis J.L., Vrang N., Jelsing J. and Rigbolt K.T.G. (2020). Human translatability of the

GAN diet-induced obese mouse model of non-alcoholic steatohepatitis. BMC Gastroentrol. 20, 210-222.

Hansen H.H., Feigh M., Veidal S.S., Rigbolt K.T., Vrang N. and Fosgerau K. (2017). Mouse models of nonalcoholic steatohepatitis in preclinical drug development. Drug Discov. Today 22, 1707-1718.

Hsu H.C., Dozen M., Matsuno N., Obara H., Tanaka R. and Enosawa S. (2013). Experimental nonalcoholic steatohepatitis induced by neonatal streptozotocin injection and a high-fat diet in rats. Cell Med. 6, 57-62.

Hussein O., Grosovski M., Lasri E., Svalb S., Ravid U. and Assy N. (2007). Monounsaturated fat decreases hepatic lipid content in non-alcoholic fatty liver disease in rats. World J. Gastroenterol. 13, 361-368.

Ibrahim S.H., Hirsova P., Malhi H. and Gores G.J. (2016). Animal models of nonalcoholic steatohepatitis: Eat, delete, and inflame. Dig. Dis. Sci. 61, 1325-1336.

Imajo K., Yoneda M., Kessoku T., Ogawa Y., Maeda S., Sumida Y., Hyogo H., Eguchi Y., Wada K. and Nakajima A. (2013). Rodent models of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. Int. J. Mol. Sci. 14, 21833-21857.

Ishimoto T., Lanaspa M.A., Rivard C.J., Roncal-Jimenez C.A., Orlicky D.J., Cicerchi C., McMahan R.H., Abdelmalek M.F., Rosen H.R., Jackman M.R., MacLean P.S., Diggle C.P., Asipu A., Inaba S., Kosugi T., Sato W., Maruyama S., Sánchez-Lozada L.G., Sautin Y.Y., Hill J.O., Bonthron D.T. and Johnson R. J. (2013). High-fat and high-sucrose (western) diet induces steatohepatitis that is dependent on fructokinase. Hepatology 58, 1632-1643.

Ishioka M., Miura K., Minami S., Shimura Y. and Ohnishi H. (2017). Altered gut microbiota composition and immune response in experimental steatohepatitis mouse models. Dig. Dis. Sci. 62, 396-406.

Itagaki H., Shimizu K., Morikawa S., Ogawa K. and Ezaki T. (2013). Morphological and functional characterization of non-alcoholic fatty liver disease induced by a methionine-choline-deficient diet in C57BL/6 mice. Int. J. Clin. Exp. Pathol. 6, 2683-2696.

Ito M., Suzuki J., Tsujioka S., Sasaki M., Gomori A., Shirakura T., Hirose H., Ito M., Ishihara A., Iwaasa H. and Kanatani A. (2007). Longitudinal analysis of murine steatohepatitis model induced by chronic exposure to high-fat diet. Hepatol. Res. 37, 50-57.

Jensen V.S., Hvid H., Damgaard J., Nygaard H., Ingvorsen C., Wulff E.M., Lykkesfeldt J. and Fledelius C. (2018). Dietary fat stimulates development of NAFLD more potently than dietary fructose in Sprague-Dawley rats. Diabetol. Metab. Syndr. 10, 4.

Kanuri G. and Bergheim I. (2013). *In vitro* and *in vivo* models of non-alcoholic fatty liver disease (NAFLD). Int. J. Mol. Sci. 14, 11963-11980.

Kasim-Karakas S.E., Vriend H., Almario R., Chow L. C. and Goodman M.N. (1996). Effects of dietary carbohydrates on glucose and lipid metabolism in golden Syrian hamsters. J. Lab. Clin. Med. 128, 208-213.

Kawasaki T., Igarashi K., Koeda T., Sugimoto K., Nakagawa K., Hayashi S., Yamaji R., Inui H., Fukusato T. and Yamanouchi T. (2009). Rats fed fructose-enriched diets have characteristics of nonalcoholic hepatic steatosis. J. Nutr. 139, 2067-2071.

Kim M.Y., Cho M.Y., Baik S.K., Park H.J., Jeon H.K., Im C.K., Won C.S., Kim J. W., Kim H.S. and Kwon S.O. (2011). Histological subclassification of cirrhosis using the Laennec fibrosis scoring system correlates with clinical stage and grade of portal hypertension. J. Hepatol. 55, 1004-1009.

Kim T.H., Choi D., Kim J.Y., Lee J.H. and Koo S.-H. (2017). Fast food diet-induced non-alcoholic fatty liver disease exerts early protective effect against acetaminophen intoxication in mice. BMC Gastroenterol. 17, 124-132.

Kirpich I.A., Gobejishvili L.N., Bon Homme M., Waigel S., Cave M., Arteel G., Barve S.S., McClain C. J. and Deaciuc I.V. (2011). Integrated hepatic transcriptome and proteome analysis of mice with high-fat diet-induced nonalcoholic fatty liver disease. J. Nutr. Biochem. 22, 38-45.

Kirsch R., Clarkson V., Shephard E.G., Marais D.A., Jaffer M.A., Woodburne V.E., Kirsch R.E. and Hall P. de la M. (2003). Rodent nutritional model of non-alcoholic steatohepatitis: species, strain and sex difference studies. J. Gastroenterol. Hepatol. 18, 1272-1282.

Kohli R., Kirby M., Xanthakos S.A., Softic S., Feldstein A. E., Saxena V., Tang P.H., Miles L., Miles M. V., Balistreri W.F., Woods S.C. and Seeley R.J. (2010). High-fructose, medium chain trans fat diet induces liver fibrosis and elevates plasma coenzyme Q9 in a novel murine model of obesity and nonalcoholic steatohepatitis. Hepatology 52, 934-944.

Kopp W. (2019). How western diet and lifestyle drive the pandemic of obesity and civilization diseases. Diabetes Metab. Syndr. Obes. 12, 2221-2236.

Kořínková L., Pražienková V., Černá L., Karnošová A., Železná B., Kuneš J. and Maletínská L. (2020). Pathophysiology of NAFLD and NASH in Experimental Models: The Role of Food Intake Regulating Peptides. Front Endocrinol. (Lausanne) 11, 597583.

Kubota N., Kado S., Kano M., Masuoka N., Nagata Y., Kobayashi T., Miyazaki K. and Ishikawa F. (2013). A high-fat diet and multiple administration of carbon tetrachloride induces liver injury and pathological features associated with non-alcoholic steatohepatitis in mice. Clin. Exp. Pharmacol. Physiol. 40, 422-430.

Larter C.Z. and Yeh M.M. (2008). Animal models of NASH: getting both pathology and metabolic context right. J. Gastroenterol. Hepatol. 23, 1635-1648.

Lau J. K., Zhang X. and Yu J. (2017). Animal models of non-alcoholic fatty liver disease: current perspectives and recent advances. J. Pathol. 241, 36-44.

Lee G.S., Yan J.S., Ng R.K., Kakar S. and Maher J.J. (2007). Polyunsaturated fat in the methionine-choline-deficient diet influences hepatic inflammation but not hepatocellular injury. J. Lipid Res. 48, 1885-1896.

Lichtman A.H., Clinton S.K., Iiyama K., Connelly P.W., Libby P. and Cybulsky M.I. (1999). Hyperlipidemia and atherosclerotic lesion development in LDL receptor-deficient mice fed defined semipurified diets with and without cholate. Arterioscler Thromb. Vasc. Biol. 19, 1938-1944.

Lieber C.S., Leo M.A., Mak K.M., Xu Y., Cao Q., Ren C., Ponomarenko A. and DeCarli L.M. (2004). Model of nonalcoholic steatohepatitis. Am. J. Clin. Nutr. 79, 502-509.

Liu X., Wang Y., Ming Y., Song Y., Zhang J., Chen X., Zeng M. and Mao Y. (2015). S100A9: A potential biomarker for the progression of non-alcoholic fatty liver disease and the diagnosis of non-alcoholic steatohepatitis. PLoS One 10, e0127352.

London R.M. and George J. (2007). Pathogenesis of NASH: animal models. Clin. Liver Dis. 11, 55-74.

Matsumoto M., Hada N., Sakamaki Y., Uno A., Shiga T., Tanaka C., Ito T., Katsume A. and Sudoh M. (2013). An improved mouse model that rapidly develops fibrosis in non-alcoholic steatohepatitis. Int. J. Exp. Pathol. 94, 93-103.

Matthews D.R., Li H., Zhou J., Li Q., Glaser S., Francis H., Alpini G. and Wu C. (2021). Methionine- and choline-deficient diet-induced nonalcoholic steatohepatitis is associated with increased intestinal inflammation. Am. J. Pathol. 191, 1743-1753.

Miura K., Kodama Y., Inokuchi S., Schnabl B., Aoyama T., Ohnishi H., Olefsky J.M., Brenner D.A. and Seki E. (2010). Toll-like receptor 9 promotes steatohepatitis by induction of interleukin-1beta in mice. Gastroenterology 139, 323-334. e327.

Montandon S.A., Somm E., Loizides-Mangold U., de Vito C., Dibner C. and Jornayvaz F.R. (2019). Multi-technique comparison of atherogenic and MCD NASH models highlights changes in sphingolipid metabolism. Sci. Rep. 9, 16810.

Nakae D., Mizumoto Y., Andoh N., Tamura K., Horiguchi K., Endoh T., Kobayashi E., Tsujiuchi T., Denda A., Lombardi B. and Konishi Y. (1995). Comparative changes in the liver of female Fischer-344 rats after short-term feeding of a semipurified or a semisynthetic L-amino acid-defined choline-deficient diet. Toxicol. Pathol. 23, 583-590.

Nakamura A. and Terauchi Y. (2013). Lessons from mouse models of high-fat diet-induced NAFLD. Int. J. Mol. Sci. 14, 21240-21257.

Neuman M.G., Cohen L.B. and Nanau R.M. (2014). Biomarkers in nonalcoholic fatty liver disease. Can J. Gastroenterol. Hepatol. 28, 607-618.

Nigro D., Menotti F., Cento A. S., Serpe L., Chiazza F., Dal Bello F., Romaniello F., Medana C., Collino M., Aragno M. and Mastrocola R. (2017). Chronic administration of saturated fats and fructose differently affect SREBP activity resulting in different modulation of Nrf2 and Nlrp3 inflammasome pathways in mice liver. J. Nutr. Biochem. 42, 160-171.

Ou T.H., Tung Y.T., Yang T.H. and Chien Y.W. (2019). Melatonin improves fatty liver syndrome by inhibiting the lipogenesis pathway in hamsters with high-fat diet-induced hyperlipidemia. Nutrients 11, 748.

Pickens M.K., Ogata H., Soon R.K., Grenert J.P. and Maher J.J. (2010). Dietary fructose exacerbates hepatocellular injury when incorporated into a methionine-choline-deficient diet. Liver Int. 30, 1229-1239.

Radhakrishnan S., Ke J.Y. and Pellizzon M.A. (2020). Targeted nutrient modifications in purified diets differentially affect nonalcoholic fatty liver disease and metabolic disease development in rodent models. Curr. Dev. Nutr. 4, nzaa078.

Roeb E. and Weiskirchen R. (2021). Fructose and non-alcoholic steatohepatitis. Front Pharmacol. 12, 634344.

Sahai A., Malladi P., Melin-Aldana H., Green R. M. and Whitington P. F. (2004). Upregulation of osteopontin expression is involved in the development of nonalcoholic steatohepatitis in a dietary murine model. Am. J. Physiol. Gastrointest. Liver Physiol. 287, G264-273.

Samuel V.T., Liu Z.X., Qu X., Elder B.D., Bilz S., Befroy D., Romanelli A.J. and Shulman G.I. (2004). Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. J. Biol. Chem. 279, 32345-32353.

Savard C., Tartaglione E.V., Kuver R., Haigh W.G., Farrell G.C., Subramanian S., Chait A., Yeh M.M., Quinn L.S. and Ioannou G.N. (2013). Synergistic interaction of dietary cholesterol and dietary fat in inducing experimental steatohepatitis. Hepatology 57, 81-92.

Savari F., Badavi M., Rezaie A., Gharib-Naseri M.K. and Mard S.A. (2019a). Evaluation of the therapeutic potential effect of Fas receptor gene knockdown in experimental model of non-alcoholic steatohepatitis. Free Radic. Res. 53, 486-496.

Savari F., Mard S.A., Badavi M., Rezaie A. and Gharib-Naseri M.K. (2019b). A new method to induce nonalcoholic steatohepatitis (NASH) in mice. BMC Gastroenterol. 19, 125.

Sberna A. L., Bouillet B., Rouland A., Brindisi M. C., Nguyen A., Mouillot T., Duvillard L., Denimal D., Loffroy R., Vergès B., Hillon P. and Petit J.M. (2018). European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD) and European Association for the Study of Obesity (EASO) clinical practice recommendations for the management of non-alcoholic fatty liver disease: evaluation of their application in people with Type 2 diabetes. Diabet. Med. 35, 368-375.

Schuppan D. and Schattenberg J.M. (2013). Non-alcoholic steatohepatitis: pathogenesis and novel therapeutic approaches. J. Gastroenterol. Hepatol. 28 (Suppl 1), 68-76.

Schuppan D., Surabattula R. and Wang X.Y. (2018). Determinants of fibrosis progression and regression in NASH. J. Hepatol. 68, 238-250.

Soret P.A., Magusto J., Housset C. and Gautheron J. (2020). *In vitro and* in *vivo* models of non-alcoholic fatty liver disease: A critical appraisal. J. Clin. Med. 10, 36.

Stephenson K., Kennedy L., Hargrove L., Demieville J., Thomson J., Alpini G. and Francis H. (2018). Updates on dietary models of nonalcoholic fatty liver disease: Current studies and insights. Gene Exp. 18, 5-17.

Svendsen P., Graversen J.H., Etzerodt A., Hager H., Røge R., Grønbæk H., Christensen E.I., Møller H.J., Vilstrup H. and Moestrup S.K. (2017). Antibody-directed glucocorticoid targeting to CD163 in M2-type macrophages attenuates fructose-induced liver inflammatory changes. Mol. Ther. Methods Clin. Dev. 4, 50-61.

Szczepaniak L.S., Nurenberg P., Leonard D., Browning J.D., Reingold J.S., Grundy S., Hobbs H.H. and Dobbins R.L. (2005). Magnetic resonance spectroscopy to measure hepatic triglyceride content: Prevalence of hepatic steatosis in the general population. Am. J. Physiol. Endocrinol. Metab. 288, E462-468.

Tashiro K., Takai S., Jin D., Yamamoto H., Komeda K., Hayashi M., Tanaka K., Tanigawa N. and Miyazaki M. (2010). Chymase inhibitor prevents the nonalcoholic steatohepatitis in hamsters fed a methionine- and choline-deficient diet. Hepatol. Res. 40, 514-523.

Tetri L.H., Basaranoglu M., Brunt E.M., Yerian L.M. and Neuschwander-Tetri B.A. (2008). Severe NAFLD with hepatic necroinflammatory changes in mice fed trans fats and a high-fructose corn syrup equivalent. Am. J. Physiol. Gastrointest. Liver Physiol. 295, G987-995.

Tølbøl K.S., Kristiansen M.N., Hansen H.H., Veidal S.S., Rigbolt K.T., Gillum M.P., Jelsing J., Vrang N. and Feigh M. (2018). Metabolic and hepatic effects of liraglutide, obeticholic acid and elafibranor in diet-induced obese mouse models of biopsy-confirmed nonalcoholic steatohepatitis. World J. Gastroenterol. 24, 179-194.

Tolosa L., Bonora-Centelles A., Teresa Donato M., Pareja E., Negro A., López S., Castell J.V. and José Gómez-Lechón M. (2011). Steatotic liver: a suitable source for the isolation of hepatic progenitor cells. Liver Int. 31, 1231-1238.

Torres Silva G., Di Pietro Fernandes C., Hiane P.A., Freitas K.C., Figueiredo P.S., Inada A.C., Filiú W.F., Maldonade I.R., Nunes Â.A., Oliveira L.C.S., Caires A.R.L., Michels F., Candido C.J., Cavalheiro L.F., Arakaki Asato M., Rodrigues Donadon J., Bacelar de Faria B., Tatara M.B., Rosa Croda J.H., Pott A., Nazário C.E.D. and Guimarães R.C.A. (2020). Caryocar brasiliense cambess. Pulp oil supplementation reduces total cholesterol, LDL-c, and Non-HDL-c in Animals. Molecules 25, 4530.

Tzeng T.F., Liou S.S., Chang C.J. and Liu I.M. (2015). [6]-gingerol

dampens hepatic steatosis and inflammation in experimental nonalcoholic steatohepatitis. Phytomedicine 22, 452-461.

van der Heijden R.A., Sheedfar F., Morrison M.C., Hommelberg P.P., Kor D., Kloosterhuis N.J., Gruben N., Youssef S.A., de Bruin A., Hofker M.H., Kleemann R., Koonen D.P. and Heeringa P. (2015). High-fat diet induced obesity primes inflammation in adipose tissue prior to liver in C57BL/6j mice. Aging (Albany NY) 7, 256-268.

Van Herck M.A., Vonghia L. and Francque S.M. (2017). Animal models of nonalcoholic fatty liver disease - A starter's guide. Nutrients 9, 1072.

Verbeek J., Lannoo M., Pirinen E., Ryu D., Spincemaille P., Vander Elst I., Windmolders P., Thevissen K., Cammue B.P. and van Pelt J. (2015). Roux-en-y gastric bypass attenuates hepatic mitochondrial dysfunction in mice with non-alcoholic steatohepatitis. Gut 64, 673-683.

Veteläinen R., van Vliet A. and van Gulik T. M. (2007). Essential pathogenic and metabolic differences in steatosis induced by choline or methione-choline deficient diets in a rat model. J. Gastroenterol. Hepatol. 22, 1526-1533.

Vial G., Dubouchaud H., Couturier K., Cottet-Rousselle C., Taleux N., Athias A., Galinier A., Casteilla L. and Leverve X.M. (2011). Effects of a high-fat diet on energy metabolism and ROS production in rat liver. J. Hepatol. 54, 348-356.

Weltman M.D., Farrell G.C. and Liddle C. (1996). Increased hepatocyte CYP2E1 expression in a rat nutritional model of hepatic steatosis with inflammation. Gastroenterology 111, 1645-1653.

Yamazaki Y., Kakizaki S., Takizawa D., Ichikawa T., Sato K., Takagi H. and Mori M. (2008). Interstrain differences in susceptibility to non-alcoholic steatohepatitis. J. Gastroenterol. Hepatol. 23, 276-282.

Ying-Rong L., Zhang M.-H., Jian-Guang L. and Zhang H. (2021). Pathogenesis of NASH and promising natural products. Chin. J. Nat. Med. 19, 12-27.

Younossi Z., Anstee Q.M., Marietti M., Hardy T., Henry L., Eslam M., George J. and Bugianesi E. (2018). Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. Nat. Rev. Gastroenterol. Hepatol. 15, 11-20.

Younossi Z.M. (2019). Non-alcoholic fatty liver disease - A global public health perspective. J. Hepatol. 70, 531-544.

Accepted April 27, 2022