Targeted Nutrient Modifications in Purified Diets Differentially Affect Nonalcoholic Fatty Liver Disease and Metabolic Disease Development in Rodent Models

Sridhar Radhakrishnan, Jia-Yu Ke, and Michael A Pellizzon

Research Diets, Inc., New Brunswick, NJ, USA

ABSTRACT

Nonalcoholic fatty liver disease (NAFLD) is a complex spectrum of disorders ranging from simple benign steatosis to more aggressive forms of nonalcoholic steatohepatitis (NASH) and fibrosis. Although not every patient with NAFLD/NASH develops liver complications, if left untreated it may eventually lead to cirrhosis and hepatocellular carcinoma. Purified diets formulated with specific nutritional components can drive the entire spectrum of NAFLD in rodent models. Although they may not perfectly replicate the clinical and histological features of human NAFLD, they provide a model to gain further understanding of disease progression in humans. Owing to the growing demand of diets for NAFLD research, and for our further understanding of how manipulation of dietary components can alter disease development, we outlined several commonly used dietary approaches for rodent models, including mice, rats, and hamsters, time frames required for disease development and whether other metabolic diseases commonly associated with NAFLD in humans occur. *Curr Dev Nutr* 2020;4:nzaa078.

Keywords: purified diet, high-fat diet, methionine- and choline-deficient diet, fructose, cholesterol, nonalcoholic fatty liver disease, metabolic disease, mice, rats, hamsters

Manuscript received November 26, 2019. Initial review completed April 16, 2020. Revision accepted April 21, 2020. Published online April 24, 2020.
The authors' salaries, office space, computing, and manuscript preparation were provided by Research Diets, Inc., New Brunswick, NJ.
Author disclosures: SR, J-YK, and MAP are employees of Research Diets, Inc., New Brunswick, NJ.

Address correspondence to MAP (e-mail: michael.pellizzon@researchdiets.com).

Abbreviations used: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CD, choline deficient; CDAA, choline-deficient amino acid–based diet; CHOP, C/EBP homologous protein; FFA, free fatty acid; HCC, hepatocellular carcinoma; HCD, high-cholesterol diet; HFD, high-fat diet; HFHC, high-fat, high-cholesterol; HFHFr, high-fat, high-fructose; HFr, high-fructose; IR, insulin resistance/insulin resistant; MCD, methionine and choline deficient; MCP-1, monocyte chemoattractant protein 1; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; NASH, nonalcoholic steatohepatitis; PTEN, phosphatase and tensin homolog; SD, Sprague Dawley; TG, triglyceride.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is a spectrum of disorders characterized by excessive lipid accumulation in hepatocytes. Each stage of the disease spectrum has distinctive histopathological characteristics. The beginning stages include simple hepatic steatosis, which is characterized by fat droplet accumulation in hepatocytes and this is usually benign and asymptomatic (1, 2). The disease may progress further to nonalcoholic steatohepatitis (NASH), which may include hepatocellular injury, ballooning (i.e., cellular swelling), and/or inflammation. If left unchecked, NASH can lead to fibrosis, cirrhosis, and ultimately hepatocellular carcinoma (HCC), thus affecting overall liver function (3, 4). A growing body of evidence portrays NASH as the hepatic manifestation of the metabolic syndrome because the majority of patients with NASH (and those with steatosis) suffer from obesity, diabetes, and insulin resistance (IR) (2, 5). However, whether IR causes or is caused by NAFLD/NASH is yet to be clearly defined. Most obese adults have hepatic steatosis, and at least one-third of these individuals will eventually develop worsening NAFLD (6) including NASH, which is projected to be the leading cause for liver transplantation in the upcoming decade (7).

Accumulation of fat droplets in the form of triglycerides (TGs) in hepatocytes constitutes steatosis, which is a hallmark feature of NAFLD (8). NAFLD is histologically diagnosed when TG accumulation occurs in >5% of hepatocytes (9) in the absence of significant alcohol consumption. Although there are many histological scoring methods for NAFLD, some of the common ones used in the literature are those by Kleiner et al. and Brunt et al. (9–12). The extent of steatosis can be graded according to the percentage of steatotic hepatocytes: mild, 5%–33% (score: 1); moderate, 33%–66% (score: 2); and severe, >66% (score: 3). Similarly, lobular inflammation is graded as a score of 0–3 and hepatocyte ballooning as 0–2. The sum of these scores is known as the NAFLD activity score (NAS); it ranges from 0 to 8 and typically scores >5 are considered confirmed cases of NASH. Similar scoring systems are also modified and translated to be used in rodent models and it has been shown that the human scoring system is largely reproducible in rodent NAFLD (13). Fibrosis is typically scored from 0 to 4, with 0 = no fibrosis, 1–2 = mild to moderate fibrosis, 3 = ad-

vanced fibrosis, and 4 = cirrhosis, and fibrotic scores are calculated separately from NAS scores. To have a complete understanding of disease development, most researchers typically report both NAS and fibrosis scores (14).

Obesity and particularly IR are tightly associated with the genesis of NAFLD and NASH. IR can lead to an increase in the circulating free fatty acids (FFAs) through adipose tissue lipolysis. Elevated insulin (due to increased secretion) and glucose concentrations (reduced uptake by skeletal muscle) typically found in conditions of IR also promote de novo lipogenesis in the liver via multiple mechanisms (15). IR also directly inhibits β-oxidation, thereby promoting hepatic accumulation of TGs and thereby steatosis (15). IR also can contribute to development of NAFLD via multiple other mechanisms. Patients with NAFLD have IR in liver, muscle, and adipose tissue (16) and this led to NAFLD being called the hepatic manifestation of metabolic syndrome. Day and James (17) proposed the "two hit" model for NASH development, with the first hit being steatosis with IR contributing to development of steatosis. This makes the liver vulnerable to the second hit (oxidative stress, proinflammatory cytokines, etc.), thus promoting NASH and fibrosis development. However, given that nonobese individuals, including those from developing countries like China and India with little to no IR, also have NAFLD, recent research has also explored other possible mechanisms toward NAFLD development independent of IR. Currently, it is thought that there are multiple hits leading to development of advanced liver disease including increased oxidative stress and inflammation, hepatotoxicity by FFAs and ceramides, gut bacterial alterations, and increased gut permeability, compounded by the existence of the metabolic disturbance of IR (18, 19).

In spite of the enormous amount of research in the field of NAFLD/NASH in the past decade, the precise mechanisms underlying the development of NAFLD and its progression to NASH have not been completely elucidated, including its link to metabolic syndrome, requiring additional studies and models to elucidate its pathophysiology. Because of its growing worldwide prevalence, various animal models that mirror both the pathophysiology and the histopathology of each stage of NAFLD/NASH are available. Certain dietary approaches can drive NAFLD/NASH in rodent models to mimic human disease and produce different severities of disease along the NAFLD spectrum, and, depending on the dietary manipulations, likely work by unique mechanisms (**Tables 1**, **2**, **Figures 1**, **2**). This is essential in determining how the disease progresses, and also helps in evaluating different therapeutic approaches toward the treatment of specific stages of NAFLD (5).

Studies frequently use either mice or rats as animal models for studying fatty liver disease (8, 20). The most common model used for diet-induced NAFLD is the C57BL/6 mouse model, which is likely due to their increased susceptibility relative to other mouse strains (21). Rats [Sprague Dawley (SD) and Wistar] and hamsters are other commonly used models. Many diets including high-fat diets (HFDs) and methionine- and choline-deficient (MCD) diets, of which there are many variations, produce a robust model for different stages of NAFLD/NASH depending on the type of diet and length of feeding. Recently a review was published regarding the influence of diet on NAFLD in rats (22) as well as 1 in mice (23), but none to our knowledge have been done for hamsters. Therefore, we will provide a more comprehensive description of dietary strategies in these 3 rodent models to aid in the selection of the diet choice during the study design phase.

HFDs

The term "HFD" encompasses a wide variety of diet formulas with different fat types and amounts (30–60 kcal% fat), as well as other composi-

TABLE 1 Summary of commonly used diets and their expected effects on nonalcoholic fatty liver disease and nonalcoholic steatohepatitis in rats and mice[1]

Diet	Rodent model	Body weight[1]	Fasting glucose/insulin	Steatosis	Steatohepatitis	Fibrosis	Time frame (fibrosis)[2]	References
Methionine- and choline-deficient diet (MCD)	Rats and mice	↓	↓	+++	+++	++	4–8 wk	(24–26)
0.1% Methionine and choline-deficient high-fat diet	Mainly mice	↓*	No change	+++	+++	++	6–12 wk	(27–29)
Choline-deficient amino acid–based diet with 0.17% methionine (CDAA)	Rats and mice	No change	↑ (Mainly mice)	+++	++	++	4–12 wk (rats) 12 wk (mice)	(30–34)
Choline-deficient high-fat diet (CD)	Mainly mice	↑	↑	+++	++	++	12 wk	(35–38)
High-fat diet (HFD)	Rats and mice	↑	↑	+++	++	+ Mild at best	24 wk (rats) 16 wk (mice)	(39–42)
High-fructose diet (HFr)	Mainly rats	No change	↑	+++	++	++	12 wk	(43, 44)
High-fat, high-fructose, high-cholesterol diet	Rats and mice	↑	↑	+++	++	+ (Mainly mice)	16 wk (rats) 20–30 wk (mice)	(45–48)

[1] +, mild; ++, modest; +++, severe. Body weight and fasting glucose/insulin for arrows is relative to the control diet.
[2] The length depends on diet formula; length of the study; and species, strain, and gender of the animal model.
*Compared with a low-fat, methionine- and choline-sufficient group. Body weight of these animals typically remains unchanged compared with baseline.

TABLE 2 Study design factors to consider in diet-induced nonalcoholic fatty liver disease rodent models[1]

	High-fat diets	High-fructose diets	High-fat, -fructose, and -cholesterol diets	Choline-deficient high-fat diets	Methionine- and choline-deficient diets
Dietary modifications commonly used	• 30–60 kcal% fat, higher SFAs increase ER stress, higher ω-6 PUFAs increase increase oxidative stress, both increase NASH • More sucrose or fructose leads to NASH and mild fibrosis	• Usually 60–70 kcal% fructose drives steatosis, NASH • The addition of sucrose (50% fructose) also effective effective for steatosis, NASH	• 40 kcal% fat (*trans* fat or SFAs), 20–40 kcal% fructose, and 1–2% cholesterol • Fat type and cholesterol increase ER and oxidative stress/fibrosis • Fructose drives steatosis and inflammation	• Fat amount (30–60 kcal% fat) • Lard commonly used; typically can drive steatosis, but prolonged feeding (6 mo) can cause fibrosis	• Rapid onset of steatosis (1 wk), NASH/fibrosis in 6–8 wk • Addition of fat ($\leq$60 kcal% fat) • Fat type typically lard, butter (SFAs), or corn oil (PUFAs), addition of sucrose and/or cholesterol drives further NASH/fibrosis
Other metabolic effects	• Increases body weight • IR/glucose intolerance	• Increases body weight • IR/glucose intolerance • Increased plasma TGs (typically rats and hamsters)	• Increases body weight • IR/glucose intolerance • Increases plasma lipids	• Increases body weight • Less IR than choline-sufficient diet	• Reduces body weight, but 0.1% methionine maintains weight • No IR, reduced plasma lipids
Matched control diet	• Low-fat diet with matched amount of sucrose or mostly corn starch	• Low-fat diet with 60–70 kcal% as either glucose or corn starch	• Low-fat diet with 60–70 kcal% as either glucose or corn starch	• Low-fat diet with choline	• Methionine- and choline-sufficient diet

[1] ER, endoplasmic reticulum; IR, insulin resistance; NASH, nonalcoholic steatohepatitis; TG, triglyceride.

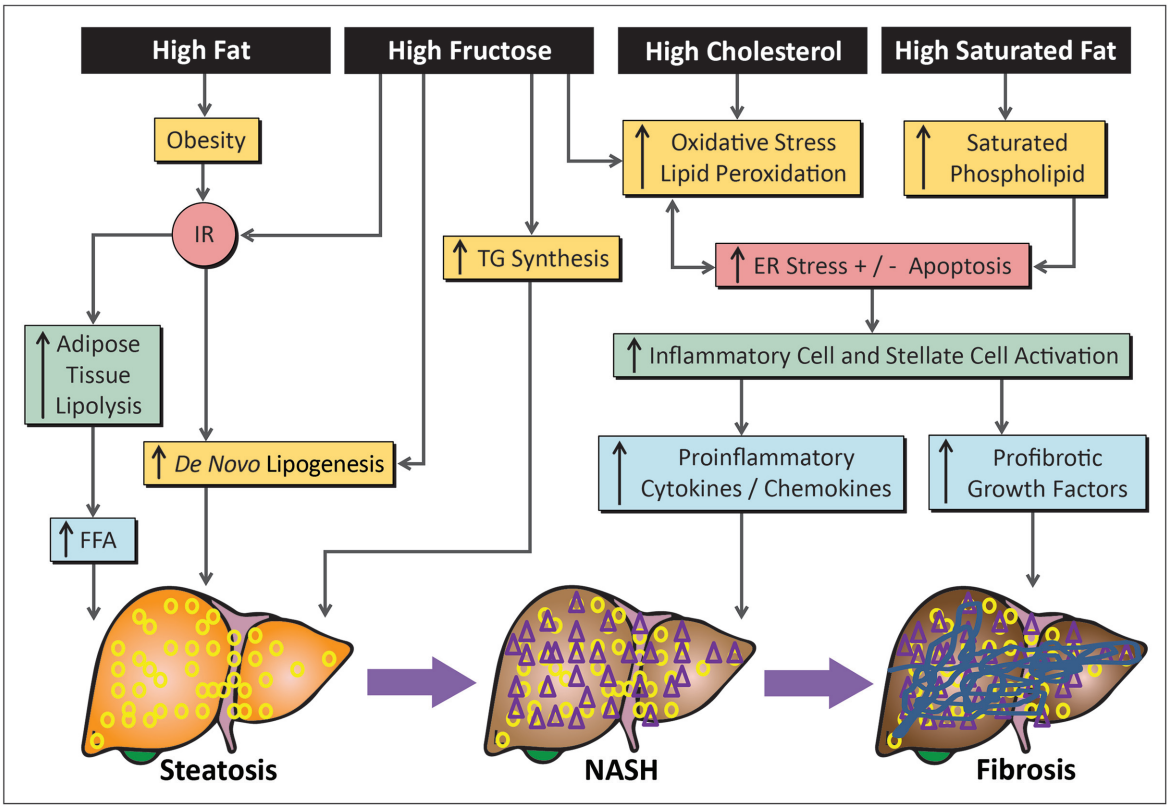

FIGURE 1 Effects of dietary fructose, total and saturated fat, and cholesterol on mechanisms affecting nonalcoholic fatty liver disease development in rodent models. ER, endoplasmic reticulum; FFA, free fatty acid; IR, insulin resistance; NASH, nonalcoholic steatohepatitis; TG, triglyceride.

tional differences such as low or high sucrose amounts or different forms (i.e., pellet or liquid). HFDs typically (depending on the fat source) increase body weight and body fat and induce IR in rodent models. One of the widely used HFDs for obesity research, D12492 (60 kcal% fat, mainly lard), induced visceral obesity and hepatic steatosis characterized by significantly increased liver and plasma FFA and TG concentrations and plasma alanine aminotransferase (ALT) in C57BL/6 mice fed for 8 wk (49). In another study, C57BL/6 mice fed diet D12492 for 16 wk exhibited increased body weight and adipose tissue weight, widespread hepatic steatosis indicated by Oil Red O staining and increased hepatic TGs, mild fibrosis, and adipose tissue inflammation (39). NAS scores of C57BL/6 animals fed D12492 for 16 wk were ~2–4 (24). When fed chronically (~52 wk), an HFD (D12492) induced NASH characterized by inflammation along with excess body weight, hyperinsulinemia, and hypercholesterolemia (50). Even a slightly lower-fat (45 kcal% fat, D12451) containing HFD induced steatosis and steatohepatitis after 6 mo in C57BL/6 mice (40). NAFLD in mice was worsened (mild fibrosis) by the addition of sucrose, as shown in a different study that compared animals fed an HFD (36 kcal% fat as mainly milk fat) with those fed a high-fat, high-sucrose diet (36 kcal% fat, 30 kcal% sucrose) (51). The type of fat in the HFD formulation also plays a role, as evidenced in the study by de Wit et al. (52) which observed that palm oil (~50% SFAs, mainly palmitic acid) in 45 kcal% fat diet increased liver TGs and body weight and reduced insulin sensitivity more rapidly (as early as week 2) than other fat sources (olive oil, safflower oil) in C57BL/6 mice.

Studies in SD rats observed that certain HFDs increase liver fat concentrations quite rapidly (within days) as well as hepatic IR before significant increases in peripheral fat deposition occur (53). Chronically, HFD-induced liver fat accumulation may not follow a linear progression and liver fat concentrations may actually decrease, then increase again during prolonged HFD feeding in rats (54). Researchers also combine HFD feeding with chemicals such as streptozotocin or carbon tetrachloride (CCl_4) to induce NASH in rat models (24, 55, 56).

Like in mice, fat type should be considered in NAFLD studies with rats, because markers of liver injury in circulation, ALT and aspartate aminotransferase (AST) (at 4 and 24 wk), and markers of endoplasmic reticulum (ER) stress including those involved with increased protein folding (X-box-binding protein 1, glucose regulated protein 78) and apoptosis [caspase-3, C/EBP homologous protein (CHOP)] in the liver were increased in rats fed a 45 kcal% fat diet with lard relative to those fed a diet high in corn oil (57). These markers were associated with higher concentrations of hepatic SFAs (after only 1 wk), and thus ER stress in combination with steatosis may set the stage for increased NASH development (57). Because the ER makes up over half the membrane composition of hepatocytes, in vitro work using primary hepatocytes confirmed that increased amounts of SFAs as palmitic acid caused increased incorporation of these fatty acids into lipotoxic phospholipids

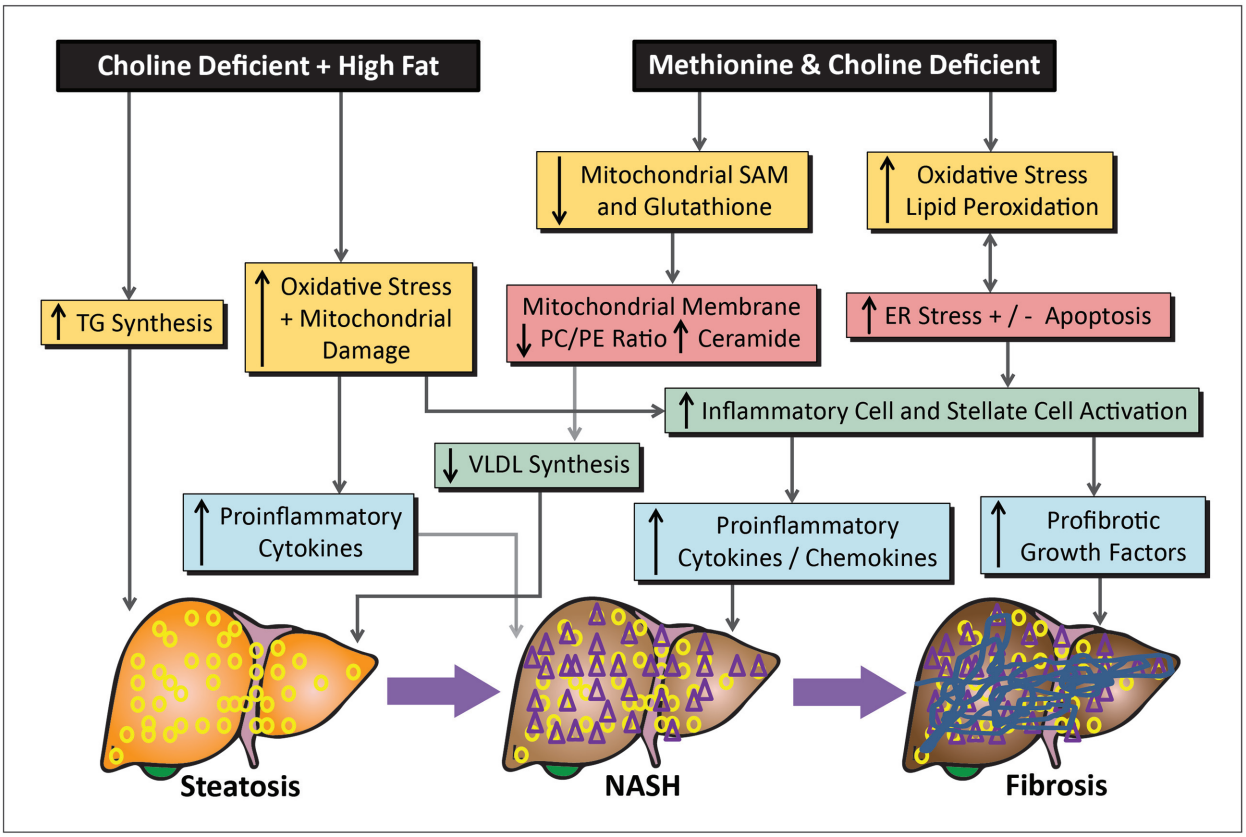

FIGURE 2 Effects of choline and methionine + choline deficiency on mechanisms affecting nonalcoholic fatty liver disease development in rodent models. ER, endoplasmic reticulum; NASH, nonalcoholic steatohepatitis; PC, phosphatidylcholine; PE, phosphatidylethanolamine; SAM, S-adenosylmethionine; TG, triglyceride.

and led to induction of ER stress markers such as CHOP (proapoptotic) in a dose-dependent manner (58). In contrast, the addition of oleate reversed this effect and allowed for more palmitic acid to be incorporated in more benign TGs (58).

Increasing the fat content of HFDs (~70 kcal% fat) is also effective in rodent models. A classic HFD-NAFLD model used SD rats fed a liquid diet composed of 71 kcal% fat (combination of olive oil, corn oil, and safflower oil), 11 kcal% carbohydrates, and 18 kcal% protein for 3 wk, as compared with control rats fed a lower-fat diet with 35 kcal% fat, 47 kcal% carbohydrates, and 18 kcal% protein (59). This diet caused hepatic steatosis, and lipid concentrations were almost 2-fold those in control rats, which was due to the increased dietary contribution of TGs. Similarly to human NAFLD patients, these rats developed IR as suggested by elevated plasma insulin concentrations (59); this diet also developed NASH and IR in male C57BL/6 mice after 16 wk (60). Even in this HFD formulation, the type of fat plays an important role because replacement (two-thirds or more) of long-chain TGs with medium-chain TGs (MCT oil) resulted in lower (or no) steatosis and lower hepatic TG and inflammation (TNF-α) in SD rats (61).

HFDs can also drive the development of NASH in hamsters. A 45 kcal% fat diet (mainly lard, D12451) increased liver and plasma lipids (total cholesterol and TGs) and steatosis score (histology) in golden Syrian hamsters fed for 10 wk relative to a 10 kcal% fat diet (62). In addition, hepatic inflammation as measured by expression of inflammatory cytokines like monocyte chemoattractant protein 1 (MCP-1), TNF-α, IL-1β, and IL-6 was elevated in the HFD group (62). Another study also reported development of steatosis (mainly microvesicular) in a hamster model fed an HFD with ~30 kcal% fat as mainly lard with 0.2% cholesterol after 8 wk, whereas those fed a low-fat purified diet (AIN-93M) had maintained normal liver histopathology. In addition to elevations in liver lipids, these hamsters also had elevated plasma total cholesterol, LDL cholesterol, and TGs (63).

It is important to note that when fed for equal lengths of time, HFD feeding results in only 10% of liver fat concentrations than what accumulates on an MCD diet (64) and induces only mild steatosis and mild/no liver fibrosis as compared with MCD diets (25, 65), highlighting an important difference between these dietary regimes. Unlike rodents fed an MCD diet, an advantage of using an HFD is that HFD-fed mice will have features of metabolic disease including weight gain, IR, and/or glucose intolerance; however, the practicality of the model is limited by length of the dietary regime, especially for fibrosis induction (41, 42).

MCD Diets

Among the different dietary approaches for diet-induced NAFLD in rodents, MCD diets produce the most severe NASH phenotype in the shortest time frame, which is highly favorable for those studying ways to slow down or reverse this phenotype. The formulation of MCD diets requires the replacement of whole protein (such as casein) in purified diets with crystalline amino acids and the removal of both methionine and choline. These diets typically induce measurable hepatic steatosis (mainly macrovesicular) in mice and rats by 1–4 wk, and if fed longer (1–2 mo), it can progress to inflammation and fibrosis (66, 67). Liu et al. (24) observed that SD rats fed an MCD diet for 7 wk exhibited significant steatosis with lobular inflammation and mild perisinusoidal fibrosis. Surprisingly, this group also observed hepatocellular ballooning, which is typically not seen in rodents fed other NAFLD diet strategies. Steatosis scores in rats consuming MCD diets range around 2–3 (24, 26). The mechanism for steatosis includes impaired VLDL secretion due to lack of phosphatidylcholine synthesis and increased lipolysis of adipose tissue (68). The progression of steatosis to steatohepatitis in MCD mouse models involves downregulation of proteins affecting methionine metabolism and oxidative stress such as peroxiredoxin, which participates in cellular defense against the development of hepatitis (69). Other mechanisms involve mitochondrial S-adenosyl-L-methionine depletion due to reduced methionine intake and this leads to mitochondrial dysfunction and mitochondrial glutathione depletion (70). This leads to increased propensity for hepatocyte injury via oxidative stress and inflammation. Some studies have shown that MCD diets more closely mimic the mechanisms implicated in the pathogenesis of human NASH, including ER stress, oxidative stress, and autophagocytic stress, relative to other dietary models, including HFDs (25).

Within the context of an MCD diet, other dietary components also affect the NASH phenotype. Sucrose is an important component of the MCD diet, because replacing it with cornstarch greatly reduces liver fat accumulation, inflammation, and injury, likely through reductions in sucrose-induced de novo lipogenesis and TG synthesis (71). Although both glucose and fructose can induce hepatic fat accumulation, fructose was more effective than glucose at inducing hepatocellular injury measured by histology, apoptosis staining, and serum ALT, in C3H/HeOuJ mice fed MCD diets for 3 wk (72). The source of dietary fat can also alter the phenotype; for example, relative to high-SFA sources (coconut oil, beef tallow), omega-6 PUFA sources such as corn oil can increase liver fat oxidation and induce expression of proinflammatory genes leading to inflammation, although this does not necessarily correlate with increased liver damage (73). Also, relative to butter fat (high in SFAs), olive oil (high in MUFAs) reduced liver TG accumulation, whereas fish oil (high in ω-3 PUFAs) reduced liver cholesterol concentrations (74). Besides fat type and sucrose, the addition of 1% cholesterol to a low-methionine (0.1%), no-choline HFD (A16092003, 45% kcal, mainly lard) accelerated development of fibrosis (~6 wk) in Wistar rats (75), as it did also in the context of an MCD (0% methionine) diet with less fat (21 kcal% fat, corn oil) in C57BL/6 mice fed for 12 wk (76).

Unlike humans with NAFLD, or other HFD-induced rodent models of NAFLD which usually are obese and have IR (among other metabolic complications), rats and mice fed MCD diets lose weight owing to a vastly lower caloric intake (24, 77–79), display cachexia, have no IR, and have low fasting serum insulin and glucose, leptin, and TG concentrations (78). This limits the extrapolation of data from this model to human NASH. Despite these differences, MCD diets are still a good candidate for screening experiments (i.e., drug compounds, genetic modifications) which can provide answers regarding how they influence the development or reversal of advanced NASH, mainly liver fibrosis phenotype, in a relatively short time frame.

Owing to its effect on weight loss and to counter this effect, some modifications have been made to traditional MCD diets. Matsumoto et al. (27) removed methionine and choline from an HFD background (60 kcal% fat, A06071301B) with the idea of providing more energy as fat to reduce weight loss. Despite an increased fat amount compared with traditional MCD diets, this diet still caused a reduction in food intake and body weight in both C57BL/6 and A/J mice. However, adding a small amount of methionine (0.1% wt:wt) back in the diet (A06071302) helped better maintain energy intake and body weight to the same level as animals fed a low-fat, methionine- and choline-sufficient diet. This diet also increased levels of liver fat accumulation, inflammatory markers, and AST and ALT within 6 wk and fibrosis progressed from week 3, week 6, and week 14; however, hepatic inflammation, TG concentrations, and ballooning did not change from week 6 to week 14. Although the modified diet arrested weight loss in both A/J and C57BL/6 mice, it increased fibrosis in only C57BL/6 mice after 6 wk, but if fed longer ($\geq$9 wk), both mouse models developed fibrosis on this diet. At week 9, the steatosis score was 3, lobular inflammation score was 1–2, ballooning score was 1, and fibrosis scores were ~2–3. Similar results were also observed by us (unpublished data) and Chiba et al. (28) in a similar diet containing 45% kcal fat, in C57BL/6 mice. However, the amounts of sucrose in the 45 kcal% diet in the aforementioned studies were 17% compared with 7% in the 60 kcal% fat diet. If the sucrose amounts are kept similar, the higher fat amount (i.e., 60 kcal% fat) provided a more robust degree of fibrosis than 45 kcal% fat or 10 kcal% fat after 8 wk in C57BL/6 mice (80). In another study, Cong et al. (81) used a modified HFD (60 kcal% fat) to simultaneously contain low amounts of methionine (0.15%) and choline (0.06%). C57BL/6 mice fed the diet for 23 wk developed obesity, IR, and dyslipidemia as well as liver steatosis, inflammation, and fibrosis. Therefore, the addition of a low methionine dose in the context of a choline-deficient HFD background can allow for an improved phenotype or, at the very least, reduce the weight loss concern associated with MCD diets. This idea of modifying so-called "standard" HFDs is powerful because it allows the researcher to "fine-tune" the phenotype to meet their needs.

The addition of 0.17% methionine in a choline-deficient amino acid–based diet (commonly known as the CDAA diet) initially formulated by Nakae et al. (30, 82) for studying HCC in rats with ~30 kcal% fat (mainly contains Primex shortening, a source of *trans* fat) also induced significant histopathological evidence of the complete NAFLD spectrum in a relatively short time frame, which includes steatosis (score: 3), inflammation (score: 1–2.4), and fibrosis (score: 2.4) (31, 32, 83). AST and/or ALT concentrations in these animals were elevated after 4 (33), 8 (31), 10 (83), or 12 (32) wk; however, these rats had no evidence of IR and gained similar or less weight and had lower blood glucose, TG, and cholesterol concentrations compared with the choline-sufficient groups. De Minicis et al. (34) also used this same CDAA diet in mice (fed for 1–9 mo) and observed relevant NASH (NAS > 5) and fibrosis after 3 mo (associated with increased mRNA expression collagen 1α, α-smooth muscle actin, and metallopeptidase inhibitor-1), which pro-

gressed further after 6 and 9 mo. Additionally, unlike in rats, in mice this group observed IR as shown by higher fasting plasma insulin concentrations and lower glucose uptake. Others who fed this diet to mice have observed significant fibrosis at 22 wk, which progressed further after 65 wk and was associated with oxidative DNA damage and HCC (66.7%) after 84 wk (84). It should be noted that the description of the CDAA diet in the methods section of some publications may lack the detail that this diet is lower in methionine than what is typically added in purified diets (typically 0.5% from casein), which is an important driver of the NASH/fibrosis phenotype. The methionine amount in CDAA diets is also lower than the NRC recommendations for mice (0.5%), but may be adequate for rats (NRC recommendation: 0.23% for methionine + cystine) (85). Furthermore, the control diet is unusual as it is also low in methionine (0.17%) and has a very high concentration of choline bitartrate (14.48 g/kg) (33), which is >7-fold higher than what is typically added in purified diets (2–2.5 g/kg) to meet NRC recommendations (85). Although it is unclear why a higher than typical amount of choline was added, the intention may have been to provide more methyl donors to the control group to compensate for the reduced amount of methionine in this diet.

In addition to rats and mice, MCD diets can drive significant steatosis and fibrosis in male F1B hamsters within 8 wk. However, in contrast to rats and mice fed an MCD diet, hamsters had similar body weight and elevated plasma TG compared with animals on a grain-based diet (86). When fed a diet containing 0.13% methionine and no added choline with 21 kcal% fat, F1B hamsters had minimal weight gain, and exhibited a high degree of steatosis (macrovesicular) and hepatocellular ballooning relative to those fed a diet with 25 kcal% fat and normal methionine and choline amounts; however, perhaps due to the study length of only 4 wk, the MCD diet–fed hamsters exhibited no fibrosis (87).

Choline-Deficient Diets

Typically, choline-deficient (CD) diets used in fatty liver disease studies tend to contain higher amounts of fat (30–60 kcal%) and these diets can induce steatosis, inflammation, and mild fibrosis over 10 wk without the reduction in body weight seen in MCD diets (35–37, 88), which makes CD diets more appealing to some researchers. However, choline deficiency, even in the context of a lard-based HFD (45 kcal% fat, mainly lard, D05010402), improved glucose tolerance compared with the choline-sufficient group in C57BL/6 mice fed for 8 wk (36). Time frame may be important as C57BL/6 mice on D05010402 showed impaired glucose tolerance if fed longer (6 mo) when compared with a grain-based diet (38), but it is not possible to conclude that the longer time frame was responsible for the differences in glucose intolerance given the many differences between these diets in addition to choline amounts. However, fibrosis in mice consuming CD diets tends to be minimal unless *1*) the diet is fed for a long time period (6–12 mo) (38) or *2*) a compound such as ethionine is added to the diet or water (36, 89). This lack of fibrosis found in mice with a choline-deficient HFD (D05010402) when fed for shorter time frames (~8 wk) may be due to an upregulation of enzymes involved with phosphatidyl choline synthesis (increased phosphate cytidylyltransferase 1, choline, alpha, *Pcyt1a* mRNA expression) and TG synthesis (increased acyl-CoA synthetase long chain family member, *Acsl-1*, acyl-CoA synthetase long chain family member 4, *Acsl-4*, and glycerol-3-phosphate acyltransferase, *Gpat*) and diglyceride esterification (increased diacylglycerol O-acyltransferase 2, *Dgat2*), which shunt potentially toxic FFAs to TGs (36). Others also found that choline deficiency in the context of an HFD (with 37 kcal% fat as lard, 46 kcal% sucrose) increased phosphatidyl choline synthesis and maintained VLDL secretion relative to those fed a choline-sufficient diet (90). Therefore, a reduced accumulation of toxic lipids and maintenance of VLDL secretion explain why (at least in the shorter term) only steatosis develops without NASH.

When considering a CD diet, significant steatosis occurs only when choline deficiency is combined with a high-fat background (60 kcal% fat, D05010403) because the low-fat CD diet (10 kcal% fat, D05010401) failed to induce any significant steatosis (91), or induced it to a mild degree relative to a higher-fat (45 kcal% fat, D05010402) diet after 8 wk (36). In contrast, an MCD diet with a lower-fat diet background (i.e., 12 kcal% fat as corn oil) was capable of initiating steatosis, NASH, and fibrosis in just 15 d, and methionine deficiency was the main driver of liver injury, likely through reduced S-adenosylmethionine and glutathione (70). After feeding either CD or MCD diets to Wistar rats for 7 wk, the MCD diet group had significantly higher scores of liver inflammation (2.9 in the MCD compared with 2.1 in the CD group) and steatosis (1.9 in the MCD compared with 0.6 in the CD group) and a rapid increase in ALT and presence of fibrosis, all of which were absent in rats fed the CD diet; a similar effect was also found in mice consuming MCD or CD diets for 15 d (26, 70). However, the CD diet–fed rats had IR and gained weight and had higher plasma lipids compared with the MCD group and those fed a grain-based diet (26).

High-Fructose Diet

Humans consuming a significant number of calories from fructose-rich foods are at increased risk of the development of obesity and NASH. Studies in rodent models to evaluate the influence of fructose on NAFLD development in rodents have used various techniques including the addition of high-fructose (HFr) corn syrup to water, which has been used in combination with HFD feeding (92) or with fructose being directly added in the context of a pelleted diet with 60–70 kcal% carbohydrate as fructose (93). In C57BL/6 mice, an HFr diet with 60 kcal% as fructose and 10 kcal% fat can drive more liver TG, steatosis, and inflammation than a low-fat matched control diet with corn starch and also an HFD with 45 kcal% fat (mainly lard) (93). These changes were accompanied by increased lipogenesis and VLDL production, antioxidant pathways suggesting oxidative stress (Nrf2, Nuclear factor erythroid 2-related factor 2), inflammation markers (IL-1β, intercellular adhesion molecule-1), and metabolic disorders such as increased plasma lipids and glucose intolerance relative to those fed the corn starch–based control diet and HFD. Findings obtained with C57BL/6 mice fed an HFD (58 kcal% fat, hydrogenated coconut oil) or high-fat, high-fructose (HFHFr) diet (combination of the same 58 kcal% fat diet and HFr corn syrup in water) for 16 wk suggested that both HFD and HFHFr diets increased body weight, IR, and hepatic steatosis similarly (92). However, only the HFHFr diet allowed for further increases in hepatic oxidative stress and the progression from liver fat deposition to inflammation, transforming growth factor-β1-driven fibrogenesis, and collagen deposition (92). Sodhi et al. (94) reported that

an HFr diet (with 20 kcal% protein, 13 kcal% fat, 66 kcal% carbohydrate, 0.1% cholesterol, "high fructose", but amount not reported) increased IR, blood pressure, markers of oxidative stress and lipogenesis, along with fibrotic markers in C57BL/6 mice fed this diet for 8 wk. However, it is not possible to determine to what extent the differences in cholesterol (only 0.02% in the control diet) or other diet background factors (30 kcal% protein, 57 kcal% carbohydrate in the control diet) contributed to the observed effects.

In the rat model, an HFr (low-fat background, ~60–70 kcal% fructose) diet may be more potent than an HFD or HFHFr diet. Kawasaki et al. (43) found that an HFr diet (73% kcal fructose) increased hepatic TGs more than high-sucrose (73% kcal), HFD (40% kcal fat), or HFHFr diets (40% kcal fat, 41% kcal fructose) in Wistar rats. In addition, the HFr diet also promoted macrovesicular and microvesicular steatosis (score: 2.6–2.9) and lobular inflammation (score: 2.4); however, fibrotic scores were not significantly different from the control group. HFr diet (70 kcal% fructose) also induced NAFLD/NASH in the SD rat model (compared with a grain-based diet) (44), including increased steatosis (score: 3), inflammation (score: 2.13), hepatic ballooning (score: 1.75), and fibrosis (score: 5.25). In this study, pericellular and perivenular fibrosis were scored separately on a range of 0–4 and then added to depict the final score (range: 0–8) (44).

Similarly to rats, an HFr diet with 60 kcal% fructose increased plasma and liver TG more than diets containing the same amount of corn starch or sucrose in male golden Syrian hamsters fed for 7 wk. In this study, the researchers also found that HFr increased body weight, adiposity, and plasma insulin and reduced glucose disappearance (glucose tolerance test) more potently than in the high-sucrose- or starch-fed groups (95).

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

As suggested in earlier sections of this review, dietary cholesterol is a critical factor in the progression of NASH and hepatic inflammation in animal models (76, 96–98). In C57BL/6 mice, a high-cholesterol diet (HCD) on a low-fat diet background (1% cholesterol, 11 kcal% fat) elevated hepatic cholesterol esters and both the HCD and an HFD (33 kcal% fat, added cocoa butter) increased steatosis and mild steatohepatitis (99). However, the combination of the high-fat content and cholesterol amount (HFHC diet) interacted synergistically to drive features of NASH because moderate steatohepatitis and mild fibrosis were observed only when 1% cholesterol was given in conjunction with an HFD. In addition, mice fed the HFHC diet allowed for greater elevations in weight gain, hepatic lipid accumulation, serum ALT concentrations, and fibrosis, and decreased adiponectin concentrations compared with animals fed a low-fat (11 kcal% fat) diet, HFD (33 kcal% fat), or HCD (11 kcal% fat, 1% cholesterol) (99).

In an effort to drive more NASH, some have added cholic acid in the context of an HFHC diet, which has been traditionally used in atherosclerosis studies in rodent models (100). In a recent study, a diet with 60 kcal% fat as mainly lard with 1.25% cholesterol and 0.5% cholic acid (called an "atherogenic diet") caused increased steatosis and fibrosis after 12 wk in male C57BL/6 mice; however, the extent of fibrosis was lesser than in those fed an MCD diet with 20 kcal% fat as corn oil (101). Although the addition of cholate in combination with higher amounts of fat and cholesterol can drive more liver TG and cholesterol accumulation, NASH, and fibrosis (102), caveats include improved insulin sensitivity and glucose tolerance and reduced liver TGs and weight gain (100, 102). The reduction in these metabolic disorders by cholic acid was independent of the presence of dietary cholesterol, which was observed in mice fed a very-high-fat diet with 60 kcal% fat as safflower oil plus 0.5% cholic acid relative to those fed the same diet without added cholic acid (102).

When C57BL/6 mice were fed a diet containing 40 kcal% fat (of which ~18% was *trans* fat), 22% fructose, and 2% cholesterol, they developed 3 stages of NAFLD (steatosis, steatohepatitis with fibrosis, and cirrhosis) as assessed by histological and biochemical methods (e.g., increased collagen content, collagen 1α1 protein, and mRNA expression of collagen-1α2 and MCP-1) in ~30 wk as shown by Clapper et al. (45) and by Trevaskis et al. (103). Animals on this diet also demonstrated metabolic dysfunction as evidenced by increased total cholesterol, fasting insulin, and HOMA-IR, and lower adiponectin. This diet formulation is widely known as the AMLN diet (D09100301) and was a preferred diet by many researchers because it caused liver damage similarly to a mechanism that occurs in the human condition. The results of the AMLN diet are similar to the ALIOS (American Lifestyle-Induced Obesity Syndrome) model used by Tetri et al. (104) and show that *trans* fats in the diet could potentially play a role in worsening NASH. The AMLN diet has also been shown to induce NAFLD/NASH in SD rats similarly to mice; however, in the rat model, the NAFLD/NASH development (steatosis, inflammation, ALT) seems to happen in a relatively shorter time frame (46, 105) (~16–20 wk).

Although the AMLN diet was widely popular, the *trans* fat source, Primex, a partially hydrogenated fat source (palm oil and partially hydrogenated soybean oil) containing *trans* fat (~25% of fat), was banned by the FDA for use in foods in 2018. Alternative fat sources are currently being evaluated and preliminary evidence suggests that replacing Primex with palm oil (D09100310) in the AMLN diet drives a similar phenotype in C57BL/6 mice (106, 107). Researchers have also used other replacement diets, such as a modified AMLN diet with non–*trans* fat Primex, which contains palm oil and fully hydrogenated soybean oil (D16022301), or with *trans* fat from corn oil shortening (D16010101), or a mixture of both (108), and observed similar (and in some cases more advanced) NASH development compared with the AMLN diet in C57BL/6 mice (47). Using the non–*trans* fat Primex worsened IR in the animals, which to our understanding has not been well characterized in the AMLN diet (47). This has also been observed when Primex shortening was replaced with palm oil (D09100310) (48). The palm oil–based diet, also known as the GAN diet, showed remarkable similarity to the AMLN diet in liver histology. Steatosis scores were 3 in both groups, and scores of inflammation (mean: 2.5) and fibrosis (range: 1.6–1.9) were also quite similar between animals in both these diet groups (48, 106). The ability of these diets to drive symptoms of metabolic disease and all stages of NASH in rodent models provides individual researchers with a more human-like model of disease development, and contract research organizations and breeders with a robust commercially available NASH model for drug discovery.

In golden Syrian hamsters, adding 0.05%–0.25% cholesterol to an HFHFr diet (30% fat, 40% fructose, 6 wk) increased liver TG and cholesterol concentrations (109). Addition of cholesterol to an HFHFr diet

also worsened glucose tolerance and insulin sensitivity in these animals and this was dose dependent. These data clearly implicate dietary cholesterol as exacerbating the effect of dietary fat and fructose and as a major determinant of the severity of metabolic disturbances in the hamster model, similarly to rats and mice.

Dietary NAFLD-Induced HCC

NASH, if unchecked, can progress to cirrhosis and can be complicated with HCC (110). Although most HCC studies involve chemically induced models, some dietary models of NASH have also shown progression toward HCC when fed for chronic periods. Wolf et al. (38) used a CD diet on an HFD background (45% kcal fat, mainly lard, D05010402) and found that along with steatosis and NASH, some C57BL/6 mice also progressed toward HCC after 1 y. The tumor incidence in HFD mice was only 2.5%, as compared with 25% (19/75 mice) in CD-HFD mice (38). In another study, C57BL/6 mice fed a choline-deficient amino acid–defined diet (CDAA as previously described) developed liver injury with biochemical features of NASH and this led to HCC. Feeding with CDAA induced IR (in ∼1 mo), hepatic steatosis (∼3 mo), inflammation (∼3 mo), and liver damage and fibrosis (∼3–6 mo). HCC developed after 9 mo of feeding (∼35% of the animals) that increased to 100% when given in combination with CCl_4 (34). Using the diet A06071302 (0.1%-methionine, no-choline HFD) in C57BL/6 mice, Ikawa-Yoshida et al. (29) observed NASH/HCC in around the same time frame (36 wk); however, others have found this same diet can drive HCC in a shorter time frame (24 wk) in 8 of 10 C57BL/6 mice (80). NASH leading to HCC development was also observed in DIAMOND mice (Diet Induced Animal Model Of Non-alcoholic fatty liver Disease) (111) consuming a Western diet (typically high-fat: ∼40–45 kcal% fat; high sugar, ∼30%; plus cholesterol at ∼0.2–1%) along with sucrose in water. This model has been shown to develop steatosis in 4–8 wk, NASH in 16–24 wk, and HCC at week 52.

Other Models of Fatty Liver Disease

Nondietary models of fatty liver disease mainly fall into 2 categories: *1*) genetic models and *2*) chemical models of liver disease induction. Some of the genetic models used earlier include the *MATO* mice, which have the gene methionine adenosyltransferase-1A removed, and mice with liver-specific phosphatase and tensin homolog (*PTEN*) deletion. Liver-specific *PTEN* deletion in mice also helps to study NASH and NASH-associated HCC [reviewed in (112, 113)]. Models which now are widely studied in concert with diet include the ob/ob mice (leptin deficiency), the db/db mice (leptin receptor deficiency), and the foz/foz mice (with a mutated *Alms1* gene). These models, particularly the ob/ob *mice*, have been used in various studies along with HFDs (including the HFHFr diet with added cholesterol, and AMLN/GAN diets) to induce various aspects of NAFLD/NASH and other comorbidities mainly because of the advantage of development of a serious disease phenotype in a relatively short time frame. Chemical models include damaging the liver using chemicals such as streptozotocin, diethylnitrosamine, CCl_4, etc. either alone or in combination with a dietary model. These types of models have been extensively reviewed elsewhere (2, 4, 23, 112, 114).

Control Diets

When designing experiments with animal models, the importance of dietary choice is typically overlooked, especially if diet is not the main focus of the study. The aforementioned diets (as are most used in NAFLD research) are all purified diets, formulated with refined ingredients, and the formulas are "open" to researchers so that they (and those that wish to repeat their work) are aware of the actual amount of each ingredient in the formulation (115, 116). The refined ingredients of purified diets allow complete control over their nutrient compositions (117), which provides an ability to manipulate the nutrients one at a time, critical to formulating diets such as the MCD or CD diets, and modifications of these diets; however, when making modifications, the choice of control diet becomes important (115). Some researchers use grain-based diets (typically available in their animal facilities) as a "control diet" for purified diets. Rather, the use of cereal grains (e.g., ground corn) and animal byproducts (e.g., fish meal) in grain-based diets, which are in stark contrast to the refined ingredients used in purified diets, makes it impossible to determine what is driving NAFLD [see references (115–117) for more information]. The lack of concern regarding the choice of control diet can be seen in the methods sections of many publications, where it is common to read vague terms such as "standard chow" or "regular diet" which do not provide readers with any useful information and typically refer to grain- or cereal-based diets (117). Instead, a defined, purified diet which only differs in the ingredients that are driving NAFLD in the experimental diet should be chosen as the control to allow for proper data interpretation (117). In this review, we cited research using purified control diets in most cases; however, in some cases, we were limited to only studies where grain-based diets were chosen or were unable to conclude what control diet was chosen because of poor reporting.

Conclusions

Owing to the ease of selectively modifying the components in purified diets, a variety of different options can drive NAFLD in rodent models and individual components of these diets can be selectively manipulated to "fine-tune" the phenotype to varying degrees of the NAFLD spectrum (see Tables 1, 2) through varying mechanisms (Figures 1, 2). Although some have limitations in representing humans with NAFLD, purified diets are powerful tools for studying the pathogenesis and progression of this disease and uncovering potential treatment targets. The combination of proper diet choice, matched control diet, and appropriate rodent model will continue to move this field forward toward developing novel prevention or treatment therapies along the NAFLD spectrum.

Acknowledgments

The authors' responsibilities were as follows—SR: drafted the manuscript; J-YK: created Table 1; MAP: had primary responsibility for the final content; and all authors: provided edits to the manuscript and read and approved the final manuscript.

References

1. Hubscher SG. Histological assessment of non-alcoholic fatty liver disease. Histopathology 2006;49(5):450–65.
2. Hansen HH, Feigh M, Veidal SS, Rigbolt KT, Vrang N, Fosgerau K. Mouse models of nonalcoholic steatohepatitis in preclinical drug development. Drug Discov Today 2017;22(11):1707–18.
3. Dowman JK, Tomlinson JW, Newsome PN. Pathogenesis of non-alcoholic fatty liver disease. QJM 2010;103(2):71–83.
4. Lau JK, Zhang X, Yu J. Animal models of non-alcoholic fatty liver disease: current perspectives and recent advances. J Pathol 2017;241(1):36–44.
5. Jouihan H, Will S, Guionaud S, Boland ML, Oldham S, Ravn P, Celeste A, Trevaskis JL. Superior reductions in hepatic steatosis and fibrosis with co-administration of a glucagon-like peptide-1 receptor agonist and obeticholic acid in mice. Mol Metab 2017;6(11):1360–70.
6. Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, Grundy SM, Hobbs HH. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology 2004;40(6):1387–95.
7. Agopian VG, Kaldas FM, Hong JC, Whittaker M, Holt C, Rana A, Zarrinpar A, Petrowsky H, Farmer D, Yersiz H, et al. Liver transplantation for nonalcoholic steatohepatitis: the new epidemic. Ann Surg 2012;256(4):624–33.
8. Van Herck MA, Vonghia L, Francque SM. Animal models of nonalcoholic fatty liver disease—a starter's guide. Nutrients 2017;9(10):1072.
9. Puri P, Sanyal AJ. Nonalcoholic fatty liver disease: definitions, risk factors, and workup. Clin Liver Dis (Hoboken) 2012;1(4):99–103.
10. Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 1999;94(9):2467–74.
11. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41(6):1313–21.
12. Kleiner DE, Brunt EM. Nonalcoholic fatty liver disease: pathologic patterns and biopsy evaluation in clinical research. Semin Liver Dis 2012;32(1):3–13.
13. Liang W, Menke AL, Driessen A, Koek GH, Lindeman JH, Stoop R, Havekes LM, Kleemann R, van den Hoek AM. Establishment of a general NAFLD scoring system for rodent models and comparison to human liver pathology. PLoS One 2014;9(12):e115922.
14. Rastogi A, Shasthry SM, Agarwal A, Bihari C, Jain P, Jindal A, Sarin S. Non-alcoholic fatty liver disease – histological scoring systems: a large cohort single-center, evaluation study. APMIS 2017;125(11):962–73.
15. Alam S, Mustafa G, Alam M, Ahmad N. Insulin resistance in development and progression of nonalcoholic fatty liver disease. World J Gastrointest Pathophysiol 2016;7(2):211–7.
16. Marchesini G, Brizi M, Morselli-Labate AM, Bianchi G, Bugianesi E, McCullough AJ, Forlani G, Melchionda N. Association of nonalcoholic fatty liver disease with insulin resistance. Am J Med 1999;107(5):450–5.
17. Day CP, James OF. Steatohepatitis: a tale of two "hits"? Gastroenterology 1998;114(4):842–5.
18. Tariq Z, Green CJ, Hodson L. Are oxidative stress mechanisms the common denominator in the progression from hepatic steatosis towards non-alcoholic steatohepatitis (NASH)? Liver Int 2014;34(7):e180–90.
19. Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. Hepatology 2010;52(5):1836–46.
20. Haczeyni F, Yeh MM, Ioannou GN, Leclercq IA, Goldin R, Dan YY, Yu J, Teoh NC, Farrell GC. Mouse models of non-alcoholic steatohepatitis: a reflection on recent literature. J Gastroenterol Hepatol 2018;33(7):1312–20.
21. Fengler VH, Macheiner T, Kessler SM, Czepukojc B, Gemperlein K, Müller R, Kiemer AK, Magnes C, Haybaeck J, Lackner C, et al. Susceptibility of different mouse wild type strains to develop diet-induced NAFLD/AFLD-associated liver disease. PLoS One 2016;11(5):e0155163.
22. Rodrigues AA, Andrade RSB, Vasconcelos DFP. Relationship between experimental diet in rats and nonalcoholic hepatic disease: review of literature. Int J Hepatol 2018:9023027.
23. Oligschlaeger Y, Shiri-Sverdlov R. NAFLD preclinical models: more than a handful, less of a concern? Biomedicines 2020;8(2):28.
24. Liu X, Wang Y, Ming Y, Song Y, Zhang J, Chen X, Zeng M, Mao Y. S100A9: a potential biomarker for the progression of non-alcoholic fatty liver disease and the diagnosis of non-alcoholic steatohepatitis. PLoS One 2015;10(5):e0127352.
25. Machado MV, Michelotti GA, Xie G, Almeida Pereira T, Boursier J, Bohnic B, Guy CD, Diehl AM. Mouse models of diet-induced nonalcoholic steatohepatitis reproduce the heterogeneity of the human disease. PLoS One 2015;10(5):e0127991.
26. Vetelainen R, van Vliet A, van Gulik TM. Essential pathogenic and metabolic differences in steatosis induced by choline or methione-choline deficient diets in a rat model. J Gastroenterol Hepatol 2007;22(9):1526–33.
27. Matsumoto M, Hada N, Sakamaki Y, Uno A, Shiga T, Tanaka C, Ito T, Katsume A, Sudoh M. An improved mouse model that rapidly develops fibrosis in non-alcoholic steatohepatitis. Int J Exp Pathol 2013;94(2):93–103.
28. Chiba T, Suzuki S, Sato Y, Itoh T, Umegaki K. Evaluation of methionine content in a high-fat and choline-deficient diet on body weight gain and the development of non-alcoholic steatohepatitis in mice. PLoS One 2016;11(10):e0164191.
29. Ikawa-Yoshida A, Matsuo S, Kato A, Ohmori Y, Higashida A, Kaneko E, Matsumoto M. Hepatocellular carcinoma in a mouse model fed a choline-deficient, L-amino acid-defined, high-fat diet. Int J Exp Path 2017;98(4):221–33.
30. Nakae D, Yoshiji H, Mizumoto Y, Horiguchi K, Shiraiwa K, Tamura K, Denda A, Konishi Y. High incidence of hepatocellular carcinomas induced by a choline deficient L-amino acid defined diet in rats. Cancer Res 1992;52(18):5042–5.
31. Chong L-W, Hsu Y-C, Lee T-F, Lin Y, Chiu Y-T, Yang K-C, Wu J-C, Huang Y-T. Fluvastatin attenuates hepatic steatosis-induced fibrogenesis in rats through inhibiting paracrine effect of hepatocyte on hepatic stellate cells. BMC Gastroenterol 2015;15:22.
32. Takeuchi-Yorimoto A, Noto T, Yamada A, Miyamae Y, Oishi Y, Matsumoto M. Persistent fibrosis in the liver of choline-deficient and iron-supplemented L-amino acid-defined diet-induced nonalcoholic steatohepatitis rat due to continuing oxidative stress after choline supplementation. Toxicol Appl Pharmacol 2013;268(3):264–77.
33. Hironaka K, Sakaida I, Uchida K, Okita K. Correlation between stellate cell activation and serum fibrosis markers in choline-deficient L-amino acid-defined diet-induced rat liver fibrosis. Dig Dis Sci 2000;45(10):1935–43.
34. De Minicis S, Agostinelli L, Rychlicki C, Sorice GP, Saccomanno S, Candelaresi C, Giaccari A, Trozzi L, Pierantonelli I, Mingarelli E, et al. HCC development is associated to peripheral insulin resistance in a mouse model of NASH. PLoS One 2014;9(5):e97136.
35. Luo F, Ishigami M, Achiwa K, Ishizu Y, Kuzuya T, Honda T, Hayashi K, Ishikawa T, Katano Y, Goto H. Raloxifene ameliorates liver fibrosis of nonalcoholic steatohepatitis induced by choline-deficient high-fat diet in ovariectomized mice. Dig Dis Sci 2015;60(9):2730–9.
36. Raubenheimer PJ, Nyirenda MJ, Walker BR. A choline-deficient diet exacerbates fatty liver but attenuates insulin resistance and glucose intolerance in mice fed a high-fat diet. Diabetes 2006;55(7):2015–20.
37. Yang YM, Noureddin M, Liu C, Ohashi K, Kim SY, Ramnath D, Powell EE, Sweet MJ, Roh YS, Hsin IF, et al. Hyaluronan synthase 2–mediated hyaluronan production mediates Notch1 activation and liver fibrosis. Sci Transl Med 2019;11(496):eaat9284.
38. Wolf MJ, Adili A, Piotrowitz K, Abdullah Z, Boege Y, Stemmer K, Ringelhan M, Simonavicius N, Egger M, Wohlleber D, et al. Metabolic activation of intrahepatic $CD8^+$ T cells and NKT cells causes nonalcoholic steatohepatitis and liver cancer via cross-talk with hepatocytes. Cancer Cell 2014;26(4):549–64.
39. Flores-Costa R, Alcaraz-Quiles J, Titos E, López-Vicario C, Casulleras M, Duran-Güell M, Rius B, Diaz A, Hall K, Shea C, et al. The

soluble guanylate cyclase stimulator IW-1973 prevents inflammation and fibrosis in experimental non-alcoholic steatohepatitis. Br J Pharmacol 2018;175(6):953–67.
40. van der Heijden RA, Sheedfar F, Morrison MC, Hommelberg PP, Kor D, Kloosterhuis NJ, Gruben N, Youssef SA, de Bruin A, Hofker MH, et al. High-fat diet induced obesity primes inflammation in adipose tissue prior to liver in C57BL/6j mice. Aging 2015;7(4): 256–68.
41. Hill-Baskin AE, Markiewski MM, Buchner DA, Shao H, DeSantis D, Hsiao G, Subramaniam S, Berger NA, Croniger C, Lambris JD, et al. Diet-induced hepatocellular carcinoma in genetically predisposed mice. Hum Mol Genet 2009;18(16):2975–88.
42. Svegliati-Baroni G, Candelaresi C, Saccomanno S, Ferretti G, Bachetti T, Marzioni M, De Minicis S, Nobili L, Salzano R, Omenetti A, et al. A model of insulin resistance and nonalcoholic steatohepatitis in rats: role of peroxisome proliferator-activated receptor-α and n-3 polyunsaturated fatty acid treatment on liver injury. Am J Pathol 2006;169(3): 846–60.
43. Kawasaki T, Igarashi K, Koeda T, Sugimoto K, Nakagawa K, Hayashi S, Yamaji R, Inui H, Fukusato T, Yamanouchi T. Rats fed fructose-enriched diets have characteristics of nonalcoholic hepatic steatosis. J Nutr 2009;139(11):2067–71.
44. Svendsen P, Graversen JH, Etzerodt A, Hager H, Roge R, Gronbaek H, Christensen EI, Moller HJ, Vilstrup H, Moestrup SK. Antibody-directed glucocorticoid targeting to CD163 in M2-type macrophages attenuates fructose-induced liver inflammatory changes. Mol Ther Methods Clin Dev 2017;4:50–61.
45. Clapper JR, Hendricks MD, Gu G, Wittmer C, Dolman CS, Herich J, Athanacio J, Villescaz C, Ghosh SS, Heilig JS, et al. Diet-induced mouse model of fatty liver disease and nonalcoholic steatohepatitis reflecting clinical disease progression and methods of assessment. Am J Physiol Gastrointest Liver Physiol 2013;305(7):G483–95.
46. Jensen VS, Hvid H, Damgaard J, Nygaard H, Ingvorsen C, Wulff EM, Lykkesfeldt J, Fledelius C. Dietary fat stimulates development of NAFLD more potently than dietary fructose in Sprague-Dawley rats. Diabetol Metab Syndr 2018;10:4.
47. Drescher HK, Weiskirchen R, Fülöp A, Hopf C, de San Román EG, Huesgen PF, de Bruin A, Bongiovanni L, Christ A, Tolba R, et al. The influence of different fat sources on steatohepatitis and fibrosis development in the Western diet mouse model of non-alcoholic steatohepatitis (NASH). Front Physiol 2019;10:770.
48. Boland M, Oró D, Tølbøl KS, Thrane S, Nielsen JC, Cohen T, Tabor D, Fernandes F, Tovchigrechko A, Veidal SS, et al. Towards a standard diet-induced and biopsy-confirmed mouse model of non-alcoholic steatohepatitis: impact of dietary fat source. World J Gastroenterol 2019;25:4904–20.
49. Kirpich IA, Gobejishvili LN, Bon Homme M, Waigel S, Cave M, Arteel G, Barve SS, McClain CJ, Deaciuc IV. Integrated hepatic transcriptome and proteome analysis of mice with high-fat diet-induced nonalcoholic fatty liver disease. J Nutr Biochem 2011;22(1):38–45.
50. Ito M, Suzuki J, Tsujioka S, Sasaki M, Gomori A, Shirakura T, Hirose H, Ito M, Ishihara A, Iwaasa H, et al. Longitudinal analysis of murine steatohepatitis model induced by chronic exposure to high-fat diet. Hepatol Res 2007;37(1):50–7.
51. Ishimoto T, Lanaspa MA, Rivard CJ, Roncal-Jimenez CA, Orlicky DJ, Cicerchi C, McMahan RH, Abdelmalek MF, Rosen HR, Jackman MR, et al. High-fat and high-sucrose (western) diet induces steatohepatitis that is dependent on fructokinase. Hepatology 2013;58(5): 1632–43.
52. de Wit N, Derrien M, Bosch-Vermeulen H, Oosterink E, Keshtkar S, Duval C, de Vogel-van den Bosch J, Kleerebezem M, Müller M, van der Meer R. 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 2012;303(5):G589–99.
53. Samuel VT, Liu ZX, Qu X, Elder BD, Bilz S, Befroy D, Romanelli AJ, Shulman GI. Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. J Biol Chem 2004;279(31):32345–53.
54. Gauthier MS, Favier R, Lavoie JM. Time course of the development of non-alcoholic hepatic steatosis in response to high-fat diet-induced obesity in rats. Br J Nutr 2006;95(2):273–81.
55. Hsu HC, Dozen M, Matsuno N, Obara H, Tanaka R, Enosawa S. Experimental nonalcoholic steatohepatitis induced by neonatal streptozotocin injection and a high-fat diet in rats. Cell Med 2013;6(1–2):57–62.
56. Kubota N, Kado S, Kano M, Masuoka N, Nagata Y, Kobayashi T, Miyazaki K, Ishikawa F. 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 2013;40(7):422–30.
57. Wang D, Wei Y, Pagliassotti MJ. Saturated fatty acids promote endoplasmic reticulum stress and liver injury in rats with hepatic steatosis. Endocrinology 2006;147(2):943–51.
58. Leamy AK, Egnatchik RA, Shiota M, Ivanova PT, Myers DS, Brown HA, Young JD. Enhanced synthesis of saturated phospholipids is associated with ER stress and lipotoxicity in palmitate treated hepatic cells. J Lipid Res 2014;55(7):1478–88.
59. Lieber CS, Leo MA, Mak KM, Xu Y, Cao Q, Ren C, Ponomarenko A, DeCarli LM. Model of nonalcoholic steatohepatitis. Am J Clin Nutr 2004;79(3):502–9.
60. Eccleston HB, Andringa KK, Betancourt AM, King AL, Mantena SK, Swain TM, Tinsley HN, Nolte RN, Nagy TR, Abrams GA, et al. Chronic exposure to a high-fat diet induces hepatic steatosis, impairs nitric oxide bioavailability, and modifies the mitochondrial proteome in mice. Antioxid Redox Signal 2011;15(2):447–59.
61. Lieber CS, DeCarli LM, Leo MA, Mak KM, Ponomarenko A, Ren C, Wang X. Beneficial effects versus toxicity of medium-chain triacylglycerols in rats with NASH. J Hepatol 2008;48(2):318–26.
62. Tzeng TF, Liou SS, Chang CJ, Liu IM. [6]-gingerol dampens hepatic steatosis and inflammation in experimental nonalcoholic steatohepatitis. Phytomedicine 2015;22(4):452–61.
63. Ou TH, Tung YT, Yang TH, Chien YW. Melatonin improves fatty liver syndrome by inhibiting the lipogenesis pathway in hamsters with high-fat diet-induced hyperlipidemia. Nutrients 2019;11(4):748.
64. Romestaing C, Piquet MA, Bedu E, Rouleau V, Dautresme M, Hourmand-Ollivier I, Filippi C, Duchamp C, Sibille B. Long term highly saturated fat diet does not induce NASH in Wistar rats. Nutr Metab (Lond) 2007;4:4.
65. Anstee QM, Concas D, Kudo H, Levene A, Pollard J, Charlton P, Thomas HC, Thursz MR, Goldin RD. Impact of pan-caspase inhibition in animal models of established steatosis and non-alcoholic steatohepatitis. J Hepatol 2010;53(3):542–50.
66. Sahai A, Malladi P, Melin-Aldana H, Green RM, Whitington PF. Upregulation of osteopontin expression is involved in the development of nonalcoholic steatohepatitis in a dietary murine model. Am J Physiol Gastrointest Liver Physiol 2004;287(1):G264–73.
67. Weltman MD, Farrell GC, Liddle C. Increased hepatocyte CYP2E1 expression in a rat nutritional model of hepatic steatosis with inflammation. Gastroenterology 1996;111(6):1645–53.
68. Ramadori P, Weiskirchen R, Trebicka J, Streetz K. Mouse models of metabolic liver injury. Lab Anim 2015;49(1 Suppl):47–58.
69. Lee SJ, Kang JH, Iqbal W, Kwon OS. Proteomic analysis of mice fed methionine and choline deficient diet reveals marker proteins associated with steatohepatitis. PLoS One 2015;10(4):e0120577.
70. Caballero F, Fernández A, Matías N, Martínez L, Fucho R, Elena M, Caballeria J, Morales A, Fernández-Checa JC, García-Ruiz C. Specific contribution of methionine and choline in nutritional nonalcoholic steatohepatitis: impact on mitochondrial S-adenosyl-L-methionine and glutathione. J Biol Chem 2010;285(24):18528–36.
71. Pickens MK, Yan JS, Ng RK, Ogata H, Grenert JP, Beysen C, Turner SM, Maher JJ. Dietary sucrose is essential to the development of liver injury in the methionine-choline-deficient model of steatohepatitis. J Lipid Res 2009;50(10):2072–82.
72. Pickens MK, Ogata H, Soon RK, Grenert JP, Maher JJ. Dietary fructose exacerbates hepatocellular injury when incorporated into a methionine-choline-deficient diet. Liver Int 2010;30(8):1229–39.

73. Lee GS, Yan JS, Ng RK, Kakar S, Maher JJ. Polyunsaturated fat in the methionine-choline-deficient diet influences hepatic inflammation but not hepatocellular injury. J Lipid Res 2007;48(8):1885–96.
74. Hussein O, Grosovski M, Lasri E, Svalb S, Ravid U, Assy N. Monounsaturated fat decreases hepatic lipid content in non-alcoholic fatty liver disease in rats. World J Gastroenterol 2007;13(3):361–8.
75. Bates JG, Breckenridge DGC, Liles JT, inventors; Methods of treating liver disease. Gilead Sciences, Inc., United States patent No.: US 2018 / 0280394 A1, Oct 4, 2018.
76. Tomita K, Teratani T, Suzuki T, Shimizu M, Sato H, Narimatsu K, Okada Y, Kurihara C, Irie R, Yokoyama H, et al. Free cholesterol accumulation in hepatic stellate cells: mechanism of liver fibrosis aggravation in nonalcoholic steatohepatitis in mice. Hepatology 2014;59(1):154–69.
77. Kirsch R, Clarkson V, Shephard EG, Marais DA, Jaffer MA, Woodburne VE, Kirsch RE, Hall PdlM. Rodent nutritional model of non-alcoholic steatohepatitis: species, strain and sex difference studies. J Gastroenterol Hepatol 2003;18(11):1272–82.
78. Rinella ME, Green RM. The methionine-choline deficient dietary model of steatohepatitis does not exhibit insulin resistance. J Hepatol 2004;40(1):47–51.
79. Tolosa L, Bonora-Centelles A, Teresa Donato M, Pareja E, Negro A, López S, Castell JV, Gómez-Lechón MJ. Steatotic liver: a suitable source for the isolation of hepatic progenitor cells. Liver Int 2011;31(8):1231–8.
80. Wei G, An P, Vaid KA, Nasser I, Huang P, Tan L, Zhao S, Schuppan D, Popov YV. Comparison of murine steatohepatitis models identifies a dietary intervention with robust fibrosis, ductular reaction, and rapid progression to cirrhosis and cancer. Am J Physiol Gastrointest Liver Physiol 2020;318(1):G174–G88.
81. Cong WN, Tao RY, Tian JY, Liu GT, Ye F. The establishment of a novel non-alcoholic steatohepatitis model accompanied with obesity and insulin resistance in mice. Life Sci 2008;82(19–20):983–90.
82. Nakae D, Yoshiji H, Maruyama H, Kinugasa T, Denda A, Konishi Y. Production of both 8-hydroxydeoxyguanosine in liver DNA and γ-glutamyltransferase-positive hepatocellular lesions in rats given a choline-deficient, L-amino acid-defined diet. Jpn J Cancer Res 1990;81(11):1081–4.
83. Fujita K, Nozaki Y, Yoneda M, Wada K, Takahashi H, Kirikoshi H, Inamori M, Saito S, Iwasaki T, Terauchi Y, et al. Nitric oxide plays a crucial role in the development/progression of nonalcoholic steatohepatitis in the choline-deficient, l-amino acid-defined diet-fed rat model. Alcohol Clin Exp Res 2010;34(Suppl 1):S18–24.
84. Denda A, Kitayama W, Kishida H, Murata N, Tsutsumi M, Tsujiuchi T, Nakae D, Konishi Y. Development of hepatocellular adenomas and carcinomas associated with fibrosis in C57BL/6J male mice given a choline-deficient, L-amino acid-defined diet. Jpn J Cancer Res 2002;93(2):125–32.
85. National Research Council (US) Subcommittee on Laboratory Animal Nutrition. Nutrient requirements of laboratory animals: fourth revised edition, 1995. Washington (DC): National Academies Press (US); 1995.
86. Tashiro K, Takai S, Jin D, Yamamoto H, Komeda K, Hayashi M, Tanaka K, Tanigawa N, Miyazaki M. Chymase inhibitor prevents the nonalcoholic steatohepatitis in hamsters fed a methionine- and choline-deficient diet. Hepatol Res 2010;40(5):514–23.
87. Bhathena J, Kulamarva A, Martoni C, Urbanska AM, Malhotra M, Paul A, Prakash S. Diet-induced metabolic hamster model of nonalcoholic fatty liver disease. Diabetes Metab Syndr Obes 2011;4:195–203.
88. Takayama F, Nakamoto K, Totani N, Yamanushi T, Kabuto H, Kaneyuki T, Mankura M. Effects of docosahexaenoic acid in an experimental rat model of nonalcoholic steatohepatitis. J Oleo Sci 2010;59(8):407–14.
89. Gogoi-Tiwari J, Köhn-Gaone J, Giles C, Schmidt-Arras D, Gratte FD, Elsegood CL, McCaughan GW, Ramm GA, Olynyk JK, Tirnitz-Parker JEE. The murine choline-deficient, ethionine-supplemented (CDE) diet model of chronic liver injury. J Vis Exp 2017;(128):56138.
90. Kulinski A, Vance DE, Vance JE. A choline-deficient diet in mice inhibits neither the CDP-choline pathway for phosphatidylcholine synthesis in hepatocytes nor apolipoprotein B secretion. J Biol Chem 2004;279(23):23916–24.
91. Allen PS, Brown AW, Brown MMB, Hsu WH, Beitz DC. Taurine and vitamin E supplementations have minimal effects on body composition, hepatic lipids, and blood hormone and metabolite concentrations in healthy Sprague Dawley rats. Nutr Diet Suppl 2015;7:77–85.
92. Kohli R, Kirby M, Xanthakos SA, Softic S, Feldstein AE, Saxena V, Tang PH, Miles L, Miles MV, Balistreri WF, et al. 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 2010;52(3):934–44.
93. Nigro D, Menotti F, Cento AS, Serpe L, Chiazza F, Dal Bello F, Romaniello F, Medana C, Collino M, Aragno M, et al. 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 2017;42:160–71.
94. Sodhi K, Puri N, Favero G, Stevens S, Meadows C, Abraham NG, Rezzani R, Ansinelli H, Lebovics E, Shapiro JI. Fructose mediated non-alcoholic fatty liver is attenuated by HO-1-SIRT1 module in murine hepatocytes and mice fed a high fructose diet. PLoS One 2015;10(6):e0128648.
95. Kasim-Karakas SE, Vriend H, Almario R, Chow LC, Goodman MN. Effects of dietary carbohydrates on glucose and lipid metabolism in golden Syrian hamsters. J Lab Clin Med 1996;128(2):208–13.
96. Matsuzawa N, Takamura T, Kurita S, Misu H, Ota T, Ando H, Yokoyama M, Honda M, Zen Y, Nakanuma Y, et al. Lipid-induced oxidative stress causes steatohepatitis in mice fed an atherogenic diet. Hepatology 2007;46(5):1392–403.
97. Subramanian S, Goodspeed L, Wang S, Kim J, Zeng L, Ioannou GN, Haigh WG, Yeh MM, Kowdley KV, O'Brien KD, et al. Dietary cholesterol exacerbates hepatic steatosis and inflammation in obese LDL receptor-deficient mice. J Lipid Res 2011;52(9):1626–35.
98. Zheng S, Hoos L, Cook J, Tetzloff G, Davis H, Jr, van Heek M, Hwa JJ. Ezetimibe improves high fat and cholesterol diet-induced non-alcoholic fatty liver disease in mice. Eur J Pharmacol 2008;584(1):118–24.
99. Savard C, Tartaglione EV, Kuver R, Haigh WG, Farrell GC, Subramanian S, Chait A, Yeh MM, Quinn LS, Ioannou GN. Synergistic interaction of dietary cholesterol and dietary fat in inducing experimental steatohepatitis. Hepatology 2013;57(1):81–92.
100. Lichtman AH, Clinton SK, Iiyama K, Connelly PW, Libby P, Cybulsky MI. Hyperlipidemia and atherosclerotic lesion development in LDL receptor–deficient mice fed defined semipurified diets with and without cholate. Arterioscler Thromb Vasc Biol 1999;19(8):1938–44.
101. Montandon SA, Somm E, Loizides-Mangold U, de Vito C, Dibner C, Jornayvaz FR. Multi-technique comparison of atherogenic and MCD NASH models highlights changes in sphingolipid metabolism. Sci Rep 2019;9(1):16810.
102. Ikemoto S, Takahashi M, Tsunoda N, Maruyama K, Itakura H, Kawanaka K, Tabata I, Higuchi M, Tange T, Yamamoto TT, et al. Cholate inhibits high-fat diet-induced hyperglycemia and obesity with acyl-CoA synthetase mRNA decrease. Am J Physiol 1997;273(1 Pt 1):E37–45.
103. Trevaskis JL, Griffin PS, Wittmer C, Neuschwander-Tetri BA, Brunt EM, Dolman CS, Erickson MR, Napora J, Parkes DG, Roth JD. Glucagon-like peptide-1 receptor agonism improves metabolic, biochemical, and histopathological indices of nonalcoholic steatohepatitis in mice. Am J Physiol Gastrointest Liver Physiol 2012;302(8):G762–72.
104. Tetri LH, Basaranoglu M, Brunt EM, Yerian LM, Neuschwander-Tetri BA. Severe NAFLD with hepatic necroinflammatory changes in mice fed trans fats and a high-fructose corn syrup equivalent. Am J Physiol Gastrointest Liver Physiol 2008;295(5):G987–95.
105. Hinojosa G, Hamilton A, Lora J, Short JD, Dearth RK. Characterizing a rat model to study the role endo-immunology plays in diet-induced non-alcoholic fatty liver disease. FASEB J 2017;31(1_supplement):887.17.
106. Hansen HH, Boland ML, Oró D, Tølbøl KS, Illemann M, Veidal SS, Feigh M, Jelsing J, Vrang N, Trevaskis JL. Novel Gubra Amylin NASH (GAN) diet-induced obese mouse models of biopsy-confirmed non-alcoholic steatohepatitis. Keystone Symposia—Integrated Pathways of Disease in NASH and NAFLD. Santa Fe, NM, Jan. 20–24, 2019.
107. Hansen HB, Kristiansen MNB, Veidal SS, Rigbolt KTG, Feigh M, Tølbøl KS, Vrang N, Jelsing J. Histological, biochemical and

molecular disease progression in a diet-induced obese mouse model of non-alcoholic steatohepatitis. American Association for the Study of Liver Diseases Annual Conference, 2018, San Francisco, CA, Nov. 8–13.
108. Rom O, Xu G, Guo Y, Zhu Y, Wang H, Zhang J, Fan Y, Liang W, Lu H, Liu Y, et al. Nitro-fatty acids protect against steatosis and fibrosis during development of nonalcoholic fatty liver disease in mice. EBioMedicine 2019;41:62–72.
109. Basciano H, Miller AE, Naples M, Baker C, Kohen R, Xu E, Su Q, Allister EM, Wheeler MB, Adeli K. Metabolic effects of dietary cholesterol in an animal model of insulin resistance and hepatic steatosis. Am J Physiol Endocrinol Metab 2009;297(2):E462–73.
110. Angulo P. Nonalcoholic fatty liver disease. Rev Gastroenterol Mex 2005;70(Suppl 3):52–6.
111. Asgharpour A, Cazanave SC, Pacana T, Seneshaw M, Vincent R, Banini BA, Kumar DP, Daita K, Min HK, Mirshahi F, et al. A diet-induced animal model of non-alcoholic fatty liver disease and hepatocellular cancer. J Hepatol 2016;65(3):579–88.
112. Larter CZ, Yeh MM. Animal models of NASH: getting both pathology and metabolic context right. J Gastroenterol Hepatol 2008;23(11): 1635–48.
113. Febbraio MA, Reibe S, Shalapour S, Ooi GJ, Watt MJ, Karin M. Preclinical models for studying NASH-driven HCC: how useful are they? Cell Metab 2019;29(1):18–26.
114. Benbow JH, Thompson KJ, Cope HL, Brandon-Warner E, Culberson CR, Bossi KL, Li T, Russo MW, Gersin KS, McKillop IH, et al. Diet-induced obesity enhances progression of hepatocellular carcinoma through tenascin-C/toll-like receptor 4 signaling. Am J Pathol 2016;186(1):145–58.
115. Pellizzon M. Choice of laboratory animal diet influences intestinal health. Lab Anim 2016;45(6):238–9.
116. Pellizzon MA, Ricci MR. Effects of rodent diet choice and fiber type on data interpretation of gut microbiome and metabolic disease research. Curr Protoc Toxicol 2018;77(1):e55.
117. Pellizzon MA, Ricci MR. The common use of improper control diets in diet-induced metabolic disease research confounds data interpretation: the fiber factor. Nutr Metab (Lond) 2018;15:3.