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Pathogenesis of and major animal models used for nonalcoholic fatty liver disease

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Abstract

Nonalcoholic fatty liver disease (NAFLD) and its pathologically more severe form, nonalcoholic steatohepatitis (NASH), have become prevalent worldwide and carry an increased risk of developing hepatocellular carcinoma and other metabolic diseases. Diverse animal models have been proposed to replicate particular characteristics of NAFLD and NASH and have provided significant clues to the critical molecular targets of NASH treatment. In this review, we summarize the histopathology, pathogenesis, and molecular basis of NAFLD progression and discuss the benchmark animal models of NAFLD/NASH.

Keywords

Nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, histopathology, pathogenesis, dietary model, genetic model

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Introduction

Nonalcoholic fatty liver disease (NAFLD) represents a progressive liver disorder ranging from simple liver steatosis to nonalcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and ultimately hepatocellular carcinoma, in the absence of excessive alcohol intake.[1,2] NAFLD is becoming one of the most alarming chronic liver diseases because it is one of the fastest growing indicators for adult liver transplantation and a major cause of hepatocellular carcinoma.[3–6] Development of NAFLD has a strong association with metabolic abnormalities such as obesity, insulin resistance (IR), and type 2 diabetes, and NAFLD itself is a risk factor for cardiovascular disease. Patients with NAFLD are at high risk of dying from cardiovascular disease and other metabolic diseases.[7–9] NASH is the pathologically more severe form of NAFLD and is characterized by hepatocellular ballooning, active hepatocellular necrosis, and liver inflammation with the presence of steatosis; moreover, it is associated with more rapid progression of fibrosis and cirrhosis.[10,11] Given the rapid growth in NAFLD prevalence, further research on the exact pathogenic mechanisms and potential drug treatments for NASH is imperative. Established animal models have vividly highlighted the important aspects of each stage of NAFLD and provide significant clues to the critical molecular events during NAFLD development, which opens up new opportunities for treatment of NAFLD in humans. This review will summarize the pathogenesis and molecular basis of NAFLD and discuss the benchmark animal models that recapitulate the histopathology and pathophysiology associated with human NAFLD.

Histopathology of NAFLD and NASH

Intracytoplasmic lipid accumulation in the form of triglycerides is an iconic feature of NAFLD. Liver biopsy followed by histological analysis is the gold standard for confirming the presence and activity of NAFLD, which is histologically diagnosed when hepatic triglyceride accumulation occurs in more than 5% of hepatocytes.[12] Grading and staging systems for NAFLD consider a wide spectrum of histopathology features. In particular, semiquantitative scoring assesses 4 major histological features: steatosis (0–3), hepatocellular ballooning (0–2), inflammation (0–3), and fibrosis (0–4).[13,14] The size of fat droplets can differ; macrovesicular steatosis is the predominant pattern seen in NAFLD and is characterized by large vacuoles that occupy the whole cytoplasm and push the nucleus to one side of the cell. Some NAFLD patients, however, present with multiple small lipid vacuoles in the cytoplasm and the nucleus remains unmoved, which is termed "microvesicular steatosis."[15] Hepatocellular ballooning, which refers to cells with swollen and rarefied cytoplasm, is a distinguishing feature of progression to NASH.[16] Hepatocellular ballooning is often associated with Mallory-Denk bodies, which result from the clumping of cytokeratins and subsequent ubiquitination.[17] Inflammation is another remarkable feature of NASH development. Lobular inflammation and portal inflammation can both present in NASH. Lobular inflammation, which is characterized by the presence of small clusters of inflammatory cells near ballooned hepatocytes, reflects the dysregulation of cytokine and chemokine expression in the fatty liver.[10,14,18] Portal inflammation is

common and usually mild in NASH patients. Increased portal inflammation is associated with many clinical and pathologic features of progressive NASH and may be considered a marker of aggravation and advanced disease.[19] NASH patients often develop a typical "chicken-wire" fibrosis surrounding individual or groups of hepatocytes that is termed "pericellular fibrosis." This finding reflects progression of NASH and it can further spread to the portal areas and subsequently lead to septal fibrosis and even cirrhosis.[20] Representative haematoxylin and eosin (H&E)-stained sections of human NASH and murine steatohepatitis are shown in Figure 1.

Pathogenesis and molecular basis of NAFLD and NASH

The pathogenesis of NAFLD and the factors that promote progression from simple steatosis to NASH are complex. Lipid accumulation in hepatocytes and its interplay with inflammatory responses, cellular stress, and cell death are believed to be the major factors contributing to NAFLD development.[21–24] Genetic factors and intestinal dysbiosis are also crucial.[25]

Steatosis occurs whenever the rate of import or synthesis of lipid by hepatocytes exceeds the rate of export or degradation.[26,27] Triglyceride is the most conspicuous type of lipid in the livers of NAFLD patients, so steatosis can be graded according to the extent of triglyceride accumulation. However, the triglycerides are not hepatotoxic compared with the other types of lipids that accumulate in the fat liver (including fatty acids, diacylglycerol, oxysterols, cholesterol, and phospholipids), so steatosis grade or severity does not predict hepatic injury, inflammation, or fibrosis.[28,29] Overnutrition and particularly IR

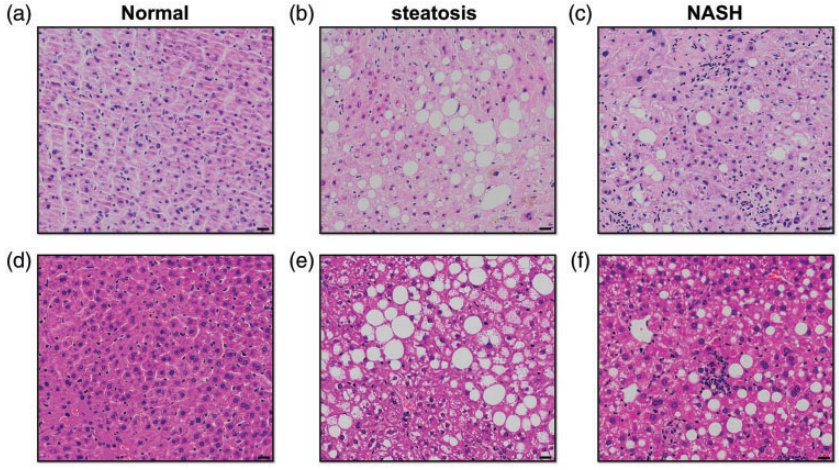

Figure 1. Histopathological features of human and murine nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) as determined by haematoxylin and eosin (H&E) staining. (a) Healthy human liver; (b) simple liver steatosis in human; (c) human NASH with hepatocellular ballooning and inflammation; (d) healthy murine liver (C57BL/6 mice fed a normal diet); (e) liver of obese *ob*/*ob* mice fed a normal diet that spontaneously developed liver steatosis; and (f) liver of C57BL/6 mice fed a methionine- and choline-deficient (MCD) diet for 4 weeks; mice developed steatosis with notable inflammation. Scale bars, 20 μm

are closely associated with the etiology of steatosis and provide the initiating and propagating damage for liver injury and resultant inflammation.[30,31] Fatty acid accumulation, in turn, exacerbates IR and hyperinsulinemia, leading to further steatosis and inflammation.[32–34] Overnutrition increases free acid influx from diets and consequent uptake by the liver, resulting in increases in *de novo* lipogenesis in the liver. Overnutrition also induces chronic inflammation and promotes IR.[35,36] IR is tightly associated with lipid accumulation in the liver and subsequent steatosis. IR promotes increased efflux of free fatty acids (FFA) from adipose tissues and overwhelms FFA uptake by the liver because insulin cannot suppress adipose tissue lipolysis via hormone-sensitive lipase when IR occurs.[36] IR promotes lipid accumulation in the liver primary by mediating uptake of FFA via the scavenger receptor CD36 and uptake of free cholesterol (FC) via CD36 and oxidised low-density lipoprotein (ox-LDL).[37] IR-associated hyperinsulinaemia and hyperglycaemia promote hepatic *de novo* lipogenesis by upregulating the key lipid synthesis regulator sterol regulatory element-binding protein isoform 1c (SREBP-1c) and the glucose metabolism regulator carbohydrate response element-binding protein (ChREBP), respectively.[38] In addition, hyperinsulinaemia can directly suppress β-oxidation of FFA.[39] More importantly, IR-associated hyperinsulinaemia is implicated in driving the accumulation of cytotoxic lipid species such as FC in the liver and activating the c-Jun N-terminal kinase (JNK) signaling pathway, resulting in mitochondrial damage and hepatocyte injury in a process called "lipotoxicity."[25,40] Molecules released from damaged hepatocytes further promote changes in signaling pathways that regulate cellular stress (such as oxidative stress and endoplasmic reticulum stress) and inflammatory responses, thus perpetuating hepatocellular injury and subsequent cell death and promoting NAFLD development.[41–43]

An increase in the production of pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-6, and activation of toll-like receptors (TLR) and the NLRP3 inflammasome are critically involved in pathophysiology of various aspects of NASH.[44,45] TLR4 links to the activation of nuclear factor (NF)-κB and macrophage recruitment in steatohepatitis.[46] The NLRP3 inflammasome, which is highly expressed in liver, is associated with IL-1β release.[47] These elements and their interaction perpetuate liver damage, inflammation, and fibrosis, resulting in progression of NASH (Figure 2).

Dietary and genetic animal models of NAFLD and NASH

High-fat diet

The high-fat diet (HFD) model is a good simulation of the modern Western diet. The main calorie intake (energy) of HFD is derived from fat (45% to 75%). Animals fed with HFD can replicate the major histopathology and pathogenesis seen in human NAFLD. With long-term HFD feeding, animals develop obesity, IR, and hepatic damage.

The classic HFD model was established in male rats and involved feeding a diet with 71% fat, 11% carbohydrates, and 18% protein for 3 weeks. A standard diet containing 35% fat, 47% carbohydrates, and 18% protein was used as the control; this diet has the same fat content as the average US diet. Rats fed HFD developed steatosis, IR, mitochondrial dysfunction, and mononuclear inflammation, accompanied by increased hepatic TNF-α and cytochrome P4502E1 (CYP2E1) induction.[48] Another frequently used HFD animal model was established in mice. Male mice (C57BL/6

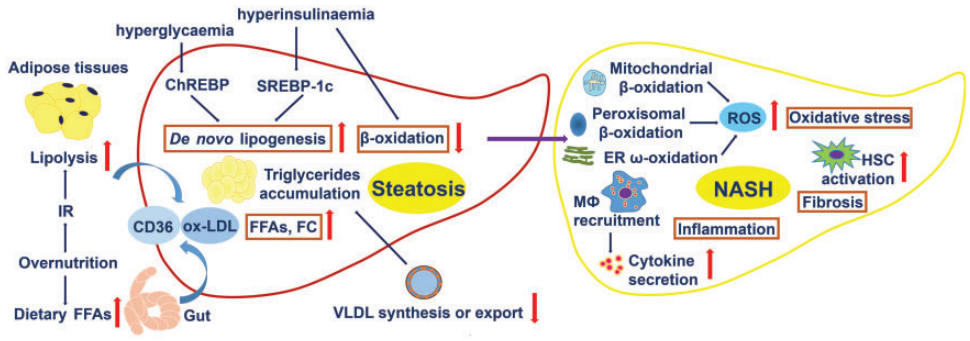

Figure 2. Major processes in pathogenesis of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH). NAFLD is mainly associated with increased hepatic *de novo* lipogenesis, increased adipose tissue lipolysis, increased efflux of dietary free fatty acids (FFAs) impaired β-oxidation and impaired synthesis or export of very-low-density lipoprotein (vLDL). NASH is mainly associated with increased oxidative stress, activated inflammatory responses, and increased hepatic fibrosis. IR = insulin resistance; ChREBP = carbohydrate response element-binding protein; SREBP-1c = sterol regulatory element-binding protein isoform 1c; FC = free cholesterol; ox-LDL = oxidised low-density lipoprotein; CD36 = cluster of differentiation 36; ER = endoplasmic reticulum; MΦ = macrophage; ROS = reactive oxygen species; HSC = hepatic stellate cells

strain) that received the same HFD for up to 16 weeks became obese and showed steatosis, hepatocyte ballooning, increased serum glucose, and decreased adiponectin, indicating hyperglycaemia and IR.[49] Similarly, our group found that male C57BL/6 mice fed a HFD (60% fat, 20% carbohydrates, and 20% protein) for 12 weeks developed steatosis (Figure 3a and 3b).

HFD diets can replicate the hallmark features of altered metabolic parameters seen in human NAFLD but the degree of hepatic pathology is not as severe. Increasingly, studies use additional elements in the HFD to more closely mimic human NAFLD, such as diets supplemented with fructose, cholesterol, or both.[50,51]

High-fat, high-fructose diet

A significantly increased consumption of calories from fructose-rich foods has been confirmed to be closely associated with development of human NAFLD and severity of fibrosis.[52,53]

Male mice (C57BL/6 strain) that were fed a high-fat, high-fructose (HFHF) diet—that is, a HFD (58 kcal% fat) supplemented with 42 g/L of carbohydrates (mixed at a ratio of 55% fructose and 45% sucrose by weight) in drinking water—for 16 weeks developed more severe hepatic oxidative stress, increased hepatic macrophage aggregation, and exacerbated liver fibrosis compared with mice fed HFD without carbohydrate supplementation. However, both groups showed gains in body weight and body fat mass and increased steatosis, fasting glucose, and IR, indicating that fructose consumption is required for NAFLD progression.[54]

Fructose can promote *de novo* lipogenesis in liver, inhibits β-oxidation, and induces hepatic insulin, which result in rapid development of intrahepatic lipid accumulation. Excessive consumption of fructose also promotes intestinal bacterial overgrowth and leads to hepatocellular damage, thus triggering NAFLD progression.[55]

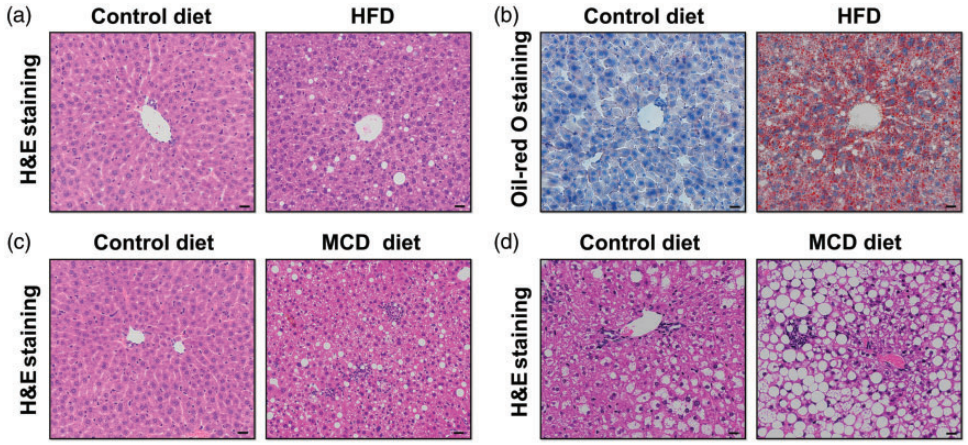

Figure 3. Histopathological features of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis in different murine models as determined by haematoxylin and eosin (H&E) and Oil-red O staining. Haematoxylin and eosin (H&E) (a) and Oil-red O (b) stained sections of C57BL/6 mice fed a control diet or high-fat diet (HFD) for 12 weeks; (c) H&E stained sections of C57BL/6 mice fed methionine- and choline-deficient (MCD) diets for 4 weeks showed steatosis and inflammation compared with those fed a control diet; (d) *ob/ob* mice fed a control diet spontaneously developed liver steatosis, and those fed MCD diets for 4 weeks developed steatosis with inflammation. Scale bars, 20 μm

High-fat, high-cholesterol diet

High cholesterol intake can induce dyslipidemia and IR, and it has been recognized as a critical factor associated with hepatic inflammation and NAFLD progression in both animal models and humans.[56–58]

Male mice (C57BL/6 strain) fed a high-fat, high-cholesterol (HFHC) diet (15% fat and 1% cholesterol) for 30 weeks became obese and developed more profound hepatic steatosis and inflammation, as well as typical perisinusoidal fibrosis compared with mice fed a single HFD or high-cholesterol diet, both of which resulted in increased hepatic steatosis with little inflammation and no signs of fibrosis. Mice fed with HFHC diets also showed hypercholesterolaemia and a significant reduction in serum adiponectin levels.[57] Obese *foz/foz* mice (deficient in the *Alms1* gene) fed a HFD containing different percentages of cholesterol (0.0%, 0.2%, or 2.0%) for 24 weeks showed different outcomes. Mice fed with 2.0% cholesterol had higher hepatic cholesterol content and much higher alanine aminotransferase (ALT) levels than other groups, suggesting that increased accumulation of free cholesterol is associated with NAFLD progression.[59]

Cholesterol increases hepatic oxidative stress and promotes hepatic apoptosis, macrophage recruitment, and fibrogenesis, thus triggering NAFLD progression.

Methionine- and choline-deficient diet

Feeding animals a methionine- and choline-deficient (MCD) diet is a commonly used nutritional model for NASH. MCD diets are usually highly enriched in sucrose (40%) and moderately enriched with fat (10%) but deficient in methionine and choline, which are essential for hepatic β-oxidation and production of very-low-density lipoprotein (vLDL).[60] Depriving animals of methionine and choline causes notable steatosis, inflammation, hepatic ballooning, reactive oxygen species

(ROS)-mediated liver damage, and fibrosis.[2]

Hepatic steatosis can be seen within 1 to 2 weeks of MCD induction.[60,61] Moreover, mice fed with MCD diets developed extensive necro-inflammation as early as 2 weeks, and the typical chicken-wire fibrosis can be seen as early as 6 weeks after MCD induction, similar to the presentation in human NASH.[62,63] MCD feeding also increases serum ALT levels and induces ballooning degeneration of hepatocytes in mice.[64] Similarly, our group found that male C57BL/6 mice fed with MCD diets developed steatosis and inflammation at 4 weeks (Figure 3c). The severity of steatohepatitis in MCD-fed mice is associated with impaired hepatic adiponectin action and adipogenic transformation of hepatocytes.[64] The responsiveness that develops in mice fed the MCD diet depends on sex and strain. For example, C57BL/6 mice exhibited more pronounced release of transaminases than did DBA/2J mice, whereas long-term MCD induction caused more severe liver injury, even hepatocarcinogenesis, in DBA/2J mice, but did not result in carcinogenesis in C57BL/6 mice.[61,65] The notable inflammation observed in MCD-induced steatohepatitis is associated with increased macrophage infiltration in the liver, activation of the NF-κB signaling pathway, and concomitant increases in downstream pro-inflammatory cytokines, such as TNF-α, monocyte chemoattractant protein (MCP)-1, transforming growth factor (TGF)-β, and IL-6.[66–68] MCD diets also promote induction of adhesion molecules, such as intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1, which are essential for polymorph recruitment.[68–70]

Animal models using MCD diets can replicate the hallmark pathological features of severe human NASH more closely than other dietary-based animal models. The steatosis, inflammation, and fibrosis induced by MCD diets develop more quickly than with HFD and other Western diet models. Cellular stress, such as endoplasmic reticulum (ER) stress, oxidative stress, and auto-phagocytic stress, is more pronounced in the MCD model than in other dietary-based NAFLD models.[71]

However, the MCD diet model has obvious disadvantages. Mice fed MCD diets always exhibit significant loss of body weight and the liver decreases proportionally in size, which go against effects seen in overweight and obese individuals with NAFLD.[2,72] In addition, the metabolic profile in the MCD model is opposite to that seen in NAFLD patients: serum levels of triglyceride, insulin, leptin, and fasting glucose are dampened, whereas serum adiponectin is not decreased.[72] Therefore, *db*/*db* (deficient in leptin receptor activity) or *ob*/*ob* (deficient in leptin) mice are often used in the MCD model to better imitate human NASH. Findings suggest that *db*/*db* mice fed with MCD diet for 4 weeks show remarkable hepatic inflammation and fibrosis.[73]

ob/ob and db/db mice

Leptin-deficient (*ob*/*ob*) mice, which carry an autosomal recessive mutation in the leptin gene, develop spontaneous liver steatosis under normal chow feeding. *ob*/*ob* mice are grossly overweight and show the altered metabolic parameters seen in human NAFLD, such as hyperinsulinaemia, hyperglycaemia, and IR.[60,74,75] However, *ob*/*ob* mice are resistant to hepatic fibrosis, given that leptin is essential for the hepatic fibrogenic response to liver injury.[76] In addition, the *ob*/*ob* mouse model is limited to spontaneous steatohepatitis unless secondary insults are added (such as a HFD or MCD diet or administration of small doses of lipopolysaccharide endotoxin).[77] Our group found that male *ob*/*ob* mice (C57BL/6 strain) fed MCD diets developed

Table 1. Commonly used animal models of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH)

Model	Diet formula (kcal%)	Obesity	Insulin resistance	Steatosis	Steatohepatitis	Fibrosis
Methionine- and choline-deficient diet (MCD)	40% sucrose, 10% fat, methionine (−), choline (−)	No	Hepatic insulin resistance	Yes	Yes	Yes
High-fat diet (HFD)	45% to 75% fat. Classic model is 71% fat, 18% protein, and 11% carbohydrates	Yes	Yes	Yes	Yes, but mild	Yes, but mild
High-fat, high-fructose diet (HFHF)	HFD supplemented with fructose (usually 23 g/L in drinking water)	Yes	Yes	Yes	Yes, but mild	Yes, but mild
High-fat, high-cholesterol diet (HFHC)	Approximately 1% cholesterol fed in conjunction with HFD (usually 15% to 40% fat)	Yes	Yes	Yes	Yes	Yes
ob/*ob* mice	—	Yes	Yes	Yes	No[1]	No[1]
db/*db* mice	—	Yes	Yes	Yes	No[1]	No[1]
foz/*foz* mice	—	Yes	Yes	Yes	No[1]	No[1]

[1]Although these mice do not develop steatohepatitis and fibrosis spontaneously, additionally feeding with MCD diets or HFD can promote development of steatohepatitis and fibrosis (not in *ob*/*ob* mice)

steatosis and inflammation at 4 weeks (Figure 3d).*db*/*db* mice are homozygous for the autosomal recessive diabetic gene (*db*), which encodes a point mutation in the leptin receptor and leads to defective leptin signaling.[63] *db*/*db* mice are obese and diabetic and develop macrovesicular hepatic steatosis accompanied by hyperglycaemia and hyperinsulinaemia.[74,75] Unlike *ob*/*ob* mice, *db*/*db* mice exhibit normal or elevated levels of leptin but are resistant to its effects. Similarly, *db*/*db* mice do not spontaneously develop inflammation or show features of NASH without further insult.The *ob*/*ob* and *db*/*db* mice are good genetic models of NAFLD because they develop pronounced hepatic steatosis and show the significant altered metabolic characteristics seen in human NAFLD. *db*/*db* mice can also be used to study the progression of steatosis to NASH in the presence of secondary insults such as a MCD diet. However, congenital leptin deficiency or leptin resistance caused by gene mutations is not prevalent in obese humans or NASH patients, so the *ob*/*ob* and *db*/*db* mice models are limited in their ability to reflect the genesis of human obesity or NASH.[63,78]

foz/*foz* mice

Obese *foz*/*foz* mice, which carry a mutated *Alms1* gene, spontaneously develop hepatic steatosis, obesity, diabetes, and IR, and show significant upregulation of cholesterol levels. HFD feeding can accentuate transition of simple steatosis to steatohepatitis by aggravating metabolic abnormalities, resulting in severe hepatocyte ballooning, inflammation, and fibrosis, accompanied by significant decreases in adiponectin levels and increases in cholesterol levels. However, despite upregulation of hepatic triglyceride content, serum triglyceride levels remain unchanged in *foz*/*foz* mice, even those fed with HFD.[79,80] All *foz*/*foz* mice are obese but the severity of NASH is strain dependent. Serum ALT levels and NAFLD activity score were higher (worse) in *foz*/*foz* C57BL6/J mice than in *foz*/*foz* BALB/c mice fed with HFD. Moreover, HFD-induced fibrosis was severe in *foz*/*foz* C57BL6/J mice but absent in *foz*/*foz* BALB/c mice.[81]

To date, diverse animal models have been proposed to mimic particular characteristics of human NAFLD, such as the American lifestyle–induced obesity syndrome (ALIOS) model, the diet-induced animal model of nonalcoholic fatty liver disease (DIAMOND) model, and the $ldlr^{-/-}$ mice model.[82–84] Recently, a murine NASH model was proposed that showed rapid progression of extensive fibrosis and hepatocellular carcinoma. The model used a Western diet, which contained high fat, high fructose, and high cholesterol, combined with a low weekly dose of intraperitoneal carbon tetrachloride (CCl_4). This model captures the progressive stages of human fatty liver disease, from simple steatosis to inflammation, fibrosis, and cancer.[85] It is important to choose the appropriate animal model to meet the research purpose (Table 1).

Conclusion

NAFLD is becoming a worldwide issue because of changes in lifestyle and resultant overnutrition. Lipid accumulation in the liver and its interplay with inflammation, oxidative stress, cell death, and autophagy is considered a major process of NAFLD progression. However, the exact mechanisms of NAFLD progression remain largely unknown. The use of animal models to replicate the important aspects of NAFLD progression provides significant clues to the critical molecular events that occur during NAFLD development and suggests a number of therapeutic targets for future treatment of NAFLD. Nevertheless, none of established animal models are

perfect and it is important to choose the appropriate animal model to meet the research purpose.

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Declaration of conflicting interest

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References

1. Farrell GC and Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. *Hepatology* 2006; 43: S99–S112. doi:10.1002/hep.20973.
2. Hebbard L and George J. Animal models of nonalcoholic fatty liver disease. *Nat Rev Gastroenterol Hepatol* 2011; 8: 35–44. doi:10.1038/nrgastro.2010.191.
3. Brunt EM, Wong VW, Nobili V, et al. Nonalcoholic fatty liver disease. *Nat Rev Dis Primers* 2015; 1: 15080, doi:10.1038/nrdp.2015.80.
4. Wong RJ, Aguilar M, Cheung R, et al. Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States. *Gastroenterology* 2015; 148: 547–555. doi:10.1053/j.gastro.2014.11.039.
5. El-Serag HB. Hepatocellular carcinoma. *N Engl J Med* 2011; 365: 1118–1127. doi:10.1056/NEJMra1001683.
6. Wong RJ, Cheung R and Ahmed A. Nonalcoholic steatohepatitis is the most rapidly growing indication for liver transplantation in patients with hepatocellular carcinoma in the U.S. *Hepatology* 2014; 59: 2188–2195. doi:10.1002/hep.26986.
7. Ma J, Hwang SJ, Pedley A, et al. Bi-directional analysis between fatty liver and cardiovascular disease risk factors. *J Hepatol* 2017; 66: 390–397. doi:10.1016/j.jhep.2016.09.022.
8. Wanless IR and Lentz JS. Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. *Hepatology* 1990; 12: 1106–1110.
9. Marchesini G, Brizi M, Morselli-Labate AM, et al. Association of nonalcoholic fatty liver disease with insulin resistance. *Am J Med* 1999; 107: 450–455.
10. Tiniakos DG, Vos MB and Brunt EM. Nonalcoholic fatty liver disease: pathology and pathogenesis. *Annu Rev Pathol* 2010; 5: 145–171. doi:10.1146/annurev-pathol-121808-102132.
11. Dowman JK, Tomlinson JW and Newsome PN. Pathogenesis of non-alcoholic fatty liver disease. *QJM* 2010; 103: 71–83. doi:10.1093/qjmed/hcp158.
12. Tandra S, Yeh MM, Brunt EM, et al. Presence and significance of microvesicular steatosis in nonalcoholic fatty liver disease. *J Hepatol* 2011; 55: 654–659. doi:10.1016/j.jhep.2010.11.021.
13. Brunt EM, Janney CG, Di Bisceglie AM, et al. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999; 94: 2467–2474. doi:10.1111/j.1572-0241.1999.01377.x.
14. Bedossa P and Patel K. Biopsy and noninvasive methods to assess progression of nonalcoholic fatty liver disease. *Gastroenterology* 2016; 150: 1811–1822.e1814, doi:10.1053/j.gastro.2016.03.008.
15. Lefkowitch JH. Morphology of alcoholic liver disease. *Clin Liver Dis* 2005; 9: 37–53. doi:10.1016/j.cld.2004.11.001.
16. Matteoni CA, Younossi ZM, Gramlich T, et al. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology* 1999; 116: 1413–1419.
17. Stumptner C, Fuchsbichler A, Heid H, et al. Mallory body—a disease-associated type of sequestosome. *Hepatology* 2002; 35: 1053–1062. doi:10.1053/jhep.2002.32674.

18. Ganz M and Szabo G. Immune and inflammatory pathways in NASH. *Hepatol Int* 2013; 7: 771–781. doi:10.1007/s12072-013-9468-6.
19. Brunt EM, Kleiner DE, Wilson LA, et al. Portal chronic inflammation in nonalcoholic fatty liver disease (NAFLD): a histologic marker of advanced NAFLD—clinicopathologic correlations from the nonalcoholic steatohepatitis clinical research network. *Hepatology* 2009; 49: 809–820. doi:10.1002/hep.22724.
20. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; 41: 1313–1321. doi:10.1002/hep.20701.
21. Cohen JC, Horton JD and Hobbs HH. Human fatty liver disease: old questions and new insights. *Science* 2011; 332: 1519–1523. doi:10.1126/science.1204265.
22. Hotamisligil GS. Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. *Cell* 2010; 140: 900–917. doi:10.1016/j.cell.2010.02.034.
23. Gual P, Gilgenkrantz H and Lotersztajn S. Autophagy in chronic liver diseases: the two faces of Janus. *Am J Physiol Cell Physiol* 2017; 312: C263–C273. doi:10.1152/ajpcell.00295.2016.
24. Farrell GC, van Rooyen D, Gan L, et al. NASH is an inflammatory disorder: pathogenic, prognostic and therapeutic implications. *Gut Liver* 2012; 6: 149–171. doi:10.5009/gnl.2012.6.2.149.
25. Wong VW, Chitturi S, Wong GL, et al. Pathogenesis and novel treatment options for non-alcoholic steatohepatitis. *Lancet Gastroenterol Hepatol* 2016; 1: 56–67. doi:10.1016/s2468-1253(16)30011-5.
26. Bradbury MW and Berk PD. Lipid metabolism in hepatic steatosis. *Clin Liver Dis* 2004; 8: 639–671, xi, doi:10.1016/j.cld.2004.04.005.
27. Machado MV and Cortez-Pinto H. Non-alcoholic fatty liver disease: what the clinician needs to know. *World J Gastroenterol* 2014; 20: 12956–12980. doi:10.3748/wjg.v20.i36.12956.
28. Puri P, Baillie RA, Wiest MM, et al. A lipidomic analysis of nonalcoholic fatty liver disease. *Hepatology* 2007; 46: 1081–1090. doi:10.1002/hep.21763.
29. Yamaguchi K, Yang L, McCall S, et al. Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. *Hepatology* 2007; 45: 1366–1374. doi:10.1002/hep.21655.
30. Kitade H, Chen G, Ni Y, et al. Nonalcoholic fatty liver disease and insulin resistance: new insights and potential new treatments. *Nutrients* 2017; 9: pii: E387. doi:10.3390/nu9040387.
31. Day CP. Pathogenesis of steatohepatitis. *Best Pract Res Clin Gastroenterol* 2002; 16: 663–678.
32. Gruben N, Shiri-Sverdlov R, Koonen DP, et al. Nonalcoholic fatty liver disease: A main driver of insulin resistance or a dangerous liaison? *Biochim Biophys Acta* 2014; 1842: 2329–2343. doi:10.1016/j.bbadis.2014.08.004.
33. Guo S. Insulin signaling, resistance, and the metabolic syndrome: insights from mouse models into disease mechanisms. *J Endocrinol* 2014; 220: T1–T23. doi:10.1530/joe-13-0327.
34. Miao H, Zhang Y, Lu Z, et al. FOXO1 involvement in insulin resistance-related pro-inflammatory cytokine production in hepatocytes. *Inflamm Res* 2012; 61: 349–358. doi:10.1007/s00011-011-0417-3.
35. Yu J, Shen J, Sun TT, et al. Obesity, insulin resistance, NASH and hepatocellular carcinoma. *Semin Cancer Biol* 2013; 23: 483–491. doi:10.1016/j.semcancer.2013.07.003.
36. Nieto-Vazquez I, Fernández-Veledo S, Krämer DK, et al. Insulin resistance associated to obesity: the link TNF-alpha. *Arch Physiol Biochem* 2008; 114: 183–194. doi:10.1080/13813450802181047.
37. Kashyap SR, Ioachimescu AG, Gornik HL, et al. Lipid-induced insulin resistance is associated with increased monocyte expression of scavenger receptor CD36 and internalization of oxidized LDL. *Obesity (Silver Spring)* 2009; 17: 2142–2148. doi:10.1038/oby.2009.179.
38. Araya J, Rodrigo R, Videla LA, et al. Increase in long-chain polyunsaturated fatty acid n–6/n–3 ratio in relation to hepatic

steatosis in patients with non-alcoholic fatty liver disease. *Clin Sci (Lond)* 2004; 106: 635–643. doi:10.1042/cs20030326.
39. Hamel FG, Bennett RG, Upward JL, et al. Insulin inhibits peroxisomal fatty acid oxidation in isolated rat hepatocytes. *Endocrinology* 2001; 142: 2702–2706. doi:10.1210/endo.142.6.8178.
40. Machado MV and Diehl AM. Pathogenesis of nonalcoholic steatohepatitis. *Gastroenterology* 2016; 150: 1769–1777. doi:10.1053/j.gastro.2016.02.066.
41. Zhang X, Shen J, Man K, et al. CXCL10 plays a key role as an inflammatory mediator and a non-invasive biomarker of non-alcoholic steatohepatitis. *J Hepatol* 2014; 61: 1365–1375. doi:10.1016/j.jhep.2014.07.006.
42. Zhang X, Han J, Man K, et al. CXC chemokine receptor 3 promotes steatohepatitis in mice through mediating inflammatory cytokines, macrophages and autophagy. *J Hepatol* 2016; 64: 160–170. doi:10.1016/j.jhep.2015.09.005.
43. Yu J, Ip E, Dela Peña A, et al. COX-2 induction in mice with experimental nutritional steatohepatitis: Role as pro-inflammatory mediator. *Hepatology* 2006; 43: 826–836. doi:10.1002/hep.21108.
44. Tilg H. The role of cytokines in non-alcoholic fatty liver disease. *Dig Dis* 2010; 28: 179–185. doi:10.1159/000282083.
45. Tilg H, Moschen AR and Szabo G. Interleukin-1 and inflammasomes in alcoholic liver disease/acute alcoholic hepatitis and nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *Hepatology* 2016; 64: 955–965. doi:10.1002/hep.28456.
46. Wu R, Nakatsu G, Zhang X, et al. Pathophysiological mechanisms and therapeutic potentials of macrophages in non-alcoholic steatohepatitis. *Expert Opin Ther Targets* 2016; 20: 615–626. doi:10.1517/14728222.2016.1125883.
47. Mridha AR, Wree A, Robertson AAB, et al. NLRP3 inflammasome blockade reduces liver inflammation and fibrosis in experimental NASH in mice. *J Hepatol* 2017; 66: 1037–1046. doi:10.1016/j.jhep.2017.01.022.
48. Lieber CS, Leo MA, Mak KM, et al. Model of nonalcoholic steatohepatitis. *Am J Clin Nutr* 2004; 79: 502–509.
49. Eccleston HB, Andringa KK, Betancourt AM, 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: 447–459. doi:10.1089/ars.2010.3395.
50. Jensen T, Abdelmalek MF, Sullivan S, et al. Fructose and sugar: A major mediator of non-alcoholic fatty liver disease. *J Hepatol* 2018; 68: 1063–1075. doi:10.1016/j.jhep.2018.01.019.
51. Ioannou GN. The role of cholesterol in the pathogenesis of NASH. *Trends Endocrinol Metab* 2016; 27: 84–95. doi:10.1016/j.tem.2015.11.008.
52. Abdelmalek MF, Suzuki A, Guy C, et al. Increased fructose consumption is associated with fibrosis severity in patients with nonalcoholic fatty liver disease. *Hepatology* 2010; 51: 1961–1971. doi:10.1002/hep.23535.
53. Ouyang X, Cirillo P, Sautin Y, et al. Fructose consumption as a risk factor for non-alcoholic fatty liver disease. *J Hepatol* 2008; 48: 993–999. doi:10.1016/j.jhep.2008.02.011.
54. Kohli R, Kirby M, Xanthakos SA, 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 non-alcoholic steatohepatitis. *Hepatology* 2010; 52: 934–944. doi:10.1002/hep.23797.
55. Lim JS, Mietus-Snyder M, Valente A, et al. The role of fructose in the pathogenesis of NAFLD and the metabolic syndrome. *Nat Rev Gastroenterol Hepatol* 2010; 7: 251–264. doi:10.1038/nrgastro.2010.41.
56. Subramanian S, Goodspeed L, Wang S, et al. Dietary cholesterol exacerbates hepatic steatosis and inflammation in obese LDL receptor-deficient mice. *J Lipid Res* 2011; 52: 1626–1635. doi:10.1194/jlr.M016246.
57. Savard C, Tartaglione EV, Kuver R, et al. Synergistic interaction of dietary cholesterol and dietary fat in inducing experimental steatohepatitis. *Hepatology* 2013; 57: 81–92. doi:10.1002/hep.25789.
58. Wouters K, van Gorp PJ, Bieghs V, et al. Dietary cholesterol, rather than liver steatosis, leads to hepatic inflammation in hyperlipidemic mouse models of nonalcoholic

steatohepatitis. *Hepatology* 2008; 48: 474–486. doi:10.1002/hep.22363.
59. Van Rooyen DM, Larter CZ, Haigh WG, et al. Hepatic free cholesterol accumulates in obese, diabetic mice and causes nonalcoholic steatohepatitis. *Gastroenterology* 2011; 141: 1393–1403, 1403.e1-5. doi:10.1053/j.gastro.2011.06.040.
60. Anstee QM and Goldin RD. Mouse models in non-alcoholic fatty liver disease and steatohepatitis research. *Int J Exp Pathol* 2006; 87: 1–16. doi:10.1111/j.0959-9673.2006.00465.x.
61. Kanuri G and Bergheim I. In vitro and in vivo models of non-alcoholic fatty liver disease (NAFLD). *Int J Mol Sci* 2013; 14: 11963–11980. doi:10.3390/ijms140611963.
62. Yamada T, Obata A, Kashiwagi Y, et al. Gd-EOB-DTPA-enhanced-MR imaging in the inflammation stage of nonalcoholic steatohepatitis (NASH) in mice. *Magn Reson Imaging* 2016; 34: 724–729. doi:10.1016/j.mri.2016.03.009.
63. Lau JK, Zhang X and Yu J. Animal models of non-alcoholic fatty liver disease: current perspectives and recent advances. *J Pathol* 2017; 241: 36–44. doi:10.1002/path.4829.
64. Larter CZ, Yeh MM, Williams J, et al. MCD-induced steatohepatitis is associated with hepatic adiponectin resistance and adipogenic transformation of hepatocytes. *J Hepatol* 2008; 49: 407–416. doi:10.1016/j.jhep.2008.03.026.
65. Kirsch R, Clarkson V, Shephard EG, et al. Rodent nutritional model of non-alcoholic steatohepatitis: species, strain and sex difference studies. *J Gastroenterol Hepatol* 2003; 18: 1272–1282.
66. Leclercq IA, Farrell GC, Sempoux C, et al. Curcumin inhibits NF-kappaB activation and reduces the severity of experimental steatohepatitis in mice. *J Hepatol* 2004; 41: 926–934. doi:10.1016/j.jhep.2004.08.010.
67. Dela Pena A, Leclercq I, Field J, et al. NF-kappaB activation, rather than TNF, mediates hepatic inflammation in a murine dietary model of steatohepatitis. *Gastroenterology* 2005; 129, 1663–1674. doi:10.1053/j.gastro.2005.09.004.
68. McCuskey RS, Ito Y, Robertson GR, et al. Hepatic microvascular dysfunction during evolution of dietary steatohepatitis in mice. *Hepatology* 2004; 40: 386–393. doi:10.1002/hep.20302.
69. Ip E, Farrell G, Hall P, et al. Administration of the potent PPARalpha agonist, Wy-14,643, reverses nutritional fibrosis and steatohepatitis in mice. *Hepatology* 2004; 39: 1286–1296. doi:10.1002/hep.20170.
70. Roebuck KA and Finnegan A. Regulation of intercellular adhesion molecule-1 (CD54) gene expression. *J Leukoc Biol* 1999; 66: 876–888.
71. Machado MV, Michelotti GA, Xie G, et al. Mouse models of diet-induced nonalcoholic steatohepatitis reproduce the heterogeneity of the human disease. *PloS One* 2015; 10: e0127991, doi:10.1371/journal.pone.0127991.
72. Rinella ME and Green RM. The methionine-choline deficient dietary model of steatohepatitis does not exhibit insulin resistance. *J Hepatol* 2004; 40: 47–51.
73. Sahai A, Malladi P, Pan X, et al. Obese and diabetic db/db mice develop marked liver fibrosis in a model of nonalcoholic steatohepatitis: role of short-form leptin receptors and osteopontin. *Am J Physiol Gastrointest Liver Physiol* 2004; 287: G1035–G1043. doi:10.1152/ajpgi.00199.2004.
74. Sanches SC, Ramalho LN, Augusto MJ, et al. Nonalcoholic steatohepatitis: a search for factual animal models. *Biomed Res Int* 2015; 2015: 574832, doi:10.1155/2015/574832.
75. Trak-Smayra V, Paradis V, Massart J, et al. Pathology of the liver in obese and diabetic ob/ob and db/db mice fed a standard or high-calorie diet. *Int J Exp Pathol* 2011; 92: 413–421. doi:10.1111/j.1365-2613.2011.00793.x.
76. Leclercq IA, Farrell GC, Schriemer R, et al. Leptin is essential for the hepatic fibrogenic response to chronic liver injury. *J Hepatol* 2002; 37: 206–213.
77. Yang SQ, Lin HZ, Lane MD, et al. Obesity increases sensitivity to endotoxin liver injury: implications for the pathogenesis of steatohepatitis. *Proc Natl Acad Sci U S A* 1997; 94: 2557–2562.
78. Paz-Filho G, Mastronardi C, Delibasi T, et al. Congenital leptin deficiency: diagnosis and effects of leptin replacement therapy. *Arq Bras Endocrinol Metabol* 2010; 54: 690–697.

79. Arsov T, Silva DG, O'Bryan MK, et al. Fat Aussie—a new Alström syndrome mouse showing a critical role for ALMS1 in obesity, diabetes, and spermatogenesis. *Mol Endocrinol* 2006; 20: 1610–1622. doi:10.1210/me.2005-0494.
80. Arsov T, Larter CZ, Nolan CJ, et al. Adaptive failure to high-fat diet characterizes steatohepatitis in Alms1 mutant mice. *Biochem Biophys Res Commun* 2006; 342: 1152–1159. doi:10.1016/j.bbrc.2006.02.032.
81. Farrell GC, Mridha AR, Yeh MM, et al. Strain dependence of diet-induced NASH and liver fibrosis in obese mice is linked to diabetes and inflammatory phenotype. *Liver Int* 2014; 34: 1084–1093. doi:10.1111/liv.12335.
82. Larner DP, Morgan SA, Gathercole LL, et al. Male 11beta-HSD1 knockout mice fed trans-fats and fructose are not protected from metabolic syndrome or nonalcoholic fatty liver disease. *Endocrinology* 2016; 157: 3493–3504. doi:10.1210/en.2016-1357.
83. Asgharpour A, Cazanave SC, Pacana T, et al. A diet-induced animal model of non-alcoholic fatty liver disease and hepatocellular cancer. *J Hepatol* 2016; 65: 579–588. doi:10.1016/j.jhep.2016.05.005.
84. Morrison MC, Verschuren L, Salic K, et al. Obeticholic acid modulates serum metabolites and gene signatures characteristic of human NASH and attenuates inflammation and fibrosis progression in Ldlr-/-.Leiden mice. *Hepatol Commun* 2018; 2: 1513–1532. doi:10.1002/hep4.1270.
85. Tsuchida T, Lee YA, Fujiwara N, et al. A simple diet- and chemical-induced murine NASH model with rapid progression of steatohepatitis, fibrosis and liver cancer. *J Hepatol* 2018; 69: 385–395. doi:10.1016/j.jhep.2018.03.011.