Review Article
High fat diet mouse model in the study of nonalcoholic fatty liver disease and hepatocellular carcinoma

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Abstract: Nonalcoholic fatty liver disease (NAFLD) is considered the hepatic manifestation of metabolic syndrome and is characterized by excessive triglyceride (TG) accumulation in the absence of "significant" alcohol consumption and is closely associated with metabolic dysregulation such as obesity, diabetes, hyperlipidemia, and cardiovascular disease. NAFLD is composed of a spectrum of liver pathology ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), which has been identified as an important risk factor for the development of hepatocellular carcinoma (HCC) and cirrhosis. Long-term high-fat (HF) diet consumption is one of the risk factors of NAFLD and feeding HF diet is widely used to produce hepatic steatosis and NASH in experimental animals. In this review, we focused on the current application of HF diet mouse model on the study of NAFLD/NASH and HCC.

Introduction

NAFLD is considered the hepatic manifestation of metabolic syndrome [1], affecting up to 20% of the population in Western countries, and 70-80% of obese individuals. Nonalcoholic fatty liver disease (NAFLD) is characterized by excessive triglyceride (TG) accumulation in the absence of "significant" alcohol consumption and is closely associated with metabolic dysregulation such as obesity, diabetes, hyperlipidemia, and cardiovascular disease [2-4]. NAFLD is composed of a spectrum of liver pathology ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), characterized of hepatocyte injury, inflammation, and fibrosis on liver biopsy [5]. NASH has been identified as an important risk factor for the development of hepatocellular carcinoma (HCC) and cirrhosis [6-9].

The prevalence of NAFLD rises in parallel with the growing epidemics of obesity and diabetes. However, the causal relationships between obesity, diabetes, NASH and liver tumorigenesis have not yet been clearly elucidated. Long-term high-fat (HF) diet consumption is one of the risk factors of NAFLD [6-9] but the mechanism is still uncertain. HF diets used in laboratory research typically contain about 32 to 60% of calories from fat, which reflects the typical western diets. Although a human diet of 60 kcal% fat would be considered extreme, diets with 60 kcal% fat are commonly used to induce obesity in rodents since animals tend to gain more weight and to display the phenotype more quickly after a shorter period of time [10]. It is generally believed that oxidative stress, mitochondrial dysfunction, and increased production of proinflammatory cytokines-combined with insulin resistance-eventually leads to the fatty degeneration in liver [11]. Feeding HF diet is widely used to produce hepatic steatosis and NASH in experimental animals. Animal models of NAFLD/NASH are used not only to elucidate the pathogenesis of NAFLD, but also to examine therapeutic effects of various agents [12]. Animal models of NAFLD include genetic and nutritional models. However, to date, there is no one model that ideally reflects hepatic histopathology and pathophysiology of human NAFLD.

Table 1. High-fat Diet Wildtype Mouse Model for NAFLD and HCC

Species	Gender	Diet	Chemical inducers	Phenotype	Reference
C57BL/6J	male	42%-45% HF < 16 weeks	N/A	Obesity, hepatic steatosis and inflammation	[14, 20]
C57BL/6J	male	42-45% HF ≥ 16 weeks	N/A	Obese, liver inflammation and severe hepatic steatosis; liver fibrosis dependent on length of the treatment	[14, 20, 23, 24, 41, 56]
C57BL/6J	male	maternal 35% HF diet till weaning, postweaning NF diet < 16 weeks	N/A	Primes steatohepatitis, mitochondrial dysfunction and altered lipogenesis gene expression	[30]
C57BL/6J	male	maternal 45% or 60% HF diet till weaning, postweaning 45% or 60% HF diet ≥ 16 weeks	N/A	Insulin resistance, NASH, significantly fibrosis depending on length of postnatal HF diet treatment	[25, 29]
NMRI	male	maternal 60% HF diet till weaning, postweaning NF diet for 9 months	NA	Insulin resistance, hyperleptinemia, hyperuricemia and hepatic steatosis	[27]
Neonatal C57BL/6J	male	32% HF for 16 weeks	STZ at day 2	Developed liver steatosis with diabetes to tumor protrusion in liver	[54]
C57BL/6J	male	40%-60% HF > 6 mon	N/A	Aggravated hepatic steatosis, in the liver, part of the mice developed tumor observed on the liver	[14, 33, 35]
C57BL/6	male	60% HF + oxLDL for 23 weeks	N/A	Obesity, NASH, liver inflammation	[34]
C57BL/6	male	45% HF + high fructose for one year	N/A	Features of early NASH at 6 months, liver inflammation and bridging fibrosis and tumor at 12 month	[41]
C57BL/6	male	60% fat + 10/20% EtOH for 42 weeks	DEN	HCC developed in DEN + HF nice, EtOH-feeding did not did not impact HCC incidence or tumor size.	[49, 50]
C57BL/6J	male	60% HF + APO10LA supplementation	DEN	Reduce the HF diet-promoted tumor multiplicity and volume	[51]

Table 2. Highfat-Diet Transgenic Mouse Model for NAFLD and HCC

Species	Gender	Diet	Chemical inducers	Phenotype	Reference
Adiponectin KO mice	male	60% HF diet for 48 weeks	N/A	Observe pericellular fibrosis around central veins	[36]
MC4R-KO	male	60% HF diet for one year	N/A	Liver inflammation, pericellular fibrosis, well-differentiated HCC after 1 year.	[37]
MPO deficient mice	male	HC-diet containing 0.2% cholesterol and 21% butter for 8 weeks	N/A	Attenuates the development of NASH and diminishes adipose tissue inflammation	[38]
Nrf2-null mice	male	45% HF diet for 24 weeks	N/A	Better insulin sensitivity more severe NASH, with cirrhosis	[39]
Irs1 KO mice	male	HF diet for more than 1 year (60 wks)	N/A	Severe insulin resistance but mild NASH and liver tumourigenesis	[42]
Irs1 KO mice	male	32% HF diet for one year	DEN	Ameliorated liver function and decreased tumor incidence	[55]
MC4R-KO mice	male	60% HF diet for one year	N/A	Obesity, insulin resistance, dyslipidemia, well-differentiated HCC	[37]

In this review, we focused on the current application of HF diet mouse model to the study of NAFLD/NASH and HCC (summarize in **Tables 1** and **2**).

Mouse models and HF diet induced NAFLD/NASH

One good reason to use the HF diet induced NAFLD rodent model is that HF diets can induce obesity, insulin resistance, fasting hyperglycemia, dyslipidemia, and altered adipokine profile, which are commonly observed in NAFLD patients [13, 14]. Outcomes of the HF-diet on NAFLD are varied depending on the degrees of steatosis, inflammation and fibrosis, which might depend on the rodent strain, the fat content of the diet, the composition of the dietary fat, and the duration of treatment [12, 15].

Although there has been no standardized diet reported for the study of NAFLD, it is widely accepted that the type and amount of fat, as well as total daily caloric intake are very important. So far, the highest amount of fat which has been applied to a rodent model is 71% of energy from fat (11% carbohydrate and 18% protein), and the fat source includes corn, olive, and safflower oils [16]. After a three-week exposure to this diet, rats developed panlobular steatosis and displayed abnormal mitochondria and mononuclear inflammation, accompanied by increased gene expression of *TNF-α* and *CYP2E1*, compared to control rats fed with the standard Lieber-DeCarli diet (35% fat, 47% carbohydrates, and 18% protein) [16]. In another study, HF emulsion diet composed of 77% of its energy from fat, 14% from total milk powder, and 9% from carbohydrates treatment on male Sprague-Dawley rats for 6 weeks was able to induce hyperlipoidemia, hyperinsulinemia, hyperglycemia and insulin resistance. Moreover, morphological evaluation revealed that rats developed hepatic steatosis, inflammation and mitochondrial lesions [17].

NAFLD/NASH researchers have been using the C57BL/6J mice model not only because they are genetically easily manipulated, but also because they are predisposed to develop insulin resistance quickly by means of diet [18-22]. Pan et al have treated these mice with a HF diet containing 42% fat for 24 weeks and observed increase hepatic inflammation marked by increased expression of pro-inflammatory cytokines such as *TNFα, MCP-1, IL-6*, and *IL-18*, and higher ratio of M1 to M2 gene expression [23].

Since many rodent models fail to replicate both metabolic syndrome (MetS) and NASH, Mells et al developed a mouse model of NASH and MetS using a solid diet containing 0.2% cholesterol, 45% calories from fat, and 30% of fat in the form of partially hydrogenated vegetable oil, combined with high-fructose corn syrup equivalent in water. After a 16-week treatment, these mice developed fibrosis and significantly increased serum leptin, IL-6, and liver α-SMA expression [24].

It is recently reported that maternal HF-diet causes liver dysfunction in male offspring mice [25-30]. In the model, pregnant mice were fed with a HF diet (45% kcal fat, 20% kcal protein, 35% kcal carbohydrate) or a standard chow diet (21% kcal fat, 17% kcal protein, 63% kcal carbohydrate) until weaning. At 30 weeks, offspring male mice developed NAFLD by histological analysis, and Kleiner proposed these mice develop insulin resistance [25]. A similar study applied an AIN-93G modified for HF (35%) content, 14-week-old offspring showed NAFAD with increased c-Jun N-terminal kinases (JNK), I kappa B kinase phosphorylation, PEPCK expression in the liver [30].

HF diet and liver fibrosis

Although simple steatosis alone is relatively benign, the presence of steatohepatitis greatly increases the risk of progression to cirrhosis [9, 31]. If not properly treated, progression of NASH leads to fibrosis and cirrhosis, as well as hepatocellular carcinoma in humans over many years [6, 7, 32]. Various animal models have established that exclusive, long-term (e.g. 12-weeks) HF diets produced NAFLD/NASH, insulin resistance, and obesity in mice; however, HF diets may not induce liver fibrosis until after more than one year of treatment. In one study, wildtype mice were fed with a high-cholesterol (HC) diet (15% milk fat, 1.5% cholesterol and 0.1% cholic acid, w/w) for 25 or 55 weeks. At week 25, these mice exhibited hypercholesterolaemia, and developed hepatic steatosis and gallstones. Till week 55, all of the mice treated with HC diet developed mild fibrosis in the liver [33]. In another study, aggravated hepatic steatosis, fibrosis, and lipid metabo-

lism were observed as early as week 21 when wildtype mice were fed with a high-fat diet (HFD) combined with oxidized low-density lipoproteins (oxLDL) [34]. These results suggest that fat composition of the diet might be more critical to induce liver fibrosis than the fat content of the diet. Different genetic backgrounds do not seem to differentially affect the outcome of HF diet induced liver fibrosis: when the C57BL/6 (BL6) and 129/SVJ mice were fed with HF high-sucrose diet [fat 35%, sucrose 40%, total carbohydrate 9% (other than sucrose) and protein 16%] for 6 months, both strains developed patchy sinusoidal fibrosis, but neither developed bridging fibrosis [35].

Although HF diet alone is seldom used in the study of liver fibrosis, it is quite often applied in genetically manipulated mice to investigate the important roles of key regulatory proteins in the progression of steatohepatitis, since a HF diet is regarded to positively relate with the progression of NASH to fibrosis. Adiponectin knockout (KO) mice were fed a HF diet containing 60% calories from fat for 24 and 48 weeks. At 48 weeks, pericellular fibrosis around central veins was observed in KO mice on the HF diet but not in wildtype mice on the HF diet [36]. In another study, melanocortin 4 receptor-deficient mice (MC4R-KO) fed with HF diet containing 60% calories from fat for 20 weeks displayed inflammatory cell infiltration, hepatocyte ballooning, and pericellular fibrosis in the liver [37]. To investigate how myeloperoxidase (MPO) contributes to the development and progression of NASH, $LDLR^{-/-}$; $MPO^{-/-}$ mice were generated by adoptively transferring bone marrow cells of $MPO^{-/-}$ to $LDLR^{-/-}$ mice. MPO deficient mice had less hepatic cholesterol accumulation, inflammation, and potentially fibrosis in response to an HC-diet containing 0.2% cholesterol and 21% butter for 8-weeks [38]. Interestingly, mice lacking the transcription factor NF-E2 p45-related factor 2 (Nrf2) exhibited better insulin sensitivity when fed a HF diet (45% fat) for 24 weeks than wildtype mice. However, they developed more severe NASH with cirrhosis [39, 40]. This result suggested the molecular mechanisms underlying the progression of NASH are independent of insulin resistance and involve mitochondrial oxidative stress as well as disruption of metabolic enzymes.

HF diet and tumorigenesis

It is widely accepted that NAFLD is a risk-factor for HCC. Therefore, it is of great significance to understand the association between HF diets and HCC. The effect of long-term HF diets leading to the development of NASH and liver tumorigenesis has been tested in in the American Lifestyle-Induced Obesity Syndrome (ALIOS) model [41]. These mice developed features of early nonalcoholic steatohepatitis at 6 months and features of more advanced nonalcoholic steatohepatitis at 12 months, including liver inflammation and bridging fibrosis. After one year on a HF diet, hepatocellular neoplasms were observed in 6 of 10 ALIOS mice. In another study, C57BL/6J male mice were fed on the HF diet composed of 22% saturated fatty acids (12.6% palmitic acid, 7.5% stearic acid) and 77% unsaturated fatty acids (64.3% oleic acid, 10.2% linoleic acid) [42]. After 60 weeks on the HF diet, all of the mice exhibited typical features of NASH, and 54% of the mice developed tumors observed on the liver surface; however, fully developed cirrhosis was rarely seen in this model. Although it is reported that hepatocellular carcinoma occurs at a rate of 1% to 4% per year after cirrhosis is established [6, 7, 32, 43], hepatocellular carcinomas (HCCs) without cirrhosis is not unusual in either humans or mice [44-46]. This result suggests that liver cirrhosis may not be a prerequisite for the development of liver tumorigenesis, especially in the presence of NASH. Consistently, a comparison study using males of two inbred strains of mice investigating the long-term effects of HF diet on liver tumorigenesis suggested a strain-diet interaction during development of HCCs [47]. The study reported that C57BL/6J but not A/J males were susceptible to NASH and HCC, and it showed involvement of Myc and NFκB signaling pathways in the HCC development.

Various studies have been focused on elucidating the association between HF diets and the progression of NASH and the initiation of HCC. However, the findings are not very consistent. Duan et al. has investigated the how a HF diet that contains 2% cholesterol and 10% lard oil affected the hepatocarcinogenesis induced by administration of diethylnitrosamine (DEN) [48]. It is interesting that this HF diet attenuated DEN-related malnutrition and fibrosis progression, in comparison with the control diet from week 10 to week 14: mice fed with HF diet developed well-differentiated HCC, and the number as well as size of tumors was much lower. In another study, DEN induced HCC for-

mation was observed in 89% of mice fed with HF diet (60% fat) for 37 weeks, while HCC formation was only found in 60% of mice fed with a control diet (10% fat) [49]. Surprisingly, EtOH-feeding (10% in drinking water) for 7 weeks did not show adjunctive impact on HCC incidence or tumor size, which is contradictory to another mouse model fed with a HF diet (60% fat) in which hepatic tumor formation was initiated by intrahepatic Hepa1-6 cell inoculation [50]. APO10LA supplementation (10 mg/kg diet) for 24 weeks was reported to significantly reduce the DEN-induced and HF diet-promoted tumor multiplicity and volume [51].

Several cohort studies have shown that diabetes mellitus and insulin resistance are risk factors of HCC [6-9, 32, 52, 53]. However, how diabetes and insulin resistance contribute to HCC development remains unknown. Neonatal male C57BL/6J mice were exposed to low-dose streptozotocin (STZ) by a single subcutaneous injection and were fed on HF diet [54]. These mice developed liver steatosis with diabetes following one week of feeding HFD to them, and they displayed tumor protrusion in their livers at 20 weeks. However, male mice treated with STZ alone showed diabetes but never developed HCC. This result suggests that a HFD promotes diabetic populations to accelerate the development of HCC. It is possible that prolonged HFD, rather than the presence of diabetes or insulin resistance, contributes to the progression of hepatic steatosis. As evidence, *Irs1* KO mice subjected to an HFD for 60 weeks were dramatically protected against NASH and liver tumourigenesis despite the presence of severe insulin resistance, in comparison to the wildtype mice [42]. Consistent with this report, *Irs1* KO mice exposed to the HF diet and DEN showed ameliorated liver function and decreased tumor incidence versus wildtype mice [55], suggesting an important role of *Irs1* in hepatic tumorigenesis. Similarly, the MC4R-KO mice, with phenotypes of obesity, insulin resistance, and dyslipidemia, developed well-differentiated HCC after being fed a HFD for 1 year [37].

Summary

These studies demonstrated that HF diets are very critical in inducing the NAFLD/NASH and in promoting the progression of HCC. Mouse models of HF diets are widely used in the research of NAFLD, are associated with liver diseases, and are valuable tools in understanding the molecular mechanisms and the pathophysiological processes involved in NAFLD and its development from NASH to HCC. To better understand and reflect the histopathology and pathophysiology of human NAFLD/NASH and HCC, it will be important to determine the most effective mouse models and the best experimental designs for the specific aims of this research. For example, when studying the effects of a drug, nutraceutical, or gene mutation on NAFLD, a HFD with 30% to 40% fat might be preferred because a very high fat content, such as 60% HFD, might leads to a more severe phenotype with is difficult to prevent or reverse. For another example, a transgenic model combined with HFD, or a HFD model combined with carcinogen treatment is more common used to induce robust HCC phenotype that is independent of the age effect, although more than 1 year HFD is also able to induce HCC.

Future studies using HFD mouse models will obtain valuable knowledge about NAFLD and HCC, which will eventually lead to effective treatment strategies for these diseases. Genetically engineered mouse models and transplant models combined with HFD are certainly an essential tool to increase our understanding of HFD-related NAFLD and HCC and to represent a valuable source for mechanism-based therapy development.

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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