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Of Mice and Men and Nonalcoholic Steatohepatitis

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Mouse models of nonalcoholic fatty liver disease (NAFLD) are required to both elucidate the pathogenesis of the disease and also to assess therapeutic interventions. The first criterion for a useful model is that it reproduces the histopathology of the disease. NAFLD is characterized by centrilobular and macrovesicular steatosis. In nonalcoholic steatohepatitis (NASH), the steatosis is accompanied by intralobular inflammation and hepatocellular ballooning. Mallory's hyaline (eosinophilic, amorphous structures in the cytoplasm) may be present. There may also be glycogenated hepatic nuclei, megamitochondria, iron deposition, and fibrosis. Fibrosis usually originates in the perisinusoidal region of zone 3. As disease progresses, bridging fibrosis and then cirrhosis develop. Given that the stage of fibrosis is the most important predictor of liver morbidity and mortality in patients with NASH, it is crucial that this aspect of the histopathology is prominent in the mouse model.

Second, a mouse model of NAFLD should reflect the pathophysiology of human NAFLD. NAFLD is regarded as the hepatic manifestation of metabolic syndrome. The concomitant abnormalities accompanying NAFLD are central obesity, insulin resistance, and hyperlipidemia. The pathogenic mechanisms associated with NAFLD are oxidative stress, inflammatory cytokines, lipotoxicity, increased free cholesterol, hyperinsulinemia, hyperleptinemia, and hypoadiponectinemia. In addition, a useful mouse model should be robust, inexpensive, advance to inflammation and fibrosis in a short time course, and respond to therapeutic interventions in a manner similar to patients with NASH.

NAFLD results from an interaction of the host genetics with the environment. In a similar way, a useful mouse model of NAFLD needs to assess multiple aspects that have been shown to be important in human NAFLD. This includes, but is not limited to, the diet, mouse strain, transgenes, microbiome, ambient temperature, circadian rhythm, and physical activity. The first approach to developing a mouse model of NASH was to assess different diets. The original diets of methionine-choline-deficient diet (MCD) and choline-deficient amino-acid-supplemented diet (CDAA) led to histological NASH with fibrosis, but did not result in weight gain or insulin resistance. Due to this failure to replicate human pathophysiology, these diets have been largely discontinued. Next, there are high-fat (HF) diets, which frequently lead to NAFL in mice, but without much inflammation or fibrosis. To this was added additional nutritional elements including high-cholesterol and high-fructose leading to Western diets (HFHCHF). The cholesterol in the diet varies from 0.2%,

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which is generally considered high cholesterol, to 2%, which is considered an atherogenic diet that is not compatible with human intake of cholesterol. With these modifications, the Western diet has resulted in the development of NASH with mild fibrosis under some circumstances (reviewed in Ibrahim et al.⁽¹⁾).

Perhaps one of the biggest surprises was how different strains of mice respond to an HF or Western diet with respect to hepatic steatosis (HS) and NASH with fibrosis. For example, an isogenic strain cross B6/129 develop NASH with fibrosis after 16 weeks on a Western diet, which is more rapid than comparison strains.⁽²⁾ The current study⁽³⁾ elegantly demonstrated the importance of mouse strains by feeding a Western diet to over 100 mouse strains. Mice that developed steatosis or NASH with fibrosis varied greatly across strains, such as HMDP mice developing HS without fibrosis and BXD19/Ty mice developing hepatic fibrosis.⁽³⁾

Many models of NASH use transgenic mice to improve the model. One approach is to use mutations that make mice hyperphagic so that they become obese and develop NASH more quickly. OB/OB mice (leptin deficient) and DB/DB mice (leptin receptor deficient) are hyperphagic and develop NAFLD. However, a concern is that leptin itself is profibrogenic, so that its defect limits the model. foz/foz mice (mutated ALMS1 gene) are hyperphagic and develop NASH with fibrosis on a Western diet.⁽⁴⁾ Patients with a mutation in this gene are also insulin resistant with NAFLD. Another approach to genetic mouse models renders mice more sensitive to Western diets or to a hepatocyte injury. These include transgenic mice expressing SREBP1-c or MUP-uPA, or knockout mice with gene deletions in PPAR alpha, MAT1A, PTEN, or AOX. The criticism of these models is that although they generate the liver pathology of NASH, the pathogenic pathways are different than in patients. A third approach to genetic models “humanizes” mice by changing the genetics to a human transgene. These approaches include expressing the transgene for human cytochrome P4502E1, the knockin of the human risk single-nucleotide polymorphism generating I148M into PNPLA3⁽⁵⁾ and, in the present study, the transgenes for human APOE*3-LEIDEN and CETP. In each case, the goal is to change the mouse into a more human pathophysiological handling of lipids and their products to more closely reflect human NASH.

The gut microbiome influences obesity and NAFLD in both humans and in colonized mouse models. In particular, fecal microbiota transplants from patients with NAFLD will confer the NASH phenotype to gnotobiotic mice on a Western diet as compared to fecal transplants from matched donors without NASH.⁽⁶⁾ Thus, a mouse model of NAFLD should be colonized with the proper microbiome.

The circadian rhythm influences the effect of the same diet on the weight and metabolism of mice.⁽⁷⁾ Although less well studied, the circadian rhythm also seems to modulate weight in people. Thus, the circadian rhythm, including the time restriction of feeding, could be incorporated into mouse models of NAFLD.

The thermoneutral temperature for mice is 30-32°C as opposed to standard housing of 20-23°C, a range chosen primarily for human comfort in the mouse house. However, housing mice at their thermoneutral temperature results in a more severe HF-diet-induced

NAFLD, particularly for female mice as compared to standard temperature.⁽⁸⁾ Thus, a more rational robust model of NAFLD may consider using thermoneutral housing at 30-32°C.

Different types of exercise training decrease HS in patients with NAFLD.⁽⁹⁾ The effect is mediated through both weight loss and through a weight-independent effect. Thus, a model of NASH may consider restricting physical activity of mice.

Some mouse models have used an additional injury to accelerate and extend the liver pathology. These models have included the STAM model in which streptozocin-induced beta-cell death produces insulin deficiency and type 1 diabetes, rendering mice more sensitive HS and inflammation on an HF diet. Another model uses a combination of HF diet and chronic CCl₄ administration. These models produce advanced NASH pathology with fibrosis, but the pathogenic mechanisms are different from human NAFLD.

Perhaps the best assessment will be when a therapeutic intervention can be shown to produce comparable results in both patients and in mouse models of NAFLD. In the meantime, many models are using a combination of genetics and environment. One can imagine developing a more robust model using a Western diet with a defined microbiome, in a thermoneutral housing, with the proper circadian rhythm in a mouse humanized by appropriate transgenes (see Fig. 1). Eventually, perhaps each patient could have an individualized avatar mouse reflecting the patient's genetic risk factors in order to assess therapies using precision medicine.

Abbreviations:

HF	high fat
HS	hepatic steatosis
NAFLD	nonalcoholic fatty Liver disease
NASH	nonalcoholic steatohepatitis

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FIG. 1.

Development of a mouse model of NASH. The model is optimized to reflect the pathology and pathophysiology of human NASH and then used to assess the effectiveness of individualized therapies.