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Modeling Fatty Liver Disease in Animals: Is There an Optimal Approach, and Is the Effort Worthwhile?

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Animal research on nonalcoholic fatty liver disease (NAFLD) is plagued by a lack of standardization in the methods used to achieve steatosis and steatohepatitis (SH). Over the last 15 years, investigators have had at their disposal (1) genetically engineered mice with predispositions to obesity and fatty liver, (2) an extensive menu of high-energy diets featuring different sugars and fats, and (3) other "tricks" such as elimination of choline or methionine/choline from the diet that accelerates and accentuates the disease process. Although much has been learned from the use of these tools individually and in combination, it remains uncertain whether they have truly brought us closer to an understanding of human NAFLD. Recognizing this dilemma, researchers have gradually moved away from disease models that produce SH but do not achieve weight gain or insulin resistance. There has also been a decline in the use of mice with genetic mutations in favor of wild-type mice with diet-induced liver disease. Still, there is tremendous heterogeneity among diets that induce obesity, with many high-fat diets (HFDs) containing 60% fat, 50–100% more than that eaten by the average American. Gaining popularity are diets with more-reasonable carbohydrate: fat ratios of roughly 40:40, which feature nutrients implicated in human NAFLD such as fructose-sweetened drinking water and trans-fat.[1–3] These diets induce SH and hepatic fibrosis when fed for 16 weeks or more.

Inasmuch as fructose- and trans-fat-enriched diets help to induce NAFLD in mice, the secret sauce in the most successful formulas appears to be cholesterol. High-energy diets containing at least 0.2% cholesterol by weight often produce liver disease that progresses beyond steatosis to SH and fibrosis; similar diets without cholesterol typically produce only steatosis. There is currently no clear explanation for this, although cholesterol feeding does increase the cholesterol content of the liver,[3] which has been observed in humans with nonalcoholic steatohepatitis (NASH).[4] There remains some debate whether 0.2% cholesterol in rodent diets is physiological or pharmacological. By my calculation, it is pharmacological, given that 0.2% is at least twice the maximum recommended concentration of cholesterol in the human diet (https://health.gov/dietaryguidelines/2015). Above this, one must take into consideration that mice consume 10 times as much food per gram of body weight per day as humans.[5] Thus, the total amount of cholesterol consumed per mouse per day on a 0.2% diet would far exceed that consumed by the average human. This does not negate the value of cholesterol-containing diets, particularly since they appear

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to be producing reliable and reproducible results; it does, however, underscore the need to understand the potential limitations of any disease model.

Knowing that any animal model of human disease has shortcomings, there has been some pushback against the maligning of dietary models that induce NASH but do not reproduce features of metabolic syndrome.[3] I am sympathetic, and have long made the argument that the methionine-choline-deficient (MCD) model of NASH, despite its imperfections, provides valuable information about factors that contribute to severe FLD. For example, studies with MCD-fed mice have yielded important insight into the importance of *de novo* lipogenesis in the pathogenesis of NASH.[6] Through their extreme phenotypes, models such as MCD support proof-of-principle or proof-of-mechanism studies, which then must be validated under conditions more relevant to humans.

As we continue the search for improved animal models of NAFLD, factors other than diet are emerging as important variables. One is eating behavior, specifically, the continuous or episodic nature of food consumption. Recent studies indicate that mice offered an HFD will suppress their natural tendency to eat only at night in favor of consuming food continuously over a 24-hour period.[7] Surprisingly, when mice are forced to eat the same HFD only at night, they consume the same number of calories per day but experience less weight gain and hepatic steatosis than those fed *ad libitum*. This time-restricted feeding has been linked to cyclical fluctuations in the gut microbiome, which may be preventing metabolic dysregulation.[8] This discovery highlights factors that will need to be taken into account when evaluating phenotypic variations among humans at risk for NAFLD. A second variable that likely impacts murine responses to high-energy diets is housing temperature. Mice fed obesogenic diets while in thermoneutral housing (30°C) develop more adipose tissue inflammation than mice housed at standard temperatures (22°C).[9] Thermoneutral housing may also facilitate the development of hepatic steatosis, although evidence to support this remains preliminary.[10] The fact that standard housing temperature constitutes a mild cold exposure for a mouse, which necessitates an increase in energy expenditure to maintain body temperature, may explain in part why mice require prolonged exposure to caloric excesses before displaying evidence of NAFLD.

Before we contemplate expanding NAFLD modeling to include not only dietary formulas, but also control of feeding times and housing temperature, we must ask whether we are fooling ourselves into thinking that any animal model will ever be able to mimic human NAFLD. This was the sobering message conveyed by Teufel et al.,[11] who compared the hepatic transcriptomes of obese humans with and without NASH to those of mice representing five different models of NAFLD (one genetic and four dietary). In their study, principal component analysis showed marked divergence between humans and mice regardless of the NASH model employed. Ironically, the model with the closest transcriptomic connection to human NASH at the single-gene level was the hepatocyte-specific phosphatase and tensin homologue knockout mouse. From a pathway perspective, there was a reasonable resemblance between humans with NAFLD/NASH and mice fed a 40% carbohydrate: 40% fat diet with 0.2% cholesterol. Teufel et al. framed their discovery positively, remarking that their analysis would permit future investigators to make informed decisions about model selection depending upon the disease pathway(s) of interest. Their

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data set, coupled with the knowledge gained from the additional research mentioned above, should move the field toward a more complete understanding of disease pathogenesis.

Thinking back on the last 15 years of animal research on NAFLD, I conclude that researchers have taken the best information from diverse sources and used it to build consensus around the factors involved in disease pathogenesis. Just as the field was beginning to feel a bit smaller as experts have begun to agree on the type of diets that model human NAFLD, other factors surfaced that will need to be taken into account in future studies. Importantly, even the best animal models fall short of faithfully reproducing human NASH. This should prompt us to continue to improve animal models, while at the same time expanding efforts to study NASH pathogenesis directly in humans whenever possible.

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Abbreviations:

HFD	high-fat diet
MCD	methionine-choline deficient
NAFLD	nonalcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
SH	steatohepatitis

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