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Commentary

Challenging the Rodent Hegemony

A New Rabbit Model of Nonalcoholic Steatohepatitis

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Since its initial description in 1980, nonalcoholic fatty liver disease (NAFLD) has become an increasingly important public health problem.[1] It is projected that NAFLD will replace hepatitis C as the most common indication for liver transplantation in the United States within the next 20 years.[2] Increasingly, NAFLD is being recognized as a problem not only in the developed countries but worldwide.[3] A strong association with metabolic syndrome and the increasing prevalence of NAFLD has paralleled the rising epidemics of obesity, insulin resistance, and diabetes mellitus.[4]

While important advances have been made in understanding the pathogenesis of NAFLD, the precise molecular events that lead to its development remain elusive. Among the crucial challenges faced is the difficulty in differentiating the presence of fat in the liver (simple steatosis) from fat associated with hepatocellular injury, inflammation, and fibrosis (steatohepatitis, NASH). The latter condition is associated with progressive fibrosis that can ultimately lead to cirrhosis, with further complications of portal hypertension and hepatocellular carcinoma.[5] It is very difficult to investigate the molecular pathogenesis of NAFLD and NASH in humans because of the heterogeneity of human populations and wide differences in their diet and lifestyle. More importantly, the inability to obtain multiple liver biopsies from patients with NAFLD or NASH and healthy volunteers adds to the difficulty of these studies.

Given these technical issues and associated ethical challenges involved in studying patients with NAFLD, considerable effort has been expended to develop animal models of fatty liver disease. In this issue of the *American Journal of Pathology*, Ogawa et al[6] report another animal model of NAFLD, which utilizes rabbits fed a high-fat diet, resulting in the development of progressive liver fibrosis and hepatic cholesterol accumulation.

Animal Models of Fatty Liver Disease

Ideally, an animal model to study NASH should replicate three important phenotypic characteristics of human disease. First, the animal should have the characteristic metabolic abnormalities, namely, insulin resistance, hyperglycemia, hyperlipidemia, and visceral adiposity. Second, the animals should develop lipid accumulation within hepatocytes along with other histological hallmarks of NASH, such as balloon degeneration and sinusoidal fibrosis. Third, there should be evidence of progressive liver injury in association with continued insult. Currently, no animal model fulfills all three criteria.

Previously, efforts to develop animal models of NAFLD have taken one of three main approaches: genetic models, dietary interventions, or a combination of the two (reviewed in Larter and Yeh[7] as well as in Nanji[8]). Most of these approaches have used rodents as the model animal. Genetic models of NAFLD include mouse models characterized by the spontaneous or targeted mutations that lead to the development of hepatic steatosis. Among the best studied of the models with spontaneous mutations are the leptin-deficient *ob/ob* mouse and leptin-receptor–deficient *db/db* mouse (reviewed in Koteish and Diehl[9]). Other models, such as the liver-specific *Pten* knockout mice or acyl-CoA oxidase knockout mice, have targeted mutations in specific genes important in lipid metabolism leading to the development of steatohepatitis but lack the metabolic features associated with human fatty liver disease.[10,11]

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The second approach of modeling fatty liver disease has been to use dietary interventions to induce hepatic lipid accumulation and abnormal liver histology. Among the most widely used diet to induce steatohepatitis is the methionine-choline deficient (MCD) diet, which causes liver histology reminiscent of human NASH but is paradoxically associated with body weight loss and insulin sensitivity.[12] This difference from the metabolic profile associated with human NASH has become a major criticism of the MCD diet and has limited its utility. To overcome some the drawbacks of the MCD diet, other approaches to induce obesity, insulin resistance, and steatohepatitis have been attempted. Feeding high-fat diet to mice and rats for 6 months or longer or overfeeding via the intragastric route results in obesity, insulin resistance, and steatohepatitis.[13,14]

The third approach to creating animal models of NAFLD has been to combine genetic models with dietary intervention. Examples of this approach including *db/db* mice, ApoE-null mice, Zucker fatty rats (*fa/fa*) with leptin-receptor defect, and the hyperphagic *foz/foz* mice fed high-fat diets for varying periods of time.[7] This combined approach has yielded a phenotype closer to human disease, with varying degrees of obesity, insulin resistance, and steatohepatitis. However, in most of the models, the accompanying fibrosis is relatively mild and does not progress to cirrhosis, arguably the most relevant clinical end point as it causes most of the morbidity and mortality associated with human fatty liver disease.

Free Cholesterol and Liver Injury: Role of the "Other" Lipid

Because hepatic triglyceride accumulation is the *sine qua non* of fatty liver disease, considerable effort has been directed toward understanding the role of free fatty acids and triglyceride in the pathogenesis of progressive liver injury in NAFLD. Much less information is available on the role of cholesterol, both free and total, in causing liver injury and progressive fibrosis. Recently, Puri et al performed lipidomic analysis in patients with NAFLD to determine the types and amounts of lipids accumulating in the liver.[15] One of the surprising findings from their study was that while the levels of hepatic free fatty acids were unaltered, there was a stepwise increase in hepatic free cholesterol as disease progressed from normal livers to steatosis and steatohepatitis. This finding is consistent with experimental data showing that free cholesterol may be an important determinant of cytotoxicity.[16]

It is in this context that the report of Ogawa et al deserves comment. In their attempt to develop a model of NASH with progressive fibrosis, the authors fed rabbits a diet supplemented with corn oil and 0.75% cholesterol for 9 months. At the end of study period, the livers of high-fat diet–fed rabbits showed a severely steatotic and nodular appearance. Surprisingly, hepatic lipid analysis showed a striking increase in total cholesterol but not triglyceride or nonesterified fatty acids. There was associated liver inflammation and alterations in expression of genes important in oxidant defense mechanisms. Most strikingly, there was significant liver fibrosis in the high-fat diet–fed group, suggesting progressive liver damage from the diet. Thus, this article highlights the often-ignored role of cholesterol in the pathogenesis of NASH and may prove to be a useful model to dissect the mechanisms of cholesterol-induced cytotoxicity *in vivo*. The bridging liver fibrosis seen in this model, a feature generally lacking in rodent models of NAFLD, may serve as a useful tool to dissect the determinants of fibrogenesis in the setting of fatty liver disease. In addition, the severe dyslipidemia seen in this model may prove to be a useful tool to study the links between NAFLD and cardiovascular risks and atherogenesis, a phenomenon of great clinical relevance. This paper also starts to focus on the role of macrophages in the pathogenesis of diet-induced liver injury. Although extensive hepatic macrophage accumulation is uncommon in human NAFLD, it is a fairly common occurrence in animals fed on an atherogenic diet. Finally, the hepatoprotective effect seen with ezetimibe supports the role of cholesterol in the pathogenesis of NASH. Ezetimibe, which is used for the treatment of hypercholesterolemia, targets Niemann-Pick C1-Like 1 (NPC1L1) and inhibits intestinal cholesterol absorption.[17,18] Interestingly, NPC1L1 is also expressed in hepatocytes and mediates the retention of biliary cholesterol by hepatocytes, and its inhibition by ezetimibe increases biliary cholesterol excretion.[19] Although many questions remain about the efficacy and safety of ezetimibe, this drug deserves further study in the management of NASH.

Putting the Pieces of the NASH Puzzle Together: The Quest Continues

Despite the exciting findings presented in the current article by Ogawa et al, several important issues and unanswered questions remain about their model. While the high-fat diet–fed rabbit model develops progressive liver fibrosis, there is no concomitant insulin resistance. Thus, like other models with similar limitations, this model recapitulates liver histology but not the metabolic profile of human NAFLD. It remains to be seen whether altering the composition of the rabbit diet, such as with the addition of fructose and/or *trans* fat, contributes to the development of obesity and metabolic syndrome in addition to the histological effects, a finding that has been seen in other animal models.[20,21] Secondly, there appears to be an as-yet-unexplained difference in the phenotype of the high-fat diet–fed rabbits. Short-term feeding with higher percentage fat causes insulin resistance but leads to death, whereas chronic feeding with lower fat content–diet causes progressive fibrosis and cholesterol accumulation without systemic insulin resistance. Finally, the cost of maintaining rabbits on the experimental diet for long periods of time, and the increased cost compared with mice experiments, will have considerable impact on the ability of researchers to use this model for NAFLD studies.

Despite the limitations of the rabbit model of Ogawa et al, the current article suggests that, perhaps, it may be time to challenge the research hegemony of rodent models of fatty liver disease. Clearly, species differences are important in the susceptibility to develop fatty liver dis-

ease and progressive fibrosis. As recently demonstrated by Lee et al[20] using the Ossabaw miniature swine, which develops metabolic features and liver histology reminiscent of human NASH, there can be crucial insights obtained by using large animal models to study fatty liver disease. It may well prove to be the case that large animal models have greater similarity to human metabolism and are more likely to develop NASH and cirrhosis than rodent models, which may significantly outweigh the drawbacks of costs and other factors involved in using large animals. Furthermore, large animals may provide an opportunity for serial liver biopsies on the same animal, a phenomenal advantage for investigating the molecular pathogenesis of NAFLD and NASH.

In conclusion, Ogawa et al report a new animal model using high-fat diet–fed rabbits, which leads to hepatic cholesterol accumulation and progressive liver fibrosis and cirrhosis. This approach may serve as a useful tool to study the role of free and total cholesterol in progressive liver injury and the molecular basis of fibrogenesis in fatty liver disease, but their model lacks symptoms of obesity and insulin resistance, thus limiting its utility. Therefore, although this model does offer a piece of the puzzle, the quest for the perfect animal model of NAFLD continues.

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