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Mouse Models for NASH: Are we there yet?

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Commentary

To date, obesity is a growing epidemic mainly caused by a combination of excess intake of fat- and/or sugar-enriched foods and a lack of exercise. As a consequence, the Metabolic Syndrome (MetS), which is a cluster of factors increasing the risk of metabolic diseases such as atherosclerosis and type 2 diabetes, may develop. Non-alcoholic fatty liver disease (NAFLD) is currently the most common liver disorder in the Western world and is considered the hepatic manifestation of MetS. NAFLD is a spectrum of liver diseases varying from excessive lipid accumulation (simple steatosis) to non-alcoholic steatohepatitis (NASH), advanced-stage fibrosis, cirrhosis and hepatocellular carcinoma (HCC). Whereas steatosis is reversible and benign, NASH is characterized by disturbed lipid metabolism that is accompanied with low-grade chronic inflammation. Important players in this key inflammatory event are the resident macrophages of liver, which are known to scavenge, internalize and subsequently accumulate oxidatively-modified lipoproteins inside the lysosomes [1]. As a consequence, macrophages transform into so-called foam cells to further induce the inflammatory process. Yet, due to a lack of mechanistic understanding, non-invasive diagnostic tools for NASH are currently poor and only few effective treatment options exist. Given that the prevalence of NAFLD/NASH rapidly increases both in children and adults, it is of great relevance to further investigate the underlying mechanisms.

During the last decade, a variety of mouse models, i.e., dietary- or chemically-induced and/or genetically modified models, substantially led to better insights into the pathophysiology of NASH. Given that obesity is a primary trigger for NASH, several diet-induced models aimed at resembling the onset of NASH. Upon feeding a high-fat (40–70% fat calories, obesogenic) or high-cholesterol (0.1–2.0% cholesterol, atherogenic) diet for 20-30 weeks, several mouse strains (including C57BL/6, BALB/c, C3H/HeN mice) were shown to develop disturbed lipid metabolism, steatosis, and moderate NASH. However, these strains displayed variability in disease onset, and also the development of fibrosis was limited in these models. As such, a novel so-called 'Amylin Liver NASH model' was generated to better mimic the Western 'fast-food' diet and its subsequent development of NASH hallmarks. Upon feeding an 'AMLN'-diet containing cholesterol (˜2%), fructose (˜20%) and trans-fatty acids (˜18%), wild-type C57/Bl6 and leptin-deficient ob/ob mice (due to a spontaneous, homozygous mutation in the leptin gene) developed marked steatosis, moderate lobular inflammation and mild-stage hepatocellular ballooning. However, also these NASH-related features were only found after a long-term period (26–30 weeks) of dieting [2].

To overcome the lack of severe hepatic fibrosis, mice were fed a non-physiological diet, deficient for or low in certain essential nutrients such as methionine and/or choline. Although it has been shown to promote murine NASH, the degree of hepatic fibrosis likely depended on a variety of factors, such as diversity in genetic background (interstrain variabilty), way of housing (interlaboratory variability) and exact diet composition (dietary variability). More importantly, instead of being obese, these models showed significant weight loss, concomitant loss in liver mass, cachexia, low serum insulin, fasting glucose, leptin and triglyceride levels and no signs of insulin resistance, therefore not resembling the human situation. Other models used for studying the progression and/or regression of liver fibrosis and subsequent development of cirrhosis and HCC are liver-targeted chemotoxin models (including carbon tetrachloride, thioacetamide and streptozotocin, in which the latter is typically given to neonatal mice, known as STAM model). Nevertheless, similar to the nutrient-deficient diets, chemotoxins are known to induce a significant reduction in body weight, and therefore do not mimic the etiology of human NASH [2].

An alternative way to gain better insight into the disease spectrum of human NAFLD is by means of genetic modifications. Although both leptin-deficient ob/ob mice and leptin-resistant db/db mice (carrying a homozygous mutation in the gene encoding for the leptin receptor) were expected to display similar features to human NASH, both models lacked the ability of spontaneously developing hepatic inflammation, thus requiring a second stimulus, such as a nutrient-deficient or 'AMLN' diet [3]. A more frequently used model to study NASH was the apolipoprotein E2 knock-in (*APOE2*ki: murine *ApoE* replaced by the human *APOE2* gene). Compared to C57Bl/6 mice, which only developed steatosis, these mice developed early hepatic inflammation and steatosis in response to high-fat diet. Nevertheless, further studies revealed that the inflammatory response was not sustained in *APOE2*ki mice, suggesting that the *APOE2* gene is not directly involved in inflammation. Relevantly, a complete deficiency in the *ApoE* gene (*ApoE*-/- mouse model) was also shown to induce hyperlipidemia in response to high-fat feeding. However, under these conditions, mice displayed lipoprotein profiles different from the human NASH situation, and furthermore, were able to spontaneously develop atherosclerotic plaques, making this model more suitable for atherosclerosis research. More recently, existing knowledge on the low-density lipoprotein receptor (*Ldlr*), a gene important for the transport of non-modified lipids into the macrophage, led to a major breakthrough in the field of NASH. By completely switching off the LDL receptor, mice fed a high fat/high cholesterol diet were able to mimic lifestyle-induced hepatic inflammation. Compared to *ApoE2*ki mice, these hyperlipidemic mice showed a sustained inflammatory response after 12 weeks of high-fat diet. As such, the *Ldlr*-/- mouse model has previously been established as a physiological model for investigating the onset of hepatic inflammation in the context of

NAFLD [4] and is currently being frequently used. Although the severity of fibrosis is rather moderate in this model, these mice have shown to develop more fibrosis compared to regular C57Bl/6 mice on a similar diet.

A relatively new genetically-modified model that became popular in NAFLD research is the obese foz/foz mouse model (carrying an 11-base pair truncating mutation in the Alström gene *ALMS1*). Due to overfeeding, these mice display features of MetS, including obesity, hyperglycemia, hyperlipidemia and insulin resistance, and can spontaneously develop steatosis, hepatic inflammation and fibrosis within 24 weeks of high-fat diet [2,3]. Nevertheless, the exact role of *Alms1* is not yet completely understood, limiting its clinical translationality. The lean polygenetic fatty liver Shionogi (FLS) is a mouse model that under normal environmental conditions has been shown to spontaneously develop hepatic inflammation, however, with rather a mild degree of fibrosis. When backcrossed with ob/ob mice, these genetically-modified mice appeared to develop severe hepatic steatosis, inflammation, advanced fibrosis, and spontaneous HCC [2]. Nevertheless, due to its uncontrollable heterogeneity in disease onset, these models are currently scarcely used. Another mouse model known to develop NASH-related HCC features is the hepatocyte-specific phosphatase and tensin homolog (PTEN)-deficient mouse model [3]. Although this model is useful for better understanding the progression from NASH to HCC, it does not exhibit human NASH features such as increased fatty acid levels and obesity, therefore also limiting its translational potential.

Instead of focusing on genetic factors, very recently, attention has been shifted towards the importance of housing conditions in preclinical NASH research [5]. In contrast to standard housing conditions (20-23°C), researchers introduced a novel concept of thermoneutral housing (30-32°C). Under these conditions, mice displayed not only an increase in pro-inflammatory immune response, but also deterioration in high-fat diet-induced NASH progression, compared to mice housed under standard condition. Furthermore, these mice also showed an increase in intestinal permeability and an alteration in gut microbiome, both hallmarks resembling the human situation. Given that these features were conserved across different mouse strains and could overcome the gender bias often observed in current NAFLD models, these data suggest that housing temperature is an important factor to be taken into account when studying human NAFLD in a preclinical setting. Yet, despite robust exacerbation of NAFLD pathogenesis, hepatic fibrosis was absent, for instance in both male and female C57BL/6 mice, suggesting that a dietary challenge is still required to promote fibrosis.

Altogether, current mouse models have become essential tools to study pathological progression from fatty liver to NASH, fibrosis, and HCC. However, each individual model addresses different aspects of the disease spectrum, implying the lack of either physiological (metabolic or biochemical) characteristics, histological features or disease progression, thus limiting its translational potential. Despite recent advances, there is still need for appropriate mouse models that better mimic the human liver disease spectrum, and therefore, this research certainly deserves further attention. It is also important to keep in mind that NASH is a multifactorial disease, involving metabolic crosstalk between different organs. As such, for each (clinically-relevant) research, the most relevant existing mouse model should be carefully selected, keeping in mind its limitations while analysing the data.

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