Animal Models of Non-alcoholic Fatty Liver Disease: from Genetics to Nutrition

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1 Introduction

Obesity is the main causal factor in the development of the metabolic syndrome. This growing multiple risk factor syndrome is characterized by a constellation of metabolic risk factors for the development of atherosclerotic cardiovascular disease and diabetes (Sarafidis & Nilsson, 2006). In addition, the metabolic syndrome was found to be a strong predictor for the development of non-alcoholic fatty liver disease (NAFLD). The term NAFLD is used to describe a variety of liver diseases of different severity, from pure steatosis to non-alcoholic steatohepatitis (NASH) and cirrhosis and, rarely, hepatocellular carcinoma (Adams & Lindor, 2007). Over recent decades, NAFLD has been increasing worldwide in line with the increased prevalence of obesity, type 2 diabetes and hyperlipidemia (Kotronen et al., 2007). Estimates of NAFLD prevalence vary between 20 and 30% in Western populations, rising to as high as 90% in morbidly obese individuals. NASH is less common, affecting only 2-3% of the general population and up to 37% of the morbidly obese. Therefore, NAFLD is now recognized to be the hepatic manifestation of the metabolic syndrome (Bedogni, et al., 2005; Browning, et al., 2004; Targher, et al., 2007).

While hepatic fat accumulation (steatosis) itself is generally considered a rather benign and reversible condition, the presence of inflammation in a fatty liver is the key feature of NASH that precedes further disease progression and enables the development of more advanced stages of the disease – such as fibrosis, cirrhosis or hepatocellular carcinoma – often leading to the need for liver transplantation (Portincasa et al., 2005). Thus, the progression from steatosis towards hepatic inflammation represents a key step in the development of NASH. The gold standard for the diagnosis of NASH is via histological assessment (Figure 1). Therefore, the Pathology Committee of the NASH Clinical Research Network designed and validated a histological feature scoring system that addresses the full spectrum of lesions of NAFLD and proposed a NAFLD activity score (NAS) for use in clinical trials. The proposed NAS is the unweighted sum of steatosis, lobular inflammation, and hepatocellular ballooning scores. A score of 5 or more correlates with a diagnosis of NASH, and biopsies with scores of less than 3 are diagnosed as "not NASH" (Kleiner et al., 2005). Therefore, this strong scoring system can be useful for assessing the range of histological features of NAFLD in humans.

Until now, it is still not clear why patients with simple steatosis not always progress into NASH (Marra et al., 2008). Although this likely results from interplay of different determinants including genetic and environmental factors, the sequence of events is not known. The so-called “two-hit hypothesis” proposed by Day et al (Day & James, 1998) postulates a sequential evolution from bland steatosis to NASH, in which insulin resistance is a first hit that is able to induce steatosis and renders the liver more susceptible to injury. Then, a second hit (drugs, ischemia, endotoxin-induced damage, etc.) would prompt steatosis progression to NASH. This theory has been reviewed several times and consideration has been given to the possibility that some subjects may be directly committed to NASH from the beginning of the disease (Petta et al., 2009). In human epidemiological studies, clinical data on the sequence of events during NASH progression are scarce, and therefore a direct correlation between the sequences of events has not really been established. The concept of “multiple parallel hits” reflects more precisely the current knowledge of this metabolic disease and could explain why this disease can also occur in rather lean subjects (Tilg & Moschen, 2010).

Knowledge of the intracellular mechanisms that trigger NASH is therefore of utmost importance. Consequently, animal models for NASH that faithfully recapitulates the pathophysiology of human NASH are necessary and may lead to the development of novel therapeutic options for the treatment of this disease.
To date, no animal model completely reflects the histopathology and pathophysiology of human NAFLD/NASH and therefore it is important to select the animal model that best conforms to the aim of the study. In this chapter, we will summarize the current knowledge on genetic and nutritional animal models of NASH, referring to their advantages and disadvantages to serve as a physiological model to study the full spectrum of liver pathology and the metabolic context that characterizes human NASH.

![Figure 1: Histological assessment of a normal liver (A), steatosis (B), lobular inflammation (C) and pericellular fibrosis (Masson’s Trichrome) around ballooned hepatocytes (D).](image)

2 Genetic Models of NAFLD

Researchers have used genetic models to better examine the role of specific genes in NAFLD. The genetic models of obesity-related liver injury can be broadly classified into two groups: first, those in which steatohepatitis develops with no or minimal features of metabolic syndrome; and second, those in which steatosis develops in the context of obesity and features of metabolic syndrome, but where there is only minor and non-progressive liver injury (Larter & Yeh, 2008).

2.1 Defective Leptin Signaling

Among the most frequently used genetic models are mice that exhibit defects in the leptin signaling pathway. Leptin is an adipokine produced by white adipose tissue which regulates food uptake and energy expenditure, and modulates cellular immunity, cell death and fibrogenesis (Friedman, 2009). In obese patients, elevated leptin levels are found and the pathophysiology is believed to occur due to resistance in leptin signaling (Halaas et al., 1997). Mice with impaired leptin signaling exist, lacking either the leptin gene (ob/ob mice) or the receptor (db/db mice). Both mouse models are extremely obese, inactive, hyperphagic and show hyperglycemia, insulin resistance and hyperinsulinemia (Bray & York, 1979). Although the metabolic abnormalities resemble NAFLD, spontaneous development of steatohepatitis is not a feature of these strains, although older males do show some mononuclear cell infiltration of liver and elevation of serum alanine transaminases (ALT). Therefore, secondary insults such as a methionine and choline deficient (MCD) diet, high fat diet (HFD) or low-dose lipopolysaccharide (endotoxin) are needed to trigger steatohepatitis in these mouse strains. A common feature of ob/ob mice is that they are protected against fibrosis, a phenomenon which led to the characterization of leptin as an essential mediator of hepatic fibrogenesis (Ikejima et al., 2001; Leclercq et al., 2002). When fed an MCD diet, db/db mice demonstrated significantly more liver fibrosis compared to ob/ob mice, pointing towards an important role of the leptin receptors (ob-Ra) in hepatic fibrogenesis in NAFLD (Sahai et al., 2004).
model has been extensively studied, although mutations in the \textit{ob} gene are not prevalent in obese subjects or NASH patients and leptin levels correlate poorly with the development of NASH (Chalasani \textit{et al.}, 2003). Hyperleptinemia, due to a loss of the leptin receptor as seen in \textit{db/db} mice, resembles the human condition more closely. However, these animals also need a secondary injurious stimulus for the induction of NASH. The advantage of \textit{ob/ob} and \textit{db/db} mice is that the phenotype of these mice stimulates the human condition of metabolic syndrome in many aspects. Therefore, these mice are useful to study the metabolic complications associated with NASH. However, these mice have the disadvantage that they do not spontaneously develop steatohepatitis or liver fibrosis and therefore cannot be used to study the pathogenesis of inflammation and later stages of the disease.

2.2 \textbf{Defective Hepatic Lipogenesis}

The sterol regulatory element binding protein (SREBP)-1c is a key transcription factor for the regulation of lipogenic enzyme genes. Targeted over-expression of SREBP-1c in adipose tissue promotes hepatic lipogenesis in mice. These mice display congenital lipodystrophy in which severe insulin resistance and diabetes develop secondary to impaired adipose differentiation (Shimomura \textit{et al.}, 1998). The restriction in adipose mass causes hepatic lipid accumulation, with marked steatosis present from as young as 8 days of age. When fed a standard chow diet, steatosis, lobular inflammation, fibrosis, ballooned hepatocytes and Mallory bodies were observed by 20 weeks of age (Nakayama \textit{et al.}, 2007). Whether these mice can be used as model for typical NAFLD is questionable. In obesity-related NAFLD, the adipose tissue is enlarged and acts as a storage compartment which is likely to contribute to perturbations of whole-body lipid homeostasis, while mice overexpressing SREBP-1c suffer from lipodystrophy and are therefore not useful to study the metabolic consequences of NASH.

2.3 \textbf{Defective \(\beta\)-oxidation}

The oxidation of fatty acids secures energy supplies independent from food intake and is regulated by a class of nuclear receptors that control the transcription of the rate limiting enzymes of this process. PPAR-\(\alpha\) is the key regulator of the genes involved in the fatty acid oxidation systems in the liver, and a significant reduction of PPAR-\(\alpha\) can be observed in HFD models (Svegliati-Baroni \textit{et al.}, 2006). Under conditions of normal feeding, PPAR-\(\alpha\) mutant mice do not accumulate fat in their liver, but fail to increase fatty acid oxidation and so develop severe steatosis when fatty acid delivery to the liver is increased by fasting conditions, indicating that defects in PPAR-\(\alpha\)-inducible fatty acid oxidation determine the severity of fatty liver phenotype to conditions reflecting energy-related stress (Kersten \textit{et al.}, 1999).

Acyl-coenzyme A oxidase (AOX) is the rate limiting enzyme of peroxisomal \(\beta\)-oxidation of long chain fatty acids (LCFA). Therefore, AOX mutant mice have defective peroxisomal \(\beta\)-oxidation of LCFA. Initially, these animals are phenotypically normal, but over time, mice develop severe steatosis and focal inflammatory cell infiltrates with hepatocyte apoptosis. However, compensative increase of fatty acid oxidation is observed by 6-8 months of age, and hepatic steatosis recovers by regeneration of hepatocytes (Cook \textit{et al.}, 2001; Fan \textit{et al.}, 1998). Loss of AOX also causes sustained hyperactivation of PPAR-\(\alpha\), leading to transcriptional up-regulation of PPAR-\(\alpha\) -regulated genes, indicating that unmetabolized substrates of AOX function as ligands of PPAR alpha (Rao & Reddy, 2001). This regeneration limits the utility of this model for studying the pathogenesis of steatohepatitis in older mice, as disease progression after 6-8 months is different from human NASH. Therefore, this model is ideal to study the early signs of liver damage, but should be used before they have reached the regeneration stage.
2.4 Defective NFκB and TNF Signaling

The nuclear factor (NF)-κB signaling pathway mediates a variety of important cellular functions by regulating immune and inflammatory responses (Karin & Lin, 2002). The I-κB kinase (IKK) complex, consisting of two catalytic subunits – IKK1 (IKKα) and IKK2 (IKKβ) – and a regulatory subunit called NF-κB-essential modulator (NEMO/IKK-γ), mediates NF-κB activation in response to a variety of stimuli by phosphorylating IκB proteins (Ghosh & Karin, 2002; Karin, 1999). Gene targeting experiments demonstrated that knockout animals for IKK2 or NEMO die during embryonic development as a consequence of liver failure due to massive hepatocyte apoptosis, highlighting the essential role of these IKK complex members for liver physiology (Li et al., 1999; Rudolph et al., 2000; Tanaka et al., 1999). By using hepatocyte-specific knockout animals for NEMO, embryonic lethality is circumvented. In the hepatocytes of these mice, NF-κB activation is blocked upon stimulation e.g. by TNF and in turn these cells undergo apoptosis (Beraza et al., 2007; Luedde et al., 2007). At around 3 weeks after birth, NEMOΔhepa animals develop severe hepatitis associated with increased transaminases, strong infiltration of immune cells, hepatocyte apoptosis and compensatory hepatocyte proliferation. At 8 weeks, the animals display a maximum in liver injury, as evidenced by a strong elevation serum aminotransferase levels, beginning lipid accumulation and first signs of fibrosis. These changes are associated with an expansion of oval cells, representing the hepatic stem cell compartment. After 9 months dysplastic nodules develop and at 12 months 100% of the animals carry macroscopically visible liver nodules, which can be classified as HCCs by histological staining (Luedde et al., 2007). Although this model is not suitable for studying the metabolic consequences of NAFLD, it is very useful for investigating the sequential development of NAFLD.

The transcription factor NF-κB can also be activated via TNF signaling. Mice with impaired shedding of the p55 TNFR (p55ΔNS) exhibit increased host defense responses, but develop spontaneous chronic active hepatitis, characterized by focal parenchymal inflammation with infiltration of lymphocytes, polymorphonuclear cells and other leukocytes, and by the presence of apoptotic hepatocytes in the majority of the inflammatory foci. This liver pathology was already apparent at 3-4 weeks of age and persisted throughout adulthood, while TNF−/− mice do not show this phenotype (Xanthoulea et al., 2004). Therefore, these data indicate that TNF-induced p55TNFR signaling is critical for disease pathogenesis and that TNFR1 receptor shedding may regulate TNF activity in vivo by defining thresholds of TNF function. Although these mice do not develop steatosis or other metabolic consequences associated with NAFLD, the advantage of using this model is that these mice develop severe and spontaneous liver injury.

By loss of the tumor suppressor gene phosphatase and tensin homolog (PTEN), via the phosphoinositide 3-kinase/Akt pathway that induces activation of the IκB-α kinase (IKK), NF-κB can also be activated (Zhang et al., 2010). Inflammation and hepatocarcinogenesis are also seen in mice with hepatocyte specific deletion of PTEN. The impaired PI3-kinases signaling in liver specific PTEN−/− mice results in extensive hepatomegaly and steatohepatitis from increased accumulation of triglycerides and spontaneous development of liver fibrosis and HCC (Watanabe et al., 2007). The advantage of this model is that the histological phenotype resembles that of human NASH, the disadvantage is that it is hypersensitive to insulin (Horie et al., 2004).

2.5 Defective Cholesterol Signaling

Based on the analogy between the mechanisms of NASH and atherosclerosis, an emerging trend in NASH research is to utilize the mouse models traditionally targeted for studies of atherosclerosis (Bieghs
et al., 2011). One of the commonly used models for atherosclerosis studies is the low density lipoprotein (LDL) receptor knock-out (LDLR<sup>−/−</sup>) mouse. The LDL receptor plays a major role in the clearance of apoB and apoE-containing lipoproteins (Choi et al., 1991). The LDLR<sup>−/−</sup> mice are therefore mildly hypercholesterolemic due to the absence of LDL receptors, which prolongs the plasma half-life of VLDL and LDL (Ishibashi et al., 1993). By using a physiological HFD rich in cholesterol (0.2%) for 3 months, LDLR<sup>−/−</sup> mice developed hepatic steatosis, inflammation, apoptosis and mild fibrosis (Bieghs et al., 2012). In addition, insulin resistance is induced upon prolonged HFD feeding (5.5 months) (Merat et al., 1999). Therefore, the LDLR<sup>−/−</sup> mouse model is particularly useful for understanding the relationships between lipid metabolism and inflammatory recruitment in the context of NASH.

Another mouse model for atherosclerosis is the apolipoprotein E2 knock-in (APOE2ki) mouse. In APOE2ki mice, the murine apoe gene is replaced by the human APOE2 allele. The APOE2 protein has a markedly reduced affinity for the LDL receptor, leading to a plasma lipoprotein profile resembling human type III hyperlipoproteinaemia (HLP) (Sullivan et al., 1998). The APOE2ki mouse has outstanding potential as it is highly responsive to dietary factors and pharmacological interventions (Wouters et al., 2010). Upon HFD for several days, APOE2ki mice developed steatosis and inflammation (Wouters et al., 2008). Surprisingly, the inflammatory response in the liver was completely abolished upon 3 months of HFD, while steatosis sustained (Bieghs et al., 2012). Apolipoprotein E-knockout mice upon a Western-type of diet (rich in fat and cholesterol) for 10 weeks also develop severe hepatic alterations, such as micro- and macrovesicular steatosis, macrophage proliferation and inflammatory nodules (Tous et al., 2005). However, this pro-inflammatory phenotype is also reduced upon prolonged feeding of 32 weeks, due to activation of anti-inflammatory genes (Tous et al., 2006). Therefore, the use of the hyperlipidemic APOE2ki and APOE<sup>−/−</sup> should be carefully considered, as these mice are only useful in studying the early stages of the disease. Since the inflammatory response is decreasing over time, it is also possible to use these mice for investigating regression in the context of NASH.

The advantage of using hyperlipidemic mice as model for NASH is the humanized lipoprotein profile. Unlike the lipoprotein profile in wild-type (WT) profile, in which most cholesterol is present in the HDL fraction, the profile of the hyperlipidemic mice is more comparable with the human plasma lipoprotein profile, in which cholesterol is mainly confined to the LDL fraction (Wouters et al., 2005). A disadvantage of using these hyperlipidemic mice is that NASH development does not occur spontaneously and these mice do not develop later stages of the disease spectrum.

3 Nutritional Models of NAFLD

3.1 Methionine- and Choline- Deficient Model

The most classical dietary model of NASH is the methionine- and choline deficient (MCD) model. The MCD diet is high in sucrose and fat (40% sucrose, 10% fat), but lacks methionine and choline, which are essential for hepatic β-oxidation and the production of very low density lipoprotein (VLDL). This results in the accumulation of intra-hepatic lipid and decreased VLDL synthesis (Anstee & Goldin, 2006). Mice fed a MCD diet may develop hepatic inflammation as early as 3 days after feeding. Severe pericentral steatosis may develop by 1 to 2 weeks, and necro-inflammation may occur after 2 weeks, followed by progressive pericellular and pericentral fibrosis. Enhanced oxidative stress can be observed from 3 weeks after intake of the MCD diet (J. G. Fan & Qiao, 2009). Although the MCD model displays all of the
hallmarks of NASH, from steatosis to inflammation, fibrosis development and elevated plasma ALT levels (Rinella et al., 2008), there is little evidence to support the assertion that this model replicates either the phenotype or the pathogenic mechanisms of metabolic syndrome-related NAFLD. In contrast to human fatty liver disease, animals fed the MCD diet tend to lose weight, have low plasma triglyceride levels and reduced liver weight/body weight ratio (Koteish & Mae Diehl, 2002). Moreover, a major disadvantage of the MCD diet is that the metabolic profile is opposite to that seen in NASH patients, namely, that insulin, leptin and glucose levels are reduced and the animals are peripherally insulin sensitive (Larter et al., 2008; Rinella et al., 2008). In addition, the severity of MCD-induced NASH in rodents may depend on the gender, strain and species used, reflecting the large inter-individual phenotypic variation seen in NASH patients.

The choline-deficient, L-amino acid defined (CDAA) diet has also been used as a rodent model of NASH, characterized by steatosis, inflammatory cell infiltration and fibrosis (Nakae et al., 1995). Although it requires a longer time frame compared to the MCD diet, NASH can result by inhibition of the fatty acid oxidation in hepatocytes. Mice on the CDAA diet do not gain weight or have changes in peripheral insulin sensitivity (Kodama et al., 2009). Since animals on the MCD/CDAA diets do not reflect the phenotype or metabolic status observed in patients, they should not be used to examine these parameters. Nevertheless, this model is ideal to study the inflammatory and fibrotic elements of the NAFLD spectrum.

3.2 High Fat Diet

In analogy to Western diets, high-fat diets (HFD) (with 45-75% of calories derived from fat) are sufficient to induce obesity, insulin resistance, dyslipidemia, hepatic steatosis, oxidative stress, mild fibrosis and increased expression of pro-inflammatory cytokines in the white adipose tissue in mice and rats; however, the degree of liver injury is not as severe as observed with the MCD model (Deng et al., 2005; Omagari et al., 2008; Varela-Rey et al., 2009). Thus, although HFD models require longer feeding periods and do not develop the same severity of steatohepatitis as observed with the MCD diet, it can be viewed as advantageous as it allows researchers working with knockout mice to judge whether liver injury is aggravated by the HFD. In addition, these HFD models do more closely resemble the pathophysiology observed in human NAFLD, with the white adipose tissue being the central organ of pro-inflammatory and metabolic abnormalities (Hotamisligil, 2006). Furthermore, several research groups have demonstrated that C57BL/6J, but not A/J inbred mice, are susceptible to NASH and HCC upon prolonged high fat feeding (>1 year), as chronic changes induced by the prolonged consumption of a HFD alone culminate in the development of primary liver dysplasias (Hill-Baskin et al., 2009; Nakamura et al., 2012; VanSaun et al., 2009). Moreover, obesity-promoted HCC development was dependent on enhanced production of the tumor-promoting cytokines IL-6 and TNF, which cause hepatic inflammation and activation of the oncogenic transcription factor STAT3 (Park et al., 2010). Therefore, the variable results regarding the pathophysiology of NASH upon HFD depend on rodent species and strain, the fat content in the diet, the composition of the dietary fat, and the duration of treatment. Thus, although the hepatic pathological outcome is not as severe compared to MCD diet, HFD can replicate the altered metabolic parameters seen in human fatty liver disease.
3.3 Atherogenic Diet

Diets with increased cholesterol content (2-5%) and cholic acids are called atherogenic diets and have initially been employed to study atherosclerosis. However, rodents receiving this diet also developed steatosis, inflammation, fibrosis and oxidative stress in a time-dependent manner, which can be exacerbated by increasing the fat content (Jeong et al., 2005; Paigen et al., 1985). Thus, a combination high fat, cholesterol and cholate in animals would cause histological features reminiscent of human NASH. However, mice fed this diet were systematically insulin sensitive, albeit hepatic insulin resistance, and lost 9% body weight, had small epididymal fat pads and low plasma triglyceride levels compared to control mice (Matsuzawa et al., 2007). Thus, although the atherogenic with increased fat content does replicate human pathology, the metabolic status differs.

3.4 Fructose

Increased fructose consumption in humans, primarily in the form of corn syrup in soft drinks, is often associated with NAFLD and fibrosis severity (Abdelmalek et al., 2010; Ouyang et al., 2008). Fructose can promote de novo lipogenesis, weight gain, hypertension, hypertriglyceridemia, the formation of reactive oxygen species, pro-inflammatory responses and insulin resistance (Dhingra et al., 2007; Kelley et al., 2004). In addition, fructose has been shown to cause ATP depletion in humans, promoting hepatic necro-inflammation. In mice, the addition of 30% fructose to drinking water caused a marked increase in steatosis, weight and intestinal bacterial overgrowth over 8 weeks, which lead to increased endotoxin levels in the portal blood, activation of Kupffer cells and hepatic inflammation (Spruss et al., 2009). In summary, these data strengthen the case for adding fructose to dietary animal models of NAFLD, as it promotes steatosis, pro-inflammatory responses and intestinal bacterial overgrowth.

3.5 Overnutrition

The most common cause of overweight and obesity in humans is overnutrition, which is chronic energy intake surfeit to the daily energy requirements of the individual, resulting in insulin resistance and NAFLD (Larter & Yeh, 2008). In rodents, overnutrition is induced by high fat feeding. However, a common problem is that rodents may adapt to high-fat feeding and become resistant to the development of obesity and/or other metabolic abnormalities (Romestaing et al., 2007). This self-correcting mechanism can be prevented by the use of forced feeding via gavage, implanted gastrostomy tube or total enteral nutrition (Larter & Yeh, 2008). Intragastric overfeeding of mice up to 85% in excess of standard intake for 9 weeks is associated with histopathological and pathogenic features of NASH (Deng et al., 2005; Gaemers et al., 2011). Overfeeding of C57Bl6 mice with a HFD was causing obesity, increased visceral fat, hyperglycemia, hyperinsulinemia, hyperleptinemia, glucose intolerance, insulin resistance and hepatic ER stress. In addition, plasma ALT levels were increased, together with neutrophil infiltration and perisinusoidal fibrosis. In conclusion, although overfeeding of mice is a cumbersome procedure, the overfed mouse model displays the characteristics of human NAFLD within the appropriate metabolic setting, i.e. obesity through overcaloric intake of a HFD.
4 Conclusion

Many animal models of NAFLD have been developed to date. This chapter has explored some of the advantages and disadvantages of genetic and nutritional models of NAFLD. The available models do not replicate the full spectrum of the disease in humans, as they only mimic certain disease aspects and differ with regards to the degree of hepatocellular injury and their metabolic context. The combination of naturally occurring genetic mutations or targeted gene modifications with dietary or chemical challenges would more closely resemble the histopathology and pathophysiology of human NAFLD. Therefore, in order to address a defined research hypothesis, an appropriate model has to be chosen carefully, based on the specific aims. With continuing improvement of the current models and the advent of novel models, scientists will be able to accumulate sufficient knowledge of disease pathogenesis, which will eventually lead to a full understanding of human NASH and development of efficient therapy options for this disease.

References


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<td>no</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
<td>+ : Good liver injury model</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- : No metabolic risk factors</td>
</tr>
<tr>
<td>High fat diet (HFD)</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes (mild)</td>
<td>yes (mild)</td>
<td>yes</td>
<td>+ : Metabolic risk factors</td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>- : Only mild steatohepatitis</td>
</tr>
<tr>
<td>Atherogenic diet</td>
<td>no</td>
<td>Hepatic insulin resistance</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>+ : Severe liver injury with oxidative stress</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- : Weight loss</td>
</tr>
<tr>
<td>Fructose</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>+ : Metabolic risk factors</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- : No severe liver injury</td>
</tr>
<tr>
<td>Overnutrition</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
<td>+ : Metabolic risk factors</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- : Metabolic risk factors and liver injury</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>- : Forced feeding</td>
</tr>
</tbody>
</table>

**Table 1:** Overview of the most common nutritional and genetic models of non-alcoholic steatohepatitis.


Merat, S., Casanada, F., Sutphin, M., Palinski, W., & Reaven, P. D. (1999). Western-type diets induce insulin resistance and hyperinsulinemia in LDL receptor-deficient mice but do not increase aortic atherosclerosis compared with normoinsulinemic mice in which similar plasma cholesterol levels are achieved by a fructose-rich diet. Arterioscler Thromb Vasc Biol, 19(5), 1223-1230.


