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Review Article

Non-alcoholic fatty liver disease: pathogenesis and models

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Abstract: Non-alcoholic fatty liver disease (NAFLD) is a complex disease characterized by a massive accumulation of lipids in the liver, with a continuous progression of simple steatosis, non-alcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma. Non-alcoholic fatty liver disease is associated with obesity, insulin resistance, and metabolic syndrome; it is a severe public health risk and is currently the most common liver disease of the world. In addition to the fatty infiltration of the liver in non-alcoholic fatty liver disease patients, the field of liver transplantation faces similar obstacles. NAFLD and NASH primarily involve lipotoxicity, inflammation, oxidative stress, and insulin resistance. However, the precise mechanisms and treatments remain unclear. Therapeutic approaches encompass exercise, weight control, as well as treatments targeting antioxidants and anti-inflammatory pathways. The role of animal models in research has become crucial as a key tool to explore the molecular mechanisms and potential treatments for non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. Here, we summarized the current understanding of the pathogenesis of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis and discussed animal models commonly used in recent years.

Keywords: non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), histopathology, pathogenesis, treatment, animal models

Introduction

Non-alcoholic fatty liver disease (NAFLD), also known as fatty liver or steatosis, is characterized by excessive accumulation of fat in the liver; it is an acquired metabolic stress liver injury that is not caused by alcohol or other apparent hepatoprotective factors, and it is closely related to insulin resistance (IR) and genetic susceptibility [1]. NAFLD-induced liver damage is manifested by metabolic syndromes, such as dyslipidemia, hypertension, hyperglycemia, and obesity [2].

NAFLD is not a simple disease progression. IR and an unhealthy diet produce large amounts of free fatty acids, accumulating fat droplets in hepatocytes, manifesting early as steatosis. In the ongoing oxidative stress and inflammation, further liver damage is triggered, progressing to non-alcoholic steatohepatitis (NASH) [3]. Approximately 40% of NASH patients progressively deteriorate and develop liver fibrosis and cirrhosis [4].

NAFLD is gradually replacing hepatitis B as the most common liver disease because of the massive availability of the hepatitis B vaccine, accompanied by a significant decrease in the proportion of hepatitis B patients and the increasing number of obese and IR patients in our society [5]. As a major public health issue, exploring its pathogenesis and potential drug therapies has become crucial.

However, the specific mechanisms underlying the development of NAFLD and NASH are still unclear, and an international consensus on treatment options has not yet been reached. Therefore, animal models are crucial for exploring the molecular mechanisms and potential therapeutic approaches for NAFLD and NASH. Studies using experimental animal models of NASH have identified several potential thera-

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peutic pathways, most of which have also demonstrated promising results.

Existing animal models can mimic the histopathological and pathophysiological features of different stages of human NAFLD by altering genetic characteristics or intervening with diet. However, no animal model can fully simulate all aspects of human NAFLD and NASH, and each model has strengths and limitations. Therefore, proper animal model selection is crucial for investigators.

In the present review, we aim to summarize the current understanding of the pathogenesis of NAFLD and NASH and discuss animal models commonly used in recent years. We compare the characteristics and applications of different animal models and provide some insights for future research.

Histopathology and pathogenesis of NAFLD

Hepatic steatosis is also an important feature of NAFLD, i.e., excessive accumulation of lipid droplets in hepatocytes as triglycerides. NAFLD is diagnosed histologically when >5% of hepatocytes are steatotic [1].

Hepatic steatosis is multifactorial and closely related to obesity, IR, and adipose tissue (AT) dysfunction.

Unhealthy lifestyle habits and a chronic diet high in sugar and fat can lead to obesity and increased hepatic fat accumulation and de novo lipogenesis (DNL) of liver fat [6, 7], directly leading to hepatic steatosis. Tumor necrosis factor-α (TNF-α) overexpressed in the liver of obese patients continuously activates IκB kinase β, which mediates the phosphorylation of insulin receptor substrate (IRS) IRS-1 and IRS-2, disrupting the ability of insulin to bind to its receptor and affecting downstream cell signaling, leading to IR [8, 9]. And insulin resistance is thought to be closely associated with the development and progression of NAFLD [10, 11].

IR is a weak response to insulin, which does not function as a normal glucose uptake regardless of normal or elevated insulin levels [12]. Under normal physiological conditions, insulin binds to the insulin receptor and mediates IRS phosphorylation through the receptor tyrosinase, activating sterol regulatory element binding protein-1 (SREBP-1) to stimulate hepatic DNL [13]. The IR condition leads to SREBP-1 overexpression, which triggers DNL upregulation [14], resulting in impaired adipose tissue (AT) lipolytic inhibition, a large amount of free fatty acid production, and finally, accumulation in the liver and kidney [15], further aggravating NAFLD.

Furthermore, adipose tissue dysfunction is also an important factor in the development of NAFLD. Adipose tissue mediates fat storage and mobilization, and the proper functioning of this process is closely related to the dynamic balance of adipokines and cytokines. Excessive fat accumulation, adipose tissue inflammation, and fibrosis lead to disruption of cytokine and adipokine (adiponectin and leptin) secretion, which cannot maintain the dynamic balance of fat synthesis and degradation properly, manifesting as adipose tissue dysfunction.

Lipocalin and leptin [16] secreted by normal adipose tissue are indispensable for maintaining hepatic homeostasis. Lipocalin is thought to have anti-inflammatory, antioxidant, anti-obesity, anti-fibrotic [17, 18], and hepatoprotective effects [19]. Studies have found that lipocalin secretion decreases when hepatic fat accumulation and fibrosis occur, directly leading to mitochondrial dysfunction, IR, and obesity [19, 20]. The role of leptin is mainly pro-inflammatory. It reduces food intake and increases energy, which can prevent fat accumulation in organs other than the AT (e.g., the liver) [21]. In obese and NAFLD patients, leptin does not function adequately because of decreased leptin levels or leptin resistance, leading to abnormal fat accumulation and further accelerating the NAFLD process (**Figure 1**).

Obesity, IR, and adipose tissue dysfunction, all of which promote each other, eventually manifest as the continued progression of NAFLD.

Histopathology and pathogenesis of NASH

Usually, one-third of NAFLD patients [22] deteriorate to NASH, diagnosed when hepatocellular steatosis is complicated by a necrotic inflammatory response of the liver and swollen hepatocytes.

For the mechanism of NASH progression, Day and James [23] proposed a second-strike

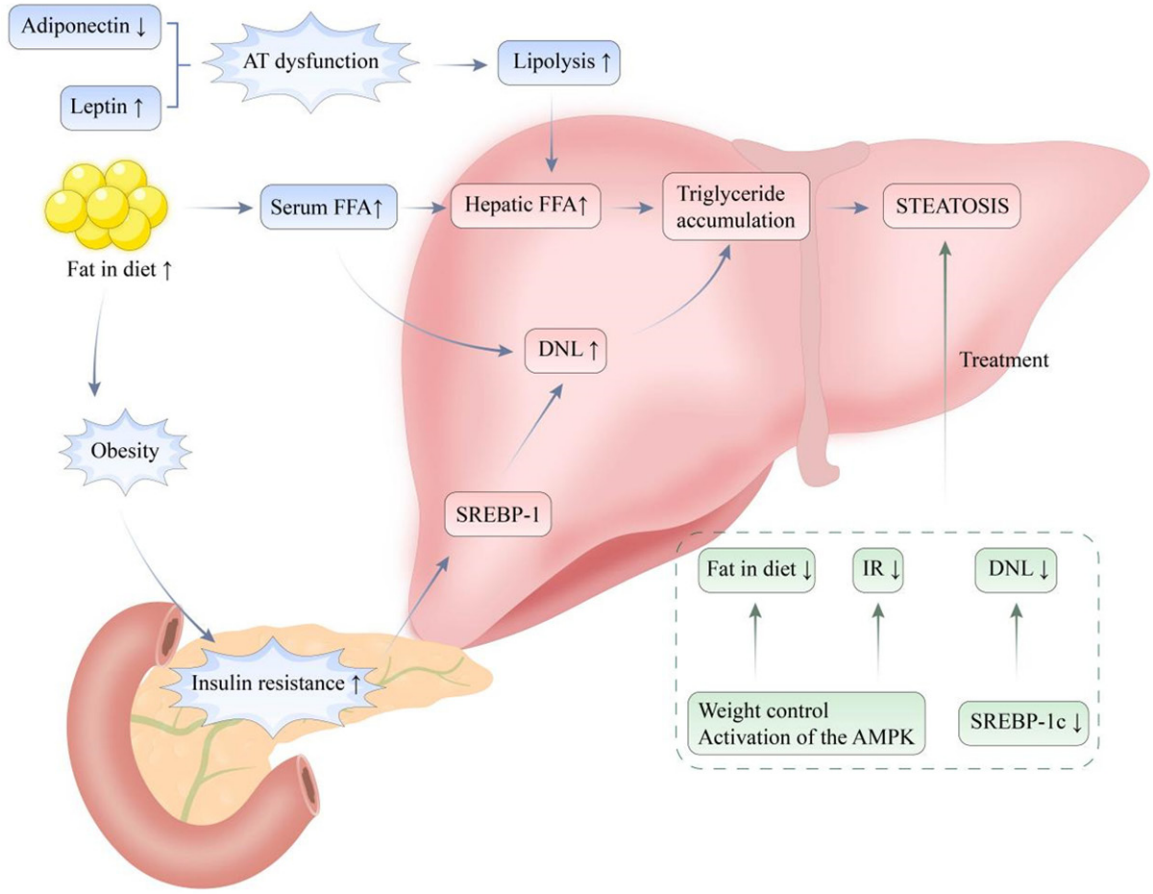

Figure 1. Mechanisms underlying non-alcoholic fatty liver disease (NAFLD) include adipose tissue (AT) dysfunction, obesity, increased hepatic free fatty acids (FFAs) levels, impaired β-oxidation, and insulin resistance. AT dysfunction leads to low adiponectin and high leptin levels, leading to hepatic insulin resistance and increased lipolysis. Increased lipolysis in the adipose tissue and excessive consumption of fats in the diet lead to increased free fatty acids in the liver. Hepatic insulin resistance leads to increased de novo lipogenesis and increasing triglyceride levels. The main pathways for the treatment of steatosis include weight control, activation of the AMPK and the inhibition of the SREBP-1c. AT, adipose tissue; DNL, de novo lipogenesis; FFAs, free fatty acids; IR, insulin resistance; TG, triglycerides; AMPK, AMP-activated protein kinase.

model to provide an initial explanation for NASH occurrence, based on which Buzzetti and Pinzani [11] proposed a multiple-strike model in an attempt to refine the progression model of NASH further. After the onset of NAFLD, hepatocytes continue to experience a series of blows, including oxidative stress, inflammatory damage, and lipotoxicity, leading to a continuous deterioration of the intrahepatic environment and accumulation of severe liver damage, i.e., NASH. The first two, oxidative stress and inflammatory damage, are the key mechanisms for the development of NASH.

Studies have shown that large amounts of saturated fatty acids in hepatocytes can trigger endoplasmic reticulum stress [24], leading to c-JNK activation, further worsening the hepatic IR and inflammatory status [25], creating dangerous positive feedback. In this process, large amounts of free fatty acids increase the permeability of the inner mitochondrial membrane, leading to the production of reactive oxygen species (ROS), which in turn triggers mitochondrial dysfunction [26, 27], leading to NASH progression and changes in intrahepatic biomarker expression.

In addition to oxidative stress, lipotoxicity and inflammation are also closely associated with the development of NASH.

When high levels of free fatty acids (FFAs) enter the circulation and the liver, they activate c-JNK

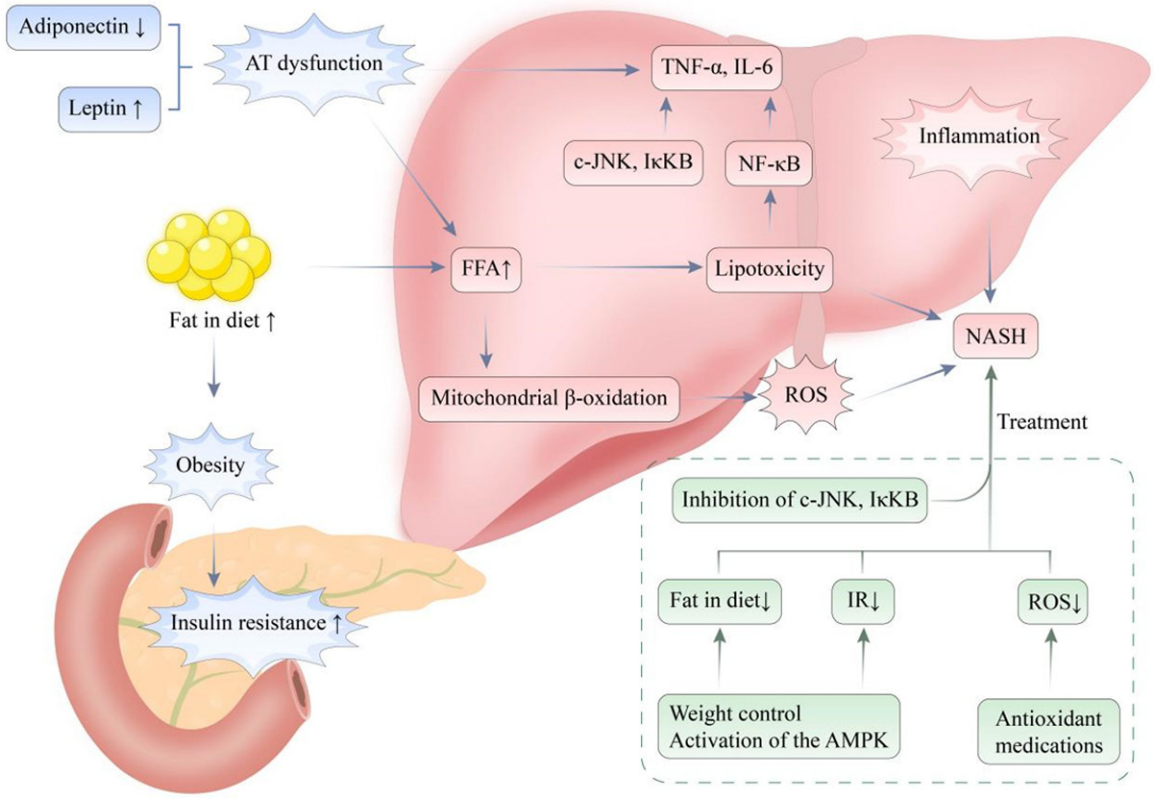

Figure 2. In non-alcoholic steatohepatitis (NASH), the mechanisms include reactive oxygen species (ROS) and inflammation. The increase in free fatty acids (FFA) delivered to the liver leads to lipotoxic molecules and mitochondrial β-oxidation. The main pathways for the treatment of NASH include weight control, activation of the AMPK, antioxidant medications and inhibition of relevant inflammatory pathways, such as c-JNK, IκKβ. AT, adipose tissue; DNL AT, adipose tissue; c-JNK, c-Jun NH2-terminal kinase; FFAs, free fatty acids; IκKβ, inhibitor of nuclear factor kappa-B kinase subunit beta; IL-6, interleukin 6; IR, insulin resistance; NASH, non-alcoholic steatohepatitis; ROS, reactive oxygen species; TNF-α, tumor necrosis factor alpha; AMPK, AMP-activated protein kinase; NF-κB, Nuclear Factor Kappa B.

and IκKB serine/threonine kinases in the liver, which are involved in the hepatic IR process, and the concomitant lipotoxicity brings direct damage and inflammatory response to the liver [12]. Because of the onset of injury, there is a sustained activation of the NF-κB pathway [28, 29] and overproduction of inflammatory factors such as TNF-α and IL-6, exhibiting a sustained inflammatory response. The mitochondrial ClpXP protease system accelerates TUFM protein degradation and impairs cellular autophagy while activating NLRP3 inflammatory vesicles, further leading to uncontrolled liver inflammation [30].

As previously stated, dysfunctional adipose tissue is characterized by reduced levels of lipocalin [31] and increased leptin levels. Reduced secretion of lipocalin, which has anti-inflammatory effects, and further increased expression of leptin, which has pro-inflammatory effects, lead to a more uncontrollable development of inflammation.

Uncontrolled intrahepatic inflammation leads to direct inflammatory damage, as evidenced by immediate histological changes, such as scattered lobular microgranulomas (Kupffer cell aggregates) and fatty granulomas around hepatocytes, and in severe cases, fibrosis. At the cellular level, this inflammation is manifested by ballooning, apoptosis, or lysis necrosis. Large, deeply stained nuclei are usually visible within the cells, often accompanied by distinct nucleoli, Mallory ring lamellar vesicles, giant mitochondria, glucose progenitors, and iron precipitates [32, 33]. At this point, the intrahepatic environment is extremely deteriorated, the progression of NASH has entered an advanced stage, and more severe liver fibrosis or hepatocellular carcinoma is highly likely to occur along with it (**Figure 2**).

Treatment of NAFLD

The prevailing view suggests that the primary threats associated with NAFLD are currently cardiovascular disease and malignancy [34-37]. Consequently, the primary focus in NAFLD treatment is directed towards mitigating the drivers of steatosis and systemic inflammation, with the aim of reducing the risk of cardiovascular disease.

Weight control and dietary optimization are recognized as pivotal in ameliorating the progression of NAFLD and NASH. Beyond its crucial role in addressing insulin resistance, weight loss has proven effective in diminishing liver fat content and curtailing the development of steatohepatitis. Achieving weight loss exceeding 10% has even demonstrated a reduction in the incidence of liver fibrosis. However, sustaining weight loss remains a formidable challenge, necessitating a well-defined plan, personal perseverance, and overcoming various medical obstacles. While bariatric surgery is not the primary treatment choice due to associated surgical risks, it can yield substantial (15-25%) and enduring weight loss in severely obese patients, leading to improved histological features in NASH and fibrosis. Weight loss further contributes to the enhancement of NAFLD and all its related cardiometabolic comorbidities, offering favorable impacts on cardiovascular and malignancy-related risks.

The activation of the AMPK (AMP-activated protein kinase) pathway and the inhibition of the SREBP-1c (sterol regulatory element-binding protein-1c) pathway are deemed effective strategies in the improvement of fatty liver [38, 39]. Activating AMPK enhances lipid oxidation and glucose uptake, promotes fatty acid oxidation, and reduces fat accumulation in the liver. Additionally, AMPK activation inhibits insulin resistance, contributing to the amelioration of the insulin signaling pathway, thereby mitigating the development of fatty liver. Conversely, inhibiting SREBP-1c disrupts a key step in lipid synthesis, resulting in diminished fat synthesis and accumulation in the liver. The synergistic interplay between these two pathways offers a potential multi-faceted intervention for the treatment of fatty liver.

Furthermore, antioxidant medications, such as vitamin E, have exhibited significant benefits in managing NAFLD. These advantages have been substantiated through various randomized controlled trials. Vitamin E usage has proven effective in mitigating hepatic steatosis and necroinflammation, preventing hepatic decompensation, and reducing mortality rates in patients with advanced liver fibrosis [40, 41]. These findings illuminate a promising avenue for NAFLD treatment, underscoring the potential efficacy of antioxidant drugs as active participants in future treatment strategies.

Animal models

There are three types of mainstream animal models of NAFLD, namely dietary models, genetic models, and chemically induced models, corresponding to several key factors in the development of NASH (**Table 1**).

Dietary model

Poor dietary habits primarily induce NAFLD in humans. Therefore, diet is an important component in the development of the disease. Based on diet, the pathogenesis of NAFLD and NASH can be simulated in animals by simulating poor dietary habits, such as high fat, high sugar, and high cholesterol, to establish a relevant model.

Methionine and choline deficient (MCD) diet model: Choline is a crucial nutrient mainly metabolized and stored by the liver. Corbin et al. [42] found that choline deficiency triggers abnormal phospholipid synthesis, abnormal lipoprotein secretion, and mitochondrial dysfunction in hepatocytes, leading to fatty liver and liver injury. Lee et al. [43] found that the transition from simple steatosis to NASH was closely associated with decreased methionine metabolism. Dietary choline and methionine deficiency can significantly decrease hepatocyte LDL levels, accompanied by hepatic steatosis, oxidative stress, hepatocyte necrosis, and severely affected cytokine levels.

The classical MCD diet model contains 10% fat and no choline or methionine components. Therefore, mice fed MCD diets develop extensive hepatocellular inflammation within 2 weeks and significant liver fibrosis within 6 weeks [44, 45]. The NAFLD model is usually completed after 2-4 weeks of feeding, and the

Table 1. Animal models of non-alcoholic fatty liver diseases

Model	Diet composition	Obesity	Steatosis	NASH	Fibrosis	HCC	NASH induction period (weeks)
High-fat (HF) diet	71% fat, 11% carbohydrates, and 18% protein	+	+	Mild	+	-	12-16
Methionine- and choline-deficient (MCD) diet	Diet usually consists of sucrose (40% of energy) and fat (10%). But, it is deficient in methionine and choline	-	+	+	+	-	5-8
High-cholesterol (HC) diet	Often fed in conjunction with high fat (15%) or high cholate (0.5%)	+	+	+	+	-	>20
HF+HC diet	/	+	+	+	Mild	-	6-42
Carbon tetrachloride	/	-	+	+	+	-	0.5-8
Thioacetamide	/	-	+	+	+	+	4-8
High-fat diet + streptozotocin	24.8% protein, 46.7% nitrogen-free extract, and 14.4% fat, with 200-μg streptozotocin injection	+	+	+	+	+	2-16
foz/foz mice	HF+HC diet	+	+	+	+	-	16-28
ob/ob mice or db/db mice	MCD	-	+	+	Mild	-	4-10

HC, high-cholesterol diet; HCC, hepatocellular carcinoma; HF, high-fat diet; MCD, methionine- and choline-deficient diet.

NASH model can be completed after more than 5 weeks of continuous feeding.

The main advantage of the MCD diet model is the high consistency in modeling the pathological outcomes of human NASH compared to other dietary models. The MCD diet model is faster and more severe in modeling time than other models, such as HFD, and can also be used to model NASH infection [46].

However, in practice, the metabolic profile of the MCD model and normal human NASH differed significantly. Model rats did not exhibit obesity characteristics and no insulin resistance profile, with only low levels of downregulation of serum pancreatic hormones, fasting glucose, leptin, and triglycerides [47, 48].

Therefore, this model is considered to be mainly suitable for studying the disease progression, inflammatory damage, and fibrosis mechanisms in NASH.

High-fat diet (HFD) model: The high-fat diet (HFD) model was developed by Charles S Lieber [49], who fed mice a high-fat diet to induce obesity, and obesity-induced hepatic steatosis is an essential factor in modern obesity.

The most typical HFD diet - 71% of energy from fat, 11% from carbohydrates, and 18% from protein - was fed continuously for 12-16 weeks to establish the NAFLD model. The model animals exhibit increased plasma insulin and NASH symptoms, such as mild inflammation and mitochondrial abnormalities in hepatocytes, but do not show fibrosis progression compared to human NASH pathology.

A high-fat diet results in a higher percentage of lipid droplets accumulating in hepatocytes compared to other dietary models. Wistar male rats were fed the same amount (15 g/day) of diets with different compositions, high fat, medium fat, high sucrose, and high fructose, for sixteen consecutive weeks. The high-fat group had the largest body weight and liver weight, and the highest rate of hepatic fat degeneration (40%) [50, 51].

The advantage of the HFD model is that it effectively mimics the cytopathological structure and pathogenesis of human NAFLD, which is reflected by the fact that they induce the hallmark features of human NAFLD, such as obesity and insulin resistance. However, compared with the MCD model, the liver damage in the HFD model is not as severe, and there is no significant fibrosis in the pathology, while its modeling time is much longer than that of the MCD model.

High cholesterol diet model (HCD): Morales and Caballeria [52] were the first to find that high-cholesterol foods lead to a selective depletion of mitochondrial glutathione (mGSH), which promotes the expression of TNF-α in the liver, increases the intrahepatic inflammatory response, and promotes the development of NASH. Meanwhile, studies have found that low dietary cholesterol reduces VLDL synthesis and fatty acid oxidation of β, accelerating cellular scorching and hepatic antioxidant response [52].

After finding that cholesterol is a crucial cause of steatohepatitis and liver inflammation development in animal models, Christopher and Erica [53] further synergized cholesterol with fat to establish animal models. Rats fed only a high cholesterol diet (HCD) (1%) had significantly increased serum insulin levels, while liver weight, triglycerides, free fatty acids, and serum ghrelin were only mildly increased. However, the mice showed a distinct NASH profile after high cholesterol, protein, and bile acid intake.

Further optimization of the feed formulation, using an animal model of HFHC with 15% fat and 1% cholesterol, demonstrated more significant weight gain, a higher percentage of hepatic fat accumulation, and higher levels of human serum ALT, corresponding to a significant reduction in lipocalin, and the appearance of cellulite and fibrosis, characteristics that made the disease more pronounced and severe in HFHC experimental mice compared with HFD or HCD mice [53].

To show more realistic changes of NASH in humans, François and Emmanuel et al. [54] improved the high-fat-high-cholesterol experiment by using a modified multi-fat-high-cholesterol diet (40.8% lipids, 14.8% protein, 44.4% carbohydrates and 0.5% cholesterol) and a drink enriched with 10% fructose to feed golden rats. Insulin resistance, lipid abnormalities and NASH-related disease features (microcys-

tic lipid degeneration, inflammation, hepatocyte swelling, and bridging fibrosis near the hepatic sinusoids) were observed after 20 weeks. Further elevated cholesterol ratios were manifested by more severe steatosis, inflammation, hepatocyte swelling, and fibrosis [33, 55].

Combined high-fat and high-cholesterol dietary models can respond exceptionally well to human NAFLD and NASH (obesity and insulin resistance) and are superior to comparable dietary or chemically induced or genetic models. However, after a high-fat diet, these dietary models appear less prone to inflammation, hepatocellular swelling, and fibrosis.

In terms of experimental animal selection, the use of C57BL6/J mice modeled NASH with only mild steatosis and little inflammatory cell infiltration. By contrast, the selection of golden Syrian hamsters better promotes NASH with significant inflammation, hepatocyte swelling, and fibrosis and with lipoprotein and bile acid metabolism levels closer to those of humans [54], making it a highly relevant model of NASH.

High fructose diet: Basaranoglu [56] and Nomura and Yamanouchi [57] reviewed the driving role of fructose in the process of NAFLD and NASH. This effect is primarily caused by the liver's efficient uptake and metabolic processing of fructose. Solute carrier family 2 facilitated glucose transporter protein member 5 (Slc2a5 or Glut5) is a membrane transporter protein that explicitly facilitates the uptake of dietary fructose from the intestinal lumen into the portal circulation. Slc2a5, together with family member Slc2a2/Glut2, efficiently transfers fructose from the blood to hepatocytes in an insulin-independent manner. In the liver, fructose is rapidly metabolized via glycolytic and lipogenic pathways, bypassing the major rate-limiting steps in glucose metabolism. Abdelmalek [58] and Ouyang [59] have further validated that there is a direct association between high fructose intake and fatty liver disease in humans.

High fructose is not usually used as a standalone modeling tool but instead increases the severity of model liver injury by adding the presence of fructose to a high-fat diet.

Genetic models

The relative homeostasis of cellular signaling pathways is fundamental to maintaining individual health and abnormalities in signaling pathways, such as PI3K/AKT [60]. For instance, it drives hepatic steatosis toward NASH and eventual evolution to liver fibrosis and liver cancer. Therefore, modifying alterations of specific genes can likewise serve the purpose of constructing animal models of NASH.

Leptin-related mouse model: The primary effects of leptin are reducing food intake and increasing energy use. It prevents fat accumulation in organs other than the AT and promotes inflammation and liver fibrosis. NAFLD and NASH patients typically exhibit leptin resistance, triggering a compensatory increase in leptin levels [61]. Therefore, leptin is often considered to have a direct association with NAFLD.

Mouse models associated with leptin hormone signaling usually consist of db/db mice and ob/ob mice.

Db/db mice, which are pure-sibling mice with an autosomal recessive diabetes mellitus (Db) gene, have a nodal mutation in the Db gene encoding the leptin receptor (Ob-Rb) that impairs leptin hormone [33] signaling. Db/db mice typically exhibit obesity, insulin resistance, and hypertension but do not spontaneously develop NASH and liver fibrosis [62].

Unlike db/db mice, ob/ob mice have only autosomal recessive deformations of the leptin gene, which directly lead to difficulties in leptin synthesis and consequently affect leptin hormone signaling. Ob/ob mice usually exhibit extreme overweight, hyperphagia, hyperinsulinism, hyperglycemia, and insulin resistance and continue developing spontaneous hepatic steatosis [62] but do not progress to steatohepatitis. Notably, as leptin is required for hepatic fibrosis [63], ob/ob mice are essentially resistant to hepatic fibrosis.

Notably, Suriano et al. [64] found that differences in the metabolic profile of db/db and ob/ob mice were determined by their intestinal environment, including intestinal flora composition, intestinal flora fractions (e.g., LPS), intestinal-derived metabolites (e.g., SCFAs), and bile

acid profile. In addition to metabolic profiles, inflammatory profiles also differ significantly. Adipose tissue is more affected by inflammation in db/db mice, whereas in ob/ob mice, this inflammation is transferred to the liver.

As a class of genetic models, the db/db and ob/ob mouse models do not show significant manifestations of inflammation and fibrosis without providing additional stimuli and serve only as a valid model of NAFLD.

When further given dietary changes, such as MCD feeding, db/db mice and ob/ob mice exhibit NASH progression and can be used for related studies. Notably, genetic mutations resulting in congenital leptin deficiency and leptin resistance are infrequent in the human obese population [65], so db/db and ob/ob mouse models are of limited applicability in reflecting human obesity, IR, and hepatic steatosis. However, most obese and diabetic patients develop simple steatosis without severe necroinflammation or extensive fibrosis and, therefore, can be used as animal models to characterize most NAFLD conditions.

Foz/Foz mice: Foz/Foz mice possess a mutated Alms1 gene, which encodes a protein in the primary ciliary matrix that plays an important function in intracellular transport and appetite regulation [66, 67].

Foz/foz mice exhibited mainly overweight and overeating and also showed IR, significantly decreased lipocalin levels, significantly increased cholesterol levels, and steatosis. Additional HFD feeding to foz/foz mice increased metabolic complications and contributed to the metastasis of steatosis and NASH with severe fibrosis, which decreased lipocalin capacity and increased cholesterol.

There is no spontaneous steatohepatitis in the Foz/Foz mouse model, but there are only spontaneous alterations in fat levels that must be subjected to food and chemical conditions before they can evolve into steatohepatitis or some severe disease. The severity of NASH induced by the diet of Foz/Foz animals depends mainly on the mouse strain. IR, hyperinsulinemia, and liver fibrosis can be observed in Foz/Foz animals on C57BL6/J background but rarely in Foz/Foz animals on BALB/C background [68].

Compared with other animal models, the main advantage of Foz/Foz mice is that they possess more pronounced lipid-like deposition. Therefore, Foz/Foz mice are more valuable for developing lipid-reducing drugs.

Chemically induced models

The main chemical toxins commonly used in chemically induced models are carbon tetrachloride (CCL4), thioacetamide (TAA), and streptozotocin (STZ), which are mainly seen in cirrhosis and HCC.

The mechanism of hepatotoxicity of CCL4 and TAA is not fully understood; however, studies have found that the hepatotoxicity of CCL4 is mainly related to its activation of NLRP3 inflammatory vesicles [69].

Similar to the MCD model, the disadvantage of the chemically induced model is that it causes weight loss in mice and does not correspond to the clinical manifestations of human NASH; the advantage is that steatosis and inflammation can occur quickly. Therefore, the current chemically induced models are mainly used to study the therapeutic targets of antifibrosis.

Conclusion

The triggers of NAFLD are diverse, and it is difficult for the various model animals to mimic all pathophysiological processes completely; therefore, there are individual peculiarities, some focusing on mimicking the cytopathological processes of NAFLD without biological features and others on the contrary. Although these models cannot fully generalize the processes of NAFLD and NASH, which is the main drawback of the models, a directional selection of representative models according to different experimental purposes can somewhat avoid these problems.

In addition to exploring the mechanism of NAFLD, these models are also an important tool to explore potential targets for treating NAFLD to some extent. After more in-depth exploration and further deepening of the understanding of fatty liver disease, it is believed that more complete models of NAFLD and HASH will undoubtedly be established shortly.

Discussion

Currently, the closest model to human NAFLD is the HFHD model, which can show the hallmark features of human NAFLD, such as obesity and insulin resistance. This model lacks the pathological features of NASH, such as inflammation and fibrosis, and can fully represent NAFLD by combining it with the high cholesterol model; however, its drawback of long production time has to be solved.

By contrast, the MCD model can mimic the pathological outcome of human NASH in a short time, and by combining it with a leptin-related model db/db mice [70], it can also better replicate the human clinical presentation. A study found that injecting glucagon-like peptide-1 analogs into db/db mice fed an MCD diet was effective in improving steatohepatitis and could reduce free fatty acid levels in the liver, suggesting exendin-4 as a possible therapeutic target for non-obese NASH patients [71]. In the HFD model, by adding lard and cholesterol to the feeding, significant fibrosis could be observed in SD rats at 24 weeks, significantly improving the problem of low liver injury in a single HFD model. Therefore, the combined use of multiple models is more efficient in modeling time and reliability compared with single means and, to some extent, compensates each other for the defects between models.

Several mechanisms relating to the development of NASH remain to be discovered, including the effect of the mitochondrial MRG15 TUFM regulatory pathway [30] on NASH and the role of CXCR3 (chemokine receptor 3) in the development of NASH [72]. With the progressive discovery of these mechanisms, the development of models for NASH will inevitably continue to spiral upward.

Disclosure of conflict of interest

None.

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