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IN-VIVO MODELS OF NON-ALCOHOLIC FATTY LIVER DISEASE

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ABSTRACT: Non-alcoholic fatty liver disease (NAFLD), a common chronic liver condition, is high in developed nations. One of the factors leading to chronic liver disease and cryptogenic cirrhosis has been identified as NAFLD. Multiple risk factors, including obesity, insulin resistance, diabetes mellitus, hyperlipidemia, and hypertension-related cardiovascular disease, contribute to the development of NAFLD. The pathogenesis of NAFLD involves a complex interplay among several key pathological processes, including insulin resistance, abnormal lipid metabolism, oxidative stress, inflammation, apoptosis, and fibrosis. The initial stage of NAFLD, known as hepatic steatosis, manifests as excessive fat accumulation within the liver. Although extensive research efforts have led to the identification of these risk factors, there remains a limited understanding of the disease initiation and the underlying molecular mechanisms driving its progression. Animal models of NAFLD give crucial information, not only in elucidating the pathogenesis of NAFLD but also in examining the therapeutic effects of various agents. An ideal model of NAFLD should correctly reflect both hepatic histopathology and the pathophysiology of human NAFLD. This review summarizes diet-induced and genetic animal models, used in recent years to add to the understanding of the mechanisms involved in NAFLD.

INTRODUCTION: Non-alcoholic fatty liver disease (NAFLD) is a prevalent chronic liver condition that carries a significant metabolic risk of several health issues, including dyslipidemia, insulin resistance, obesity, and type II diabetes. NAFLD is a spectrum of fatty liver disease that includes the three basic pathological subgroups of liver steatosis, non-alcoholic steatohepatitis (NASH), and fibrosis, and it is more common than metabolic syndrome. Simple liver steatosis, which is caused by a significant accumulation of fat in liver cells, is the least serious stage [1].

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The global prevalence of NAFLD is estimated to be 25% and continues to rise worldwide in the setting of the obesity epidemic. In India, the prevalence of NAFLD ranges from 9% to 53% of the general population. More recently, a population-based study from coastal south India reported an overall NAFLD prevalence rate of 49.8%; after controlling for sex, body mass index (BMI), diabetes, and metabolic syndrome, urban residence was found to be associated with a higher risk for NAFLD [2].

There is currently no FDA approved pharmaceutical agent for the treatment of NAFLD. A recommended intervention for NAFLD is a lifestyle change, which includes calorie restriction and increased physical activity. Pharmaceutical treatments have included lipid-lowering medications, insulin sensitizers, and antioxidants [3]. Impaired insulin signaling increases fatty acid (FA) production *via* lipolysis in white adipose tissue

(WAT). The result is increased hepatic IR, substrate overload, and increased de novo lipogenesis, as well as increased triglyceride (TG) build-up. WAT-generated FA accounts for approximately 60% of FA in NAFLD with hepatic TG build up. 15% come from diet, while 25% come from enhanced de novo lipogenesis, which is controlled by PPAR and the carbohydrate response element-binding protein (CREB) [4].

Simple steatosis, the most common type of NAFLD, has long been thought to be a relatively benign state of liver injury; however, human studies show that fatty livers are more vulnerable to injury from various causes and progress more quickly to steatohepatitis, increasing the likelihood of further liver-related morbidity and mortality [5]. Due to ethical constraints in tissue collection, but also therapeutic interventions (e.g., drug testing), and because the occurrence of steatosis, but more importantly the progression to later stages of the disease (e.g., NASH, fibrosis, or cirrhosis), may require a long period to study. Animal models resembling conditions of the early stages of NAFLD in humans (e.g., steatosis and steatohepatitis). The current review will concentrate primarily on NAFLD dietary and genetic animal models [6].

Animal Models of NAFLD: NAFLD usually develops over time and is caused by a combination of risk factors such as obesity, an inappropriate dietary pattern (high fat and/or sugar intake), inactivity due to a sedentary lifestyle, and possibly genetic susceptibility. These factors cause a slew of molecular changes in the human body [7]. As a result, the following criteria should be included in animal models used to study the development of NAFLD and its progression to later stages of the disease such as NASH or even fibrosis and cirrhosis: i) the pathogenic patterns and histological changes found in various stages of human illness. (ii) The fundamental physiological changes associated with disease progression in humans (such as weight gain, insulin resistance, but also decreased intestinal barrier. In addition to steatosis, an appropriate NAFLD animal model should exhibit signs of inflammation, liver cell damage (such as ballooning hepatocytes), and fibrosis. In addition to the elevated levels of bacterial endotoxins commonly observed in NAFLD, the model should account for metabolic issues such as obesity, insulin resistance, poor glucose tolerance, dyslipidemia, and altered adipocytokine profiles [8].

Dietary Fat Animal Models of NAFLD: Nutrition plays an important role in the pathogenesis of NAFLD. It has been proposed that different dietary components influence the progression of NAFLD. Fat accumulation stimulates lipolysis in adipocytes and raises free fatty acid (FFA) levels, resulting in lower plasma lipid clearance and increased -oxidation in muscles [9]. In various preclinical animal studies of NAFLD using various sources of dietary lipids, the master role of specific types of dietary fat in NAFLD progression has been proposed and extensively discussed. Diverse dietary lipids have some distinguishing characteristics, including differences in fatty acid composition, such as degree of saturation (e.g., saturated, mono- and polyunsaturated fatty acids). Recent research suggests that elevated FFA, particularly saturated fats (SFAs), may be involved in lipotoxic mechanisms in animal models. Indeed, SFAs have long been considered the most dangerous of dietary lipids due to their toxic effect on a variety of cell types. They can directly affect hepatocytes via several mechanisms, including death receptor signaling and ER stress initiation, which results in intrinsic mitochondrial apoptosis, receptor motivation, the appearance of inflammasomes, and autophagy blockage. A small sample study discovered that people with NASH absorb more saturated fat and cholesterol and lower absorption of polyunsaturated fatty acids (PUFA) [10].

High-fat Diet Induced NAFLD: High-fat diet (HFD) associated obesity is greatly common in patients with NAFLD which is emerging as one of the most universal causes of liver disease worldwide, especially in Western countries. Despite its high prevalence, only a small proportion of those affected will become inflamed, followed by fibrosis and chronic liver diseases and the majority of patients will only show simple steatosis. According to epidemiological studies, a high-fat diet may be a risk factor for the development of obesity and insulin resistance. Following a high-fat meal, large amounts of chylomicron-TG are delivered to the liver and undergo lipolysis in the lysosomes, resulting in the

release of large amounts of FA. Steatosis, diabetes, and obesity are all caused by an increase in dietary-derived FA. These models are useful because they do not require an unphysiological procedure to produce NASH-like characteristics. As a result, high-fat diets (HFD), with 30%-75% of total calories derived from saturated fatty acids (unsaturated fatty acids), have been proposed as a useful tool for induced metabolic changes and NAFLD [11].

HFD consumption is a risk factor for NAFLD. Many animal models use HFD to cause NAFLD [12]. When a healthy individual consumes dietary fat, the lipids are converted into triglycerides in the intestine and packaged into chylomicrons for delivery to surrounding tissues (mainly muscle and adipose tissue). FAs are released by the topical effect of lipoprotein lipase when chylomicrons arrive at target tissues (LPL). Adipose tissue extracts and stores free fatty acids from chylomicrons; however, some FFAs (33-36% of total delivery) overflow into the circulatory system and become available for absorption by the liver. However, hepatic steatosis develops in animals within a few days of HFD exposure [13].

This is not surprising given that the liver accepts approximately 75% of the bloodstream from the hepatic portal vein, which provides venous blood from the esophagus, stomach, and intestines. Furthermore, the typical American diet provides the liver with up to 20 g of fat per day, accounting for roughly half of the total triglyceride content [14]. As a result, the liver is extremely vulnerable to diet-induced steatosis.

TABLE 1: HIGH FAT DIET-INDUCED NAFLD

Sr. no.	Animal	Diet composition	The route, Dosing, and Study duration	Postulated biological mechanisms	Biochemical Evaluations	Ref.
1.	C57BL/6 mice	HFD Contains 60% fat	Orally, 100 mg/kg/day, 16 weeks	Impaired Glucose and Lipid homeostasis	Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), High-density lipoprotein-cholesterol (HDL-C), Triglycerides (TG), Total cholesterol (TC)	15
2.	Wistar-albino rats	HFD contains 83% of basal fodder, 10% of lard, 5% egg yolk powder, and 2% of cholesterol	Orally,25, 50, 100, and 200 mg/kg/ day,6 weeks	Oxidative stress	Low-density lipoprotein (LDL), TG, HDL, TC	16
3.	Albino Wistar rats	HFD contains 21.3% Protein,33.7% Carbohydrate, 45% fat	Orally, 45, 90, and 180 mg/kg, 6 weeks	Visceral obesity, Altered lipid metabolism, Insulin resistance	Liver index, Malondialdehyde (MDA), Serum ALT, Free fatty acids (FFA), Insulin, Glucose, Homeostatic model assessment of insulin resistance (HOMA-IR)	17
4.	C57BL/6 J mice	HFD contains 45% fat	Orally,50 and 200mg/kg, 8 weeks	Altered lipid metabolism, Hepatic steatosis	Serum glucose,HDL-C, LDL-C, FFA, Insulin, Leptin, Adiponectin, TG, TC, ALT, AST, HOMA-IR	18
5.	Sprague Dawley rats	HFD contains 40% fat	Orally, 40 and 80 mg/kg/day, 8 weeks	Increase liver fat and Hepatic insulin resistance	Alkaline phosphatase (ALP),Albumin (ALB), AST, ALT	19
6.	Sprague Dawley rats	HFD contains 60% fat	Orally, 50 and 100mg/kg/day	Abnormal lipid accumulation, Oxidative stress	Catalase (CAT), Glutathione peroxidase (GPx), Superoxide dismutase (SOD), ALT, TG, AST, TC	20
7.	Sprague–Dawley rats	HFD contains 40% beef tallow	Orally, 125, 250, and 500 mg per kg body weight per day, 20 week	Liver injury and Insulin resistance through oxidative stress	Creatinine, Uric acid, Glucose, TC, AST, ALT, LDL, TG	21

High Fructose Diet-Induced NAFLD: Fructose is a highly lipogenic sugar present in processed foods and beverages in large amounts throughout the world. Fructose can be found in its monosaccharide form or can be bound to glucose with a disaccharide bond in sucrose. High-fructose corn syrup and sucrose (cane or beet sugar) are the primary dietary sources of fructose because they are commonly used to sweeten beverages and processed foods. Even though fructose does not raise insulin levels immediately, it does raise insulin resistance, fasting glucose, and insulin levels over time. In infusion/clamp studies, fructose induces hepatic and extra-hepatic insulin resistance in healthy adult humans, but the mechanism by which insulin resistance is induced is unknown. Consumption of high fructose increases visceral fat in animal models [22].

High-fructose diet upregulates the lipogenic pathway *via* the activation of lipogenic enzymes such as sterol regulatory element binding protein (SREBP) and fatty acid synthase (FAS) resulting in ectopic lipid accumulation and subsequent lipotoxicity. Fructose, either alone or in combination with increased de novo lipogenesis, may promote oxidative stress, in part through mitochondrial dysfunction and endoplasmic reticulum (ER) stress, both of which contribute to the development of an inflammatory process and the progression of simple steatosis to NASH. Fructose enters the liver rapidly and uncontrollably, primarily through the glucose transporter (GLUT2). This carbohydrate is preferentially converted into fructose-1-phosphate (F1P) at the cellular level by fructokinase, which has a high affinity for fructose, is not controlled by insulin, and is induced by fructose [23]. Following that phosphorizes produced from F1P by aldolase B can be converted into glucose, lactate, and fatty acids [24]. While the lipogenic pathway is minor in physiological conditions, it becomes very active after an acute fructose load as the flux of fructose carbons into lipogenic precursors increases, because F1P formation bypasses the glycolysis regulatory site of phosphofructokinase. Unregulated fructose entry and metabolism into hepatocytes explain why, in high fructose diets, significant amounts of this carbohydrate continue to enter glycolysis, resulting in excess acetyl-CoA production relative to liver oxidative capacities, promoting de novo lipogenesis. High fructose consumption also leads to an accumulation of glycolysis intermediates that can be converted to glycerol-3-phosphate for use in triglyceride (TG) synthesis by saturating the glycolytic pathway [25]. Chronic fructose consumption raises de novo lipogenesis by activating several key transcription factors, including Sterol Response Element Binding Protein 1c (SREBP1c) and Carbohydrate-Responsive Element-Binding Protein (ChREBP) [26]. As a result, their key target enzymes regulating lipid syntheses, such as Fatty Acid Synthase (FASN) and Acetyl-CoA Carboxylase (ACC), increase, as demonstrated in rodents fed a 60% high fructose diet for eight weeks or a western diet containing 30% fructose for eight weeks[27]. Fructose appears to be the most potent lipogenic carbohydrate contributing to the development of liver steatosis because it is both a substrate and an activator of de novo lipogenesis.

TABLE 2: HIGH FRUCTOSE DIET-INDUCED NAFLD

Sr. no.	Animal	Diet composition	The route, Dosing, and Study duration	Postulated biological mechanisms	Biochemical evaluations	Ref.
1.	Sprague Dawley rats	20% fructose	Orally,100mg/kg and 400mg/kg, 10week	Increase adiposity	Glucose, Insulin, TC, TG, HOMA-IR	28
2.	Sprague Dawley rats	70% fructose	Orally, 200 mg/rat/day, 5 weeks	Oxidative injury and Inflammation	Tumor necrosis factor-α (TNF-α), MDA,TG, LDL-C, TC	29
3.	Sprague Dawley rats	10% fructose	Orally, 40 mg /kg and 31 mg/kg, 5 weeks	Fatty liver, hepatotoxicity, Increase inflammatory cytokines	TC, HDL-C, LDL-C, TG, MDA, TNF-α	30-31
4.	Wistar rats	60%fructose	Orally,150 mg/kg,6 weeks	Increase hepatotoxicity	Serum AST, ALT, ALP, Total bilirubin (TB), Total protein and TC	32

Methionine- and Choline-Deficient (MCD) Diet-induced NAFLD: Mice fed an MCD diet are a frequently used nutritional model of NASH, that induces an elevation in aminotransferase and hepatic histological changes characterized by steatosis, focal inflammation, hepatocyte necrosis, and fibrosis. The MCD diet normally contains substantial amounts of sucrose (e.g., 40%) and low amounts of fat [10%] but is deficient in methionine and choline, both being essential factors in human and animal nutrition. Previous research using a choline-deficient (methionine-containing) diet suggests that the pathogenesis of hepatic steatosis may be due, at least in part, to impairment in hepatic VLDL secretion. This assumption is supported further by the fact that methionine and choline are precursors to phosphatidylcholine, the main phospholipid-coating VLDL particles. Thus, impaired VLDL secretion may play a role in MCD diet-induced hepatic lipid accumulation in mice. However, this model does not show increased FA, obesity, or IR, which are all known features of human NASH [33-34]. As a result, following an MCD diet causes hepatic steatosis. Additionally, oxidative stress and changes in cytokines and adipocytokines occur, all of which contribute to liver injury. In general, the MCD diet is easy to obtain and use, and it induces more severe histopathology of NASH than other dietary models. The degree of liver injury induced by an MCD diet depends on the species, strain, and sex of the. In recent study investigated the responses to an MCD diet of male and female Wistar, Long-Evans, and Sprague Dawley rats, as well as C57BL6 mice. The Wistar strain and male sex were linked to the highest level of steatosis in rats [35].

TABLE 3: METHIONINE AND CHOLINE-DEFICIENT (MCD) DIET-INDUCED NAFLD

Sr. no.	Animal	The route, Dosing, and Study duration	Postulated biological mechanisms	Biochemical evaluations	Ref.
1.	C57BL/6J mice	Orally, 50 and 150 mg/kg, 6 weeks	Fat accumulation and Neutrophils infiltration	ALT, AST, TG, TC	36
2.	C57BL/6J mice	Orally, 0, 0.1, 0.5, and 1 mg/kg, 12 weeks	Hepatocyte ballooning and Steatosis with mild inflammatory cell infiltration	TNF-α, Transforming growth factor-beta 1 (TGF-β1), Interleukin-1β (IL-1β), C-reactive protein (CRP), α-Smooth muscle actin (α-SMA), Matrix metalloproteinase-2 (MMP-2) and Matrix metalloproteinase -9 (MMP-9)	37
3.	C57BL/6 mice	Orally, 2 weeks	Induction of inflammatory cytokines and Oxidative stress	TG, TC, HDL, LDL, TNF-α, Interleukin -6(IL-6)	38
4.	C57BL/6J mice	Orally, 50 and 100 mg/kg/day, 4 weeks	steatohepatitis with fibrosis	TG, TC, ALT, AST	39
5.	C57BLKS/J lar Leprdb/Leprdb (db/db) mice	Orally, 10 and 100 mg/kg/day, 3 weeks	Inflammation of hepatocytes and NASH	Lactate dehydrogenase (LDH), ALT, AST, TC, HDL-C, TG, MDA, LDL-C	40
6.	C57BL/6 mice	Orally,500 mg/kg/day, 4 weeks	Histological changes such as Hepatic steatosis, Cellular inflammatory infiltrate and Hepatocyte necrosis	ALT, TG, MDA, Glutathione (GSH), Real-time PCR(RT-PCR)	41

Genetic Rodent Models of NAFLD:

Ob/ob Mice, db/db Mice, and Zucker Fatty Rats (fa): One molecule that regulates mice's energy balance is the obese (ob) gene. Ob/ob mice have been extensively studied and represent a naturally occurring model of NAFLD. These mice lack leptin due to a mutation in the ob gene, which limits the manufacturing of leptin. Ob/ob mice are hyperphagic, sedentary, and develop extreme obesity in the absence of leptin. These mice have a mutation in the ob gene, which encodes leptin, resulting in hyperphagia and obesity. The hepatocytes of these insulin-resistant mice spontaneously become steatotic, making them a valuable tool for studying. NAFLD Along with severe IR, these mice have hyperinsulinemia, which causes hyperglycemia and hyperlipidemia. Most crucially, when given a typical diet, they

acquire fatty livers on their own. However, despite having considerable obesity, ob/ob mice do not display steatohepatitis, including inflammation and fibrosis, due to their leptin insufficiency [42]. Increased inducible nitric oxide synthase in ob/ob mice muscle and liver cells and increased glucose flux *via* the hexosamine pathway in muscle are two mechanisms that can contribute to insulin resistance. Leptin has a direct effect on ob/ob mice skeletal muscle cells *in-vitro*, opposing insulin lipid-incorporating effect. This improves insulin resistance but does not cure it. Mitochondrial dysfunction can result in intracellular fat accumulation and lipotoxicity. Mitochondria are deficient in ob/ob mice adipose tissue, liver, skeletal muscle, and macrophages. Increased lipid peroxidation in the vicinity of mitochondria has been linked to the pathogenesis of lipotoxicity and ob/ob mice have been linked to an increase in hepatocyte reactive oxygen species production. In ob/ob mice, inhibiting lipid peroxidation reduces liver cell damage and it causes fatty liver and NASH [43].

The well-characterized recessive mutation of diabetes (db) also results in profound and early-onset obesity. Mice with the db mutation show an obese phenotype that is almost exactly that of ob/ob mice. Because their leptin receptor (Ob-R) is defective, db/db mice have higher serum levels of leptin. Overeating causes macrovesicular hepatic steatosis, significant obesity, and insulin resistance in these mice. These obese mice, however, do not develop fibrosis when fed a normal diet. This model requires a second hit, such as a methionine choline-deficient (MCD) diet, to induce steatohepatitis symptoms such as fibrosis [44]. The rat gene fatty (fa) is a homolog of the mouse db gene, and fa/fa rats are also thought to develop obesity and diabetes as a result of a mutation in the Ob-R gene locus. These rats, like db/db mice, develop steatohepatitis symptoms after being induced with a second hit. Obese (fa/fa) Zucker rats also have hyperphagia, which leads to hyperinsulinemia, hyperlipidemia, and the development of liver steatosis due to leptin receptor loss [45].

TABLE 4: MODEL OF OB/OB MICE, DB/DB MICE, AND ZUKAR FATTY RATS (FA)

Sr. no.	Animal	Diet	Biochemical evaluations	Ref.
1.	Ob/ob mice	AIN-93G diet	Serum glucose, Serum insulin, HOMA-IR, Total lipid, TG, FFA, ALT, GSH	46
4.	Ob/ob mice	Basal diet	RT-PCR, Total lipid, Triglycerides, TC, TG, ALT,Hepatic non-esterified fatty acid (NEFA), Serum NEFA	47
5.	Ob/ob mice and C57BL/6J mice	High-fat diet	HOMA-IR, Glucose, Insulin, RT-PCR	48
7.	Db/db Mice	Normal diet	ALT, AST, ALP, HDL-C,LDL-C, TG, TC, SOD, CAT and GSH	49
8.	C57BL/6J mice and db/db mice	Semisynthetic AIN-76 Diet	TG, TC, ALT, AST, Serum adiponectin, Insulin and Monocyte chemoattractant protein-1 (MCP-1) levels.	50
9.	Db/db mice	-	Serum TG ,FFA, ALT, AST	51
11.	Db/db mice	Normal diet	Serum levels of ALT, AST, TG, NEFA, TC, TG, Insulin, RT-PCR	52
13.	(fa/fa) Rats	High-fat diet	Glucose, Insulin, Sexual hormone binding globulin(SHBG), TC, TG, HDL, LDL, NEFAs	53
14.	(fa/fa) Rats	Standard laboratory diet	Total protein, TC,ALB, ALT, AST	54
15.	(fa/fa) Rats	AIN-93 diet	ALT,AST,ALP,TG,NEFA,Insulin,Glucose	55
16.	(fa/fa) Rats	Semisynthetic diet	TG, ALT, ALP, AST, lactate dehydrogenase (LDH)	56

Melanocortin 4 Receptor (MC4R): The melanocortin 4 receptor (MC4R) gene is mainly expressed in the feeding center of the hypothalamus and regulates food intake and energy expenditure. The MC4R gene is also known to be a cause of hereditary obesity in humans. Furthermore, MC4R gene-deficient mice given a high-fat diet are a NASH model that develops NASH-like fibrosis in 20 weeks and HCC in about a year based on obesity complications such as insulin resistance and dyslipidemia. The MC4R gene also regulates the autonomic nervous system and energy production in brown adipose tissue in mice; thus, feeding regulation is likely to be the cause of obesity in MC4R gene knockout (MC4R-KO) mice. The MC4R gene is rarely expressed in the liver, and its absence is an excellent pathological model of fatty liver and NASH [57].

TABLE 5: MODELS OF MELANOCORTIN 4 RECEPTOR (MC4R)

Sr. no.	Animal	diet	Biochemical evaluations	Ref.
1.	MC4R-KO male mice	High-fat diet	Lipase, TG, Glucose, and Blood ketone levels, Serum insulin and HOMA-IR	58
2.	MC4R-KO male mice	Standard diet	ALT, TG, FFA, TC, Adiponectin, Leptin	59
3.	MC4R-KO mice	-	AST, ALT, LDH, TG, TC	60

Sterol Regulator Element-Binding Protein 1c (SREBP): Sterol regulatory element-binding proteins (SREBPs) are a family of membrane-bound transcription factors that principally regulate lipid synthesis. An inherited lipodystrophic model with IR and steatosis, transgenic mice expressing nuclear SREBP-1c (nSREBP-1c) in adipose tissue under the control of the aP2 promoter, spontaneously developed steatohepatitis. Despite the absence of obesity, nSREBP-1c transgenic mice have IR, hypertriglyceridemia, elevated levels of transaminases, and mild inflammation, as seen on histological liver specimens, which are consistent with the findings in human NASH. In this model, however, fibrosis is not observed [61]. Steatosis, mononuclear cell infiltration, pericellular fibrosis, ballooning degeneration, and Mallory-Denk body formation are seen in the livers of these transgenic mice at 20 weeks or older, which is similar to that seen in NASH. However, the mice exhibit abnormal adipose tissue differentiation, significant insulin resistance, and diabetes mellitus; thus, the liver lesion may model steatohepatitis associated with lipodystrophy rather than normal NASH.

KKAy Mice: KKAy mice (also called lethal yellow KK mice) were originally developed by crossing KK mice with yellow obese mice (Ay mice). Because of the antagonism between melanocortin receptor 4 (MC4R) and ectopic expression of the agouti protein, KKAy mice (the yellow offspring obtained from a cross of black KK females with obese yellow Ay males) are obese, hyperglycaemic, hyperinsulinemic, hyper-triglyceridemic, hypercholesterolemic, insulin resistant, and exhibit steatosis with inflammation (steatohepatitis). The phenotype of KKAy mice, including altered adipokine expression, is similar to that of metabolic syndrome in humans, indicating that this strain could be useful as a model of metabolic syndrome-related NASH[62].

Tsumura–Suzuki obese Diabetes Mice: Tsumura–Suzuki obese diabetes (TSOD) male mice spontaneously develop diabetes mellitus, obesity, glucosuria, hyperglycemia, and hyperinsulinemia without any special treatments, such as gene manipulation. Therefore, TSOD is regarded as a polygenic model of metabolic syndrome. As NAFLD/NASH is associated with metabolic syndrome, determined whether these mice develop NAFLD/NASH. They observed microvesicular steatosis, hepatocellular ballooning, and Mallory–Denk bodies in the livers of 4-month-old mice, with increasing severity over time. Interestingly, small liver nodules with high cellularity and the absence of portal tracts were frequently observed after 12 months. Most of the nodules showed nuclear and structural atypia and mimicked human HCC. Recently, it has been suggested that splenic iron accumulation is involved in the development of NASH in TSOD mice. Although TSOD mice provide a natural model of NAFLD, the mice take a long time to develop the disorder, and the severity of steatosis and inflammation is mild [63].

CONCLUSION: As summarized and discussed in this review, many animal models, particularly rodent models of NAFLD have been developed and used in recent years to unrevealed the molecular mechanisms involved in the onset but also in the progression of this liver disease. However, the available models, be it *in-vivo* mimic certain disease aspects found in humans and markedly differ in regards to the degree of hepatocellular damage and metabolic alterations associated with the development of the disease.

Nevertheless, when chosen carefully, *in-vivo* models can be used to verify hypotheses on mechanisms underlying the development of NAFLD and as tools to test new therapeutic and prevention strategies. The future aim should be to develop animal models that more closely reflect the histopathology and pathophysiology found in humans with NAFLD, thereby, increasing the knowledge of the molecular mechanisms involved in the onset but also the progression of NAFLD and

providing the basis for the development of better therapeutic approaches to the disease.

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