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ANIMAL MODELS OF NON-ALCOHOLIC FATTY LIVER DISEASES (NAFLD)

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ABSTRACT

Animal models play a crucial role in understanding the pathogenesis and therapeutic interventions of Non-Alcoholic Fatty Liver Disease (NAFLD). Dietary models, including High Fat Diet (HFD), High Fructose Diet, Methionine and Choline Diet (MCD), Atherogenic Diet, High Fat High Cholesterol Diet (HFHC), High Fat High Fructose Diet (HFHF), and Choline Deficient L-Amino-Defined Diet (CDAA), mimic human dietary patterns and induce NAFLD phenotypes in rodents. These models replicate various aspects of NAFLD, such as hepatic steatosis, inflammation, and fibrosis, by altering lipid metabolism, insulin sensitivity, and oxidative stress. HFD and high fructose diets induce hepatic lipid accumulation, resembling the metabolic abnormalities observed in human NAFLD. MCD diet, deficient in methionine and choline, leads to severe steatohepatitis and fibrosis. Atherogenic diets rich in cholesterol and cholate promote hepatic inflammation and atherosclerosis, representing NAFLD with cardiovascular comorbidities. HFHC and HFHF diets exacerbate hepatic steatosis and dyslipidemia, mirroring the effects of Western diets. Chemical models, like CDAA, induce liver injury by depleting essential nutrients, leading to hepatocellular damage and fibrosis. These models facilitate the study of NAFLD progression and the evaluation of therapeutic interventions. Overall, animal models of NAFLD, both dietary and chemical, provide valuable tools for investigating the underlying mechanisms of the disease, identifying potential therapeutic targets, and testing novel treatment strategies. These models contribute significantly to advancing our understanding of NAFLD pathogenesis and guiding the development of effective clinical interventions.

KEYWORDS: Animal models, HFD, MCD, HFHC, HFHF, CDAA.

1. INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a group of liver diseases that develop when surplus fat builds up in the liver without being caused by excessive beverage use or another kind of liver disease. At one end of the range, simple steatosis, or the buildup of fat in the liver, evolves to the condition known as non-alcoholic steatohepatitis (NASH), which may eventually result in liver fibrosis.[1] Hepatic steatosis is present in NAFLD without any obvious symptoms of inflammation, but it is associated with nucleate inflammation and apoptosis in NASH, which can result in fibrosis and cirrhosis.[2] Triglyceride (TG) buildup in liver hepatocytes above an acceptable level of 5% is an early indication of NAFLD. Various histological anomalies are brought on by TG deposition without significant alcohol usage or other secondary causes of steatosis. This may progress to cirrhosis, fibrosis, and non-alcoholic steatohepatitis (NASH), which can lead to hepatocellular carcinoma.[3,4] NAFLD has grown to epidemic levels, affecting around 25% of the global population.[3,5,6] In several nations, NASH plays a substantial role in chronic liver disease and liver transplantation. This is due to the fact that this disease is identified by the formation of ballooning hepatocytes, which can make the condition more severe, and the development of inflammatory infiltrates in the hepatic lobules.[7,8] Obesity, dyslipidaemia, and hypertension are merely a few of the diseases associated with multiple sclerosis (MS) that are thought to be significantly influenced by insulin resistance (IR).[9,10]

In 2016, evidence from a meta-analysis of research released between 1990 and 2015 showed that NAFLD was the most predominant reason for chronic liver disease (CLD) with a worldwide incidence of approximately 25 percent.[11,12] The ensuing outcomes of the Global Burden of Disease (GBD) research have augmented this data and revealed confirmation that NAFLD is the disease burden associated with the sequelae of CLD, including cirrhosis and liver cancer, that is expanding the fastest globally.[11,13,14] A global epidemic of obesity and type-2 diabetes mellitus (T2DM) is the cause of this tremendous rise. Several ecological and inherited factors could expose people with this condition to chronic liver disease in addition to obesity and T2DM.[15,16,17] The prevalence of metabolic disorders raises the chance of developing NAFLD, advanced liver disease, and death in addition to raising the likelihood of having NAFLD.[18,19] Along with undesirable clinical consequences like higher mortality rates, NAFLD is also linked to a substantial economic burden and a reduction in the quality of life that is tied to one's health.[11,20] NAFLD incidence rates have increased globally due to the current worldwide epidemic of obesity and rise in sedentary habits brought on by the

COVID-19 pandemic, yet there is still no approved medication for dealing with the condition; instead, lifestyle changes are the primary line of treatment. In order to address the multiple pathophysiological pathways of NAFLD, there is an urgent need for a novel medicine.[21,22,23]

According to various investigations, NAFLD can be caused due to excessive intake of fats, particularly saturated fats, carbohydrates, especially refined carbohydrates, and meat-based proteins.[24-25] Additionally, increased consumption of meat and fizzy drinks have been associated to NAFLD in adults.[24] The most accepted therapy recommendations for NAFLD currently present are lifestyle and dietary changes.[26,27,28] Weight loss and enhanced physical exercise are two aspects of changing one's lifestyle.[26,29,30] Steatosis, dyslipidaemia, insulin resistance, and cardiovascular risk can all be improved, as well as hepatic inflammation and hepatocellular injury, by losing weight through reduced calorie consumption and greater physical activity.[24,27,30,31] All NAFLD patients are also counselled to strictly adhere to a number of dietary guidelines, such as abstaining from simple carbs, saturated fats, and sweetened beverages and consuming diets containing lots of fruits and vegetables.[26,27,31,32]

Several studies have examined on the association between NAFLD and dietary intakes, no comprehensive review has been done to compile the results. Therefore, the goal of this study was to comprehensively examine the observational investigations that have been conducted on the association between dietary habits, food groupings, and the development of non-alcoholic fatty liver.

2. PATHOGENESIS OF NASH/NAFLD

NASH progression is a complicated procedure that is still not entirely comprehended. Numerous studies on animals have been performed recently to better understand the pathophysiology of NAFLD and NASH, focusing mostly on the distinctions between various dietary models (methionine/choline deficient diet (MCD), high fructose or high fat).[33,34] It has been hypothesised that the occurrence of NASH involves two stages in accordance with the available body of research.[35] Fat accumulation in the liver, which will promote insulin resistance, is the initial stage of this physiological process.[33,36,37] The second stage of the process involves cellular and molecular alterations that are brought by oxidative damage and the oxidative degradation of fatty acids in the liver as a result of numerous factors, including cytokine injury, elevated insulin levels, hepatic iron and/or fatty acids peroxidation, fluctuations in the extracellular matrix, altered equilibrium of energy, and altered the

functioning of the immune system. It requires a complex procedure for insulin resistance to arise.[33] Increased accumulation of fat and differentiation of adipose cells are crucial factors in the emergence of resistance to insulin in the context of MS, as also the situation for many NASH patients.[33,36]

There are two main forms of NAFLD. The current consensus suggests that diabetes associated insulin resistance is the main pathophysiological mechanism for the first form of NAFLD, which has a restricted association with the metabolic disorder. The second form of NAFLD has an association to viral infections that might cause liver steatosis to develop.[33]

2.1 OXIDATIVE STRESS

Research studies have discovered a significant relationship between the level of cellular oxidative stress and the seriousness of NASH as well as the existence of mitochondrial stress related molecular markers in steatohepatitis humans well as animal models. The additional FFA content imposed due to obesity and IR is a significant contributor to oxidative stress in NASH.[38] Reactive oxygen species (ROS) generation may accelerate when mitochondrial βoxidation becomes over stimulated under conditions of FFA load.[38,41] The discrepancy between ROS and a cell's antioxidant defences under persistent oxidative stress causes lipid peroxidation and degradation, which in turn causes damage to cells such as damage to intracellular organelles, cell death, and activation of fibro genic stellate cells of the liver.[38,42]

2.2 PROINFLAMMATORY CYTOKINES

NASH is significantly correlated with aberrant generation of cytokines along with persistent inflammation of the liver. In NASH patients, there has been evidence of an increase in the production of proinflammatory cytokines like TNF-α and interleukin (IL)-6 etc.[38,43,45] TNF- and IL-6 both have an impact on adipokine levels. These proinflammatory cytokines lower adiponectin levels, which have anti-inflammatory, anti-atherogenic, and anti-diabetic characteristics. They also elevate leptin levels, which feeds the vicious process of persistent inflammation found in obesity.[38,46]

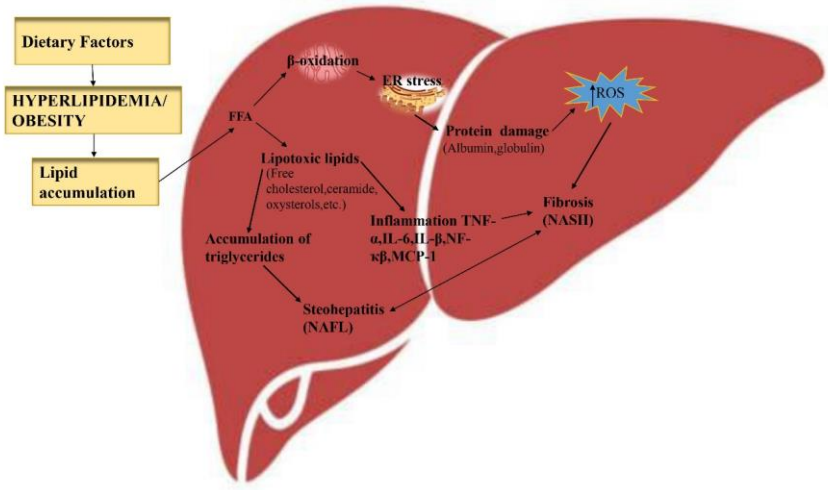

Figure 1: Pathogenesis of NAFLD/NASH. In NAFLD/NASH, In NAFLD/NASH, dietary factors such as high fat, sugar, and cholesterol intake promote oxidative stress, triggering ROS generation. These ROS, in turn, initiate lipid peroxidation and inflammation in the liver, contributing to the progression of hepatic steatosis to steatohepatitis and fibrosis. dietary factors such as high fat, sugar, and cholesterol intake promote oxidative stress, triggering ROS generation.

3. DIETARY MODELS

The primary diets used in the animal models of NAFLD are high-fat, high-fructose, cholesterol- and cholate-rich diets, methionine- and choline-deficient diets (MCD diets), choline-deficient L-amino acid-defined diets (CDAA diets), and others. Certain dietary patterns are substantially dissimilar from those that are common among people. For instance, the majority of human diets do not lack choline. The aforementioned diets frequently combine more than one dietary pattern to cause simple steatosis and steatohepatitis.[47] The hepatocellular histopathology characteristics and the biochemical impairment that are equivalent to clinical NAFLD have been attempted to be mimicked in some overnutrition-based models.[47,48]

3.1 HIGH FAT DIET ANIMAL MODEL

Ubiquitous high-fat diet (HFD) has been utilised to develop NAFLD animal models. This category of diets includes several kinds of regimens with fat contents that range from 45 to 75 kcal%.[49] Insulin resistance, pronounced pan lobular steatosis, inflammatory conditions, and cellular fibrogenesis occur in a rat model of the HFD (71% of calories come from fat, 11% from carbs, and 18% from proteins).[50,51,52] The overabundance of free fatty acids causes triglyceride buildup in the liver both directly through their dietary intake and

indirectly through enhanced lipolysis. It's interesting to note that after 1-2 weeks, steatosis appears, then fades for 6–12 weeks before returning.[38,53] A phenotype resembling the human disease is produced by the HFD, and it is signified by adiposity (after 10 weeks), diabetes, insulin resistance (hyperinsulinemia after 10 weeks and intolerance to glucose after 12 weeks), and elevated cholesterol levels (after 10 weeks).[53] The observed steatosis and inflammation are noticeably less severe than they are in rats fed the MCD diet, despite the fact that NASH typically appears within 12 weeks.[54,55] The plasma levels of alanine aminotransferase (ALT) have risen 9–10 times after 34–36 weeks.[56,57] Resulting from prolonged exposure (36–50 weeks), this diet only mildly promotes fibrosis.[57] Different strains and genders of mice exhibit various sensitivity levels to HFD. That is why dietary fat quantity, time span, and strains are factors in choosing this model. The model, which is frequently employed in metabolic research, also exhibits characteristics of real NAFLD/NASH progression and has a less severe pathogenic result than human NASH, notably in terms of the cellular fibrogenesis process.[58,59]

Mice fed a regular HFD exhibit a larger rise in body weight than mice fed trans fats (after 6–8 weeks), while examining the various kinds of fat used. However, after 8–16 weeks, mice fed trans fats have greater severity of steatosis and higher ALT levels. After 4 weeks, mice fed trans fats show considerably lower insulin sensitivity compared to mice fed a typical HFD. After up to 8 weeks, there are no alterations in the levels of cholesterol or triglycerides.[53] Trans fats appear to cause greater steatosis in rats than a typical lard-based HFD does after 13 weeks, and this is complemented by a greater degree of insulin resistance and a disordered lipid metabolism. But there are no variations in ALT levels between these two diets.[60]

3.2 HIGH FRUCTOSE DIET MODEL

Fructose is a monosaccharide sugar. The main site of metabolism of fructose is liver.[53,61] Consuming too much sugar has been linked to the emergence and worsening of NAFLD by aggravating the accumulation of fat, oxidative stress, inflammatory processes, diabetes, insulin resistance, and even fibrosis, as is the situation in the USA due to a large consumption of corn syrup.[47,62] A high-fat diet and fructose consumption promote insulin resistance, inflammatory reactions and stress in the ER by means of multiple pathways, such as free fatty acid (FFA)-derived lipotoxicity, and the generation of cytokines that are proinflammatory and chemokines.[58,63,63]

Mice supplied drinking water containing 30% fructose exhibit a 3- to 4-fold increase in hepatocellular triglyceride concentrations, an enormous rise in body mass index, as well as simple steatosis.[64,47] After 8 weeks, drinking water enriched with 20% fructose in male Wistar rats results into considerable overweight, elevated blood pressure, high blood sugar levels, and hypertriglyceridemia. Additionally, the lipid vacuoles in these rats have been formed, but there are no cells associated with inflammation or ballooning neurodegeneration.[65,47] After 8 weeks of therapy, gastrointestinal colonisation of bacteria is observed as well that is subsequently accompanied by elevated endotoxin levels in the portal bloodstream and Kupffer cell stimulation.[47] Nowadays, it is common practise to induce NAFLD in animal models and enhance the disease's progression to NASH and fibrosis of the liver in research laboratories due to fructose's substantial effects on gut and liver homeostasis.[66]

3.3 METHIONINE AND CHOLINE DIET (MCD)

The MCD diet is inadequate in methionine and choline[67], which are necessary nutrients for hepatic β-oxidation process and the generation of very low-density lipoprotein (VLDL), while having an excessive sucrose content and moderate fat amount (40% sucrose, 10% fat).[68] A deficiency in methionine can prevent the body from synthesising proteins. Lecithin contains choline, which is a potent organic base and a precursor of acetylcholine.[58,69] Phosphatidylcholine, a substance necessary for the synthesis of very low-density lipoproteins (VLDL), is derived from choline. A combination of extracellular lipoproteins called VLDL facilitates the transportation of triglycerides, which are fats, from the liver to the muscles and adipose tissue. Methionine is required for the synthesis of glutathione, a crucial protein that fights free radicals.[70] Methionine and choline are significant methyl group donors, despite the fact that the precise pathophysiology of this model is not completely clarified. It is essential for the maintenance of numerous physiological processes, including lipid metabolism, that the hepatocytes have an adequate methylation capability.[66,71]

Another consequence of choline deficiency is reduced VLDL secretion in liver, which leads to hepatic fat buildup, liver cell death, an increase in oxidative stress, and alterations in cytokines and adipokines, but not to major inflammation or fibrosis.[38,53,68,70] After a twoweek period of diet, aspartate transaminase (AST) and ALT levels dramatically and consistently rise.[53,72] Apart from weight gain, peripheral IR, and elevated cholesterol levels, the MCD model does not show any additional metabolic characteristics that are comparable

to clinical NAFLD.[54,72] Comparatively, rats on the MCD diet exhibit considerable weight loss, reduced fasting blood sugar concentrations, low peripheral tolerance to insulin, low plasma levels of insulin and leptin, lowered triglycerides in the blood and cholesterol, and unaltered or elevated blood levels of adiponectin.[47]

As a result, this model is usually recognised as being sufficient to investigate intrahepatic processes associated with NASH and the medication-based treatment of NASH, but it has been recognised as potentially insufficient for studying the multisystemic pathological entity, namely NALFD, across all of its elements.[73]

3.4 ATHEROGENIC (high cholesterol and high cholate) DIET ANIMAL MODEL

This dietary regimen imitates several epidemiologic characteristics of human NASH since it contains cholate (0.5%) and cholesterol (1-1.25%). Human NASH does not exhibit the consequent weight loss, diminished IR, or decreased plasma TG levels. On the other hand, after continued feeding, a combination with an HF diet (for example, the addition of 60% fat from cocoa butter) caused hepatocellular IR and NASH-like wounds, including MDBs[74] that are in connection with exaggerated hepatocytes.[75] The existence of cholic acid in the blood encourages the absorption of saturated fats and cholesterol and interferes with the transformation of cholesterol to bile acids in the body, leading to decreased the elimination of cholesterol and increasing the cholesterol levels, specifically non-high-density lipoprotein cholesterol in the bloodstream. Cholesterol in nutrition is a significant risk indicator for NASH because it makes the liver more vulnerable to malignant necrosis factor- and fas-induced steatohepatitis.[47,53] In addition, the Ath diet causes fibrosis (after 24 weeks), hepatocellular ballooning (after 24 weeks), inflammation (after 6 weeks), and steatosis (after 6 weeks). After six weeks, the subjected animals also exhibit elevated levels of ALT, total blood cholesterol, and, to a lesser degree, triglycerides.[76]. Additionally, when compared to mice given standard chow (SC), epididymal fat pads typically implemented as an investigational replacement for human visceral adipose tissue seem to be smaller. This should undoubtedly be viewed as a deficiency given the clear role visceral adipose tissue plays in NASH and other illnesses associated with obesity like coronary artery disease and DM2.[77]

Disease progression was associated with the down-regulation of genes for enzymes that protect against free radicals, indicating the interaction between fat intake in excess and cholesterol. In agreement with this research, Nrf2-deficient animals treated with diet

exhibited significantly higher levels of oxidative stress, proinflammatory cytokine, inherent immunity, and fibrogenesis-related gene mRNA.[78,79,80]

3.5 HIGH FAT HIGH CHOLESTEROL DIET (HFHC)

The amount of fat and other nutrients in the diets varies, but when integrated with cholesterol, sugar as well as fast food, they mimic Western-style nourishment which has been favourable to the occurrence of NAFLD.[75,81] In NAFLD/NASH study findings, the high-fat, high cholesterol diet (HFHC) paradigm is frequently employed.[82,83] After 20 weeks of feeding, the addition of 0.75% of cholesterol to a plant oil-based HFD significantly triggered proinflammatory and pro-fibrotic processes, which in turn exacerbated NASH prevalence and fibrosis of the liver on a histological stage.[83] Cholesterol has been proposed to directly contribute to the pathogenesis of NASH, and elevated cholesterol levels is considered to be a risk factor for the occurrence of NAFLD.[84,85] Hepatocyte cholesterol triggered the transcriptional regulator TAZ, which causes fibrosis of the liver and NASH. A key feature of human NASH is a ballooning-like proliferation of the cells of the liver which has been observed in mice fed diets containing 30% fat, 1.25% cholesterol, and 0.5% cholate.[86,87] A 16-week dietary schedule utilising an HFD containing 0.2% cholesterol resulted in more evident damage to the liver and fibrosis than the same HFD without cholesterol, as determined by research conducted in wild-type C57BL/6 mice.[88]

This model is capable of being used to evaluate medications, research how microbes in the intestines affect NAFLD[89], and investigate the molecular causes of NAFLD. For instance, in the NASH mouse model created by feeding mice with HFHC diet, cordycepin, possibly a new endogenous AMPK activator for treating NASH, reduces the development of hepatic steatosis, inflammation, damage to the liver, and fibrosis.[90]

3.6 HIGH FAT HIGH FRUCTOSE DIET (HFHF)

Increasing dietary intake of fructose like cholesterol, is an important risk determinant for the development of NAFLD in humans and associated with rises in disease severity, including the degree of NAFLD/NASH and obesity.[55,66,91] This method is commonly employed via incorporating 10% fructose to the HFD. Despite this, in fructokinase knockout mice, the amount of steatosis, inflammation, and fibrosis is reduced.[92] Mice feeding the HFHFD attained greater body weight and have more significant steatosis after 8 weeks. The HFHFD feeding group of mice show indications of inflammation in the liver after 16 weeks. Still, there has been no discernible difference in ALT level.[93] After 2 weeks of treatment, an

HFHFD causes considerably greater plasma triglycerides, greater ALT levels, and increased steatosis in rats than an HFD.[94] High levels of fat as well as fructose consumption promote insulin resistance, inflammatory processes, and ER stress via a variety of processes involving free fatty acid (FFA)-derived lipotoxicity and the production of cytokines and chemokines promote inflammation.[58,95]

As a result, the HFHFD model is an excellent alternative for investigating the involvement of ER stress and lipid steatosis in NAFLD. A recent research investigation evaluated the comparison in the effect of several dietary regimens on NAFLD in mice with that observed in human patients, concluding that the HFFD model best represents the human pathophysiology of NAFLD.[96]

3.7 CHOLINE DEFICIENT L-AMINO-DEFINED DIET (CDAA)

Another prominent approach to the pharmacological and epidemiological study of NAFLD is dietary choline shortage, L-amino acid defined diet (CDAA).[97,98] Because both of them lack choline, the CDAA diet has characteristics comparable to the MCD diet. The distinction is that a L-amino acid combination substitutes the equivalent and homologous proteins.[47] CDAA diet for mice, like MCD diet, exacerbated lipid generation, induced oxidative stress and inflammation, and eventually led to the fibrosis of the liver.[68] Animals administered a CDAA diet nurture a comparable or possibly a little more chronic form of NASH, and they also have a greater rise in ALT levels, although over a somewhat extended time period.[53] A significant degree of fibrosis has been observed after 20-22 weeks.[53,99] Despite the fact that they are unable to lose the weight that the MCD diet does, the metabolic hallmarks associated with human NAFLD do not manifest whenever utilised over precisely the same time range as the MCD diet.[68,100] However, mice administered a CDAA diet have an enormous rise in their body weight, plasma triglyceride and total cholesterol concentrations, and HOMA-IR (homeostatic model assessment insulin resistance) after 22 weeks, indicating enhanced resistance to insulin.[53]

It has been shown that a CDAA diet mixed with HFD may lead to accelerated progression of NASH with fibrosis (6-9 weeks) devoid of substantial reduction in weight, but it still does not provoke metabolic syndrome characteristics.[47,101]

4. CHEMICAL MODELS

The liver is frequently exposed to hepatotoxic chemicals, which disrupt liver homeostasis and cause non-alcoholic fatty liver disease. NAFLD aetiology is complicated, and environmental variables which might include chemical/pharmacological drugs serve a significant role. Streptozotocin, carbon tetrachloride (CCL4), and numerous other chemicals are routinely used for the induction of NAFLD.

4.1 CARBON TETRACHLORIDE

Carbon tetrachloride (CCl4)-induced liver injury is a well-established generic model for hepatic fibrosis. CCl4 causes an oxidative stress response in the liver, which results in hazardous protein and lipid peroxidation intermediates and a severe apoptotic reaction. [53] Male mice of the strain BALB/c received an intraperitoneal injection of CCl4 (0.4 mL/kg) twice a week for six weeks, which elevated serum levels of aminotransferase and alkaline phosphatase while disrupting the liver's anti-oxidative status. Severe fibrosis is also caused by receiving an injection of CCl4.[55]

While utilised alone, CCl4 causes fibrosis but does not induce obesity or resistance to insulin which means it is not a NAFLD model by its own identity, therefore it is commonly paired with dietary models when simulating NALFD. In this scenario, CCl4 enhances the detrimental impact of a diet rich in fats on the progression of NASH and fibrosis.[102,103] Multiple administrations of CCl4 to HFD-fed mice (8 times over a period of four weeks) causes liver steatosis along with hepatocellular inflammation, liver-specific ballooning, centrilobular fibrosis, both pericellular and perisinusoidal, elevated levels of triglycerides and substantially elevated transaminase levels after 12 weeks. The mean body weight in the HFD + CCl4 group is considerably less than in the HFD group and is different from the mean body weight in the untreated control group. Furthermore, the HFD + CCl4 group reported less total blood cholesterol and blood glucose levels than both the control and HFD groups.[104] Another group achieved equivalent outcomes in an eight-week rat model study.[105] Similarly to the couple of HFD and CCl4, thioacetamide (TAA) can also be employed[106], as well as the combination of an alternative diet including a CDAA diet with CCl4.[107]

In another recent investigation, male C57BL/6 mice receive CCl4 and a Liver X receptor (LXR) agonist together with HFD nutrition, resulting in resistance to insulin as well as histopathological signs and symptoms of NASH which include macro vesicular steatosis of the liver, ballooning hepatocytes, Mallory-Denk bodies, lobular inflammation, lipogenesis,

oxidative stress and fibrosis and enhanced serum levels of TNF-alpha and interleukin-6 (IL-6).[108]

4.2 STREPTOZOTOCIN

Non-alcoholic fatty liver disease (NAFLD) is substantially more common among diabetics and obese people. The combination of a high-fat diet (HFD) and a single low-dose streptozotocin (STZ) exposure has been used to develop type II diabetes-associated NAFLD because it precisely mimics the genuine human pathophysiology of fatty liver.[109] In streptozotocin induced diabetic rat model increase in serum ALT, AST, and ALP activity demonstrates that liver impairment appeared after 30 days of STZ injection.[110]

In another rat model, from gestational day 16, pregnant Sprague-Dawley rats have been given a diet high in fat. Streptozotocin (STZ) (180, 200, or 256 mg/kg) has been administered subcutaneously into the neonates two days after delivery. Throughout the nursing period, mothers are supplied a high-fat diet.[111] The newborn rats have been supplied exactly the same high-fat diet when they came to be weaned (4 weeks old). At the period of weaning, the survival rates are 25.6% (180 mg/kg STZ), 22.8% (200 mg/kg STZ), and 19.4% (256 mg/kg STZ).[111] The average body weight of NASH rats is approximately 20% lower in comparison to that of normal rats. NASH rats showed greater concentrations of glucose, alanine aminotransferase, and hyaluronic acid in their blood. During histology, conventional steatohepatitis characteristics including ballooning, infiltration of inflammatory cells, and perivenular and pericellular fibrosis have been identified .[111]

In mice HFD feeding commences at four weeks of age and is promptly followed by neonatal streptozotocin injection, resulting in simple steatosis at 6 weeks, NASH with inflammatory foci and ballooning at 8 weeks, and persistent pericellular fibrosis commencing at 8-12 weeks. The mice have increased transaminases and fasting glycemia as early as 6 weeks of age. Furthermore, by 20 weeks of age, mice develop hepatocellular carcinomas.[112]

4.3 THIOACETAMIDE (TAA) Administration in Combination to Fast-Food Diet

TAA serves as a conventional hepatic toxic chemical which is also a potential cancer-causing agent and mutagenic substance which may provoke oxidative stress and sterile inflammation, resulting in both temporary and permanent damage to the liver.[113,114] Thioacetamide is bioactivated in two phases by the enzyme cytochrome P450 (CYP)2E1 into TA sulfoxide (TASO) and subsequently to thioacetamide sulfoxide (TASO2), a highly reactive metabolite

which triggers necrosis of hepatocytes.[115,116] TAA is not toxic to the liver by itself but its chemically active metabolites covalently bind to proteins and lipids, resulting in oxidative stress and central lobular necrosis that damages the liver.[117] TAA causes hepatic damage in rats as well as mice at 100 mg/kg dosages.[115]

Persistent damage to the liver and fibrosis can be effectively and consistently induced by intraperitoneal administration of 150 mg/kg TAA three times per week for 11 weeks in rats and TAA administration in water used for drinking at 300 mg/L for 2-4 months in mice.[116] TAA (75 mg/kg) in combination with a fast-food diet [12% SFA and 2% cholesterol with high fructose corn syrup (42 g/L final concentration)] given intraperitoneally to C57BL/6 mice, three times a week, for eight weeks results in the development of the primary histological characteristics associated with NASH, which are inflammation of the liver, hepatocellular ballooning, accumulation of collagen and bridging fibrosis.[118]

TAA-induced liver fibrosis is more appropriate animal model to investigating the underlying causes of hepatocellular fibrosis, evaluating therapeutic medicines and assessing the accuracy of liver fibrosis immunological biomarkers.[117]

4.4 DIETHYLNITROSAMINE

DEN is widely recognised as hepatotoxic compound capable of causing numerous forms of fibrosis and hepatocellular necrosis.[119] By causing mutant mutations in DNA and enhancing the generation of ROS, DEN has been proven to cause significant hepatic damage.[120] Frequently employed in hepatocellular carcinoma (HCC) models, DEN is a well-known cancer-causing agent that causes considerable oxidative stress and Genetic alterations, promotes lipotoxicity, and promotes the development of cirrhosis and hepatocellular fibrosis.[121] In certain research, rats receiving an intraperitoneal injection of DEN once a week for around 4-6 weeks to promote fibrosis of the liver.[122,123]

Thompson et al. implemented an HFD murine model in conjunction by administering a one-time intraperitoneal dose of DEN between the ages of 21 and 25 days. At the age of 42 weeks, HFD-fed, DEN-treated mice showed the NASH phenotypic pattern in 8 out of 9 animals as compared to 6 out of 10 SC-fed, DEN-treated mice and 4 out of 10 HFD-fed, vehicle-treated mice. Meanwhile, when compared with the untreated animals, HFD-fed, DEN-treated mice reported significantly greater weight and ALT levels.[124] The 16-week MCD diet mouse model has been paired with a single intraperitoneal injection of DEN at 10

days of life. At the completion of the experiment, all MCD diet-fed, DEN-treated mice have several HCCs, whereas SC-fed, DEN-treated animals only have a few.[125]

5. GENETIC MODELS

A wide range of genetic models have been constructed for the better understanding of NASH. The fact, that a particular gene has to be mutated to isolates these models from humans who do not possess these genetic changes. The majority of these models possess a single characteristic in common: mutations in the genome cause considerable hepatic fat buildup without apparent inflammation or fibrosis.

5.1 Leptin Deficiency (The *ob/ob* Mice)

The ob gene encodes leptin, an adipocyte hormone which regulates consumption of food and susceptibility to insulin. In Lepob/Lepob (ob/ob) mice, functional leptin synthesis is impaired.[126] Ob/ob mice are sluggish, hyperphagic, and excessively obese with elevated cholesterol levels, high blood sugar levels, elevated insulin levels, and resistance to insulin.[47] Because hyperphagia i.e. excessive eating is associated with overweight or obesity, leptin insufficiency does not play a significant role in NAFLD.[70] Obesity constitutes a major contributory factor for NAFLD.[58] Ob/ob mice are commonly used genetic animal models of obesity and insulin resistance, respectively.[127]

Ob/ob mice consuming standard chow acquire a fatty liver but no hepatocellular fibrosis.[75] Fat buildup in the liver's cells promotes steatosis and hepato-lipotoxicity in ob/ob mice, but seldom develops to steatohepatitis and hepatocellular fibrosis.[99] As a result, a second stimulus must be provided to promotes fibrosis, which includes hepatotoxic substances (e.g., CCl4) or the MCD diet, so steatohepatitis might be examined in these genetic animal models consuming high-caloric dietary regimens.[70] The ob/ob mice are not susceptible to NASH until they are challenged by supplementary metabolic stimuli that include LPS exposure or consumption of specifically designed dietary regimens (i.e. diets with high-fat content (HFD)[128], or methionine and choline-deficient (MCD) diets). In addition, leptin appears to be specifically associated with hepatocellular fibrogenesis. That is why just a few research investigations are currently exist which demonstrate the progression of NAFLD-associated fibrosis of the liver in ob/ob mice.[66]

The ob/ob mice consuming high-fat diet which includes fructose and cholesterol produced fibrosis in along with major steatosis, hepatocellular inflammation with ballooning.[129]

NASH has been reduced in ob/ob mice by administration of TNF- antibodies. Additionally, norepinephrine elevates the quantity of stellate cells in the liver, raises TGFmRNA levels and enhances hepatocellular collagen mRNA expression respectively, and promotes extensive perisinusoidal hepatocellular fibrosis.[75] Due to reduced FA oxidation, PPAR-deficient ob/ob mice acquire severe weight gain along with hepatocellular steatosis without MDBs.[130]

5.2 Leptin Receptor Deficiency (The *db/db* Mice or fa/fa Rat)

Db/db mice lacking the DB gene (which codes for the leptin receptor) are leptin resistant either with or without hyperleptinemia.[58] Despite possessing a greater concentration of leptin, these mice acquire resistance to leptin due to a genetic change within its receptor.[70] Db/db Mice are homozygous for the autosomal recessive diabetes gene (db), which codes a point mutation which results in an insufficient amount of the long isoform of the leptin receptor (ObRb) which leads to impaired signalling of leptin.[38] Like ob/ob mice, db/db mice are also lethargic, hyperphagic, and extremely obese with elevated cholesterol levels, high blood glucose levels, hyperinsulinemia, and insulin resistance, as well as chronic liver steatosis but no steatohepatitis or hepatocellular fibrosis. They also require extra triggers like the MCD diet for the development of steatohepatitis signs and symptoms.[47,66,75] Db/db mice fed with conventional chow diet has characteristics similar to C57BL/6J mice fed with HFD in terms of gaining fat as well as visceral inflammation. Db/db mice are able to show manifestations of human related type 2 diabetes.[131]

It is worth noting that when db/db mice fed a normal control diet, they do not acquire inflammation on their own. On the other hand, calorie overabundance over a period of one month or more may cause severe inflammation of the liver. Db/db mice serve as viable genetic models of NAFLD but not of NASH in the absence of supplementary treatments due to the fact that they seldom highlight NASH characteristics when they are consuming a regular control diet. [55] When "second hit" in the way of modifications to their diets is given to these mice, then, they can develop signs and symptoms of NASH.[38]

5.3 Foz/foz mice model

It is a genetic mice model of type 2 Diabetes which includes a mutation in the Alms1 gene (foz/foz), which has significance in hypothalamic satiety management.[53] Patients suffering from Alström syndrome are considerably more susceptible than other syndromes to acquiring paediatric type 2 diabetes mellitus (T2DM).[58] After 20-24 weeks, this genetic change causes hyperphagic, rise in body mass index, glucose intolerance and when paired with an HFD,

NASH with hepatocellular fibrosis.[49,132] These mice also have lower amounts of adiponectin, intolerance to glucose, and elevated cholesterol levels.[75,133] Diet containing high fat accelerates strain-dependent development to NASH with life-threatening fibrosis by suppressing PPAR-mediated peroxisomal FA oxidation.[38,81]

As the pathophysiology of fatty liver disease is complex and diverse, so the mice models exhibiting comparable clinical manifestations but not usually identical pathways found in the clinic are accessible. The ARRIVE guideline, developed by the editorial boards of prominent biomedical journals, outlines a few guidelines for choosing suitable animal models for research purposes.[134]

5.4 KK-Ay/a Mice

KKAy/a mice are produced through breeding KK mice with yellow obese mice (Ay) mice. A heterozygous genetic alteration in the agouti gene leads to hypothalamic appetite impairment, hyperphagia and adiposity in these mice.[47] Weight gain, insulin resistance, high blood sugar levels, and steatosis arise in these mice. Additional triggers which include consumption of MCD diet can cause steatohepatitis.[75,81].

5.5 CD36-Deficient Mice

CD36 serves as a scavenger receptor that possesses various ligands and biological activities, like facilitating the process of free fatty acid (FFA) absorption. It's linked to the identification of nutritional fatty acids. CD36 additionally functions as a modulator of FFA transport into tissues, such as adipose, cardiac, and skeletal muscle tissues. [135] CD36 is generally known as fatty acid translocase.[136]

The CD36 knock out mice has lower fatty acid absorption as well as utilisation by the muscles of the skeleton leading to greater lipid breakdown within adipose tissue and plasma free fatty acid (FFA), which results in enhanced transportation of fatty acids to the hepatocytes for utilisation.[137] Steatosis occurs primarily as a result of inflammation, high levels of fatty acid and triglyceride in blood circulation and insulin resistance in liver.[81]

5.6 Proliferator-Activated Receptor-α Knockout Mice

The nuclear receptors known as PPARs control the genes involves in the absorption, transportation, intracellular binding, storage as well as catabolism of Fatty acids. PPAR-γ expression is very low in normal liver. It is mainly expressed in adipose tissue, epithelium

layer of colon, as well as macrophages.[75] Numerous exogenous ligands like pharmaceuticals (fibrates), can activate them in meanwhile, endogenous ligands which connect these receptors to the breakdown of lipids as well as the response against oxidative stress have been identified.[138] By decreasing the fat deposition in the hepatocytes as well as suppressing the expression of inflammatory genes, PPAR-α transcription factor mitigates the persistent inflammation associated with obesity.[75] Also, it suppresses NF-κB signalling along with its effects.[130] In mouse models, simple steatosis and SH are linked to decreased PPAR-α expression and activity, whereas in human models, PPAR-α expression and activity are linked only with the condition of steatohepatitis.[75,139,140]

Under typical feeding conditions, mice lacking PPARα are unable to build up fat in their hepatocytes. On the other hand, when they undergo a fast lasting twenty-four hours as well as a high-fat diet, they experience life-threatening steatosis[141], of the liver but are not diagnosed with NASH.[47] Mice given diets rich in fat (primarily saturated fatty acids obtained from lard) experiences weight gain, diabetes, resistance to insulin, and steatosis as well as higher levels of cytokines that promote inflammation and hepatic stellate cell activation.[75] The steatohepatitis that occurs in PPAR-α null mice nourishes with MCD diet is more severe in comparison to that of wild-type mice nourishes with MCD diet alone. This indicates that methionine and choline deficiency-induced steatohepatitis is exacerbated by null PPAR-α.[47]

5.7 Apolipoprotein E Knockout Mice

Apolipoprotein E (apo) is a versatile protein which plays crucial functions in neuroscience, disorders of the brain, as well as the metabolism of lipids. Mediating the interaction of lipoproteins or lipid complexes in the bloodstream or interstitial fluids to particular receptors on the cell surface constitutes one of apoE primary roles. Because these receptors are able to internalise lipoprotein fragments which includes apoE, apoE performs an integral part in the distribution as well as redistribution of lipids among the cells and tissues in the human body.[142] ApoE knockout mice that are given a western diet exhibit personality characteristic associated with metabolic syndrome and NASH which makes them a valuable asset for NASH research. Mice without ApoE and given regular standard diet only show symptoms associated with steatosis.[47]

ApoE knockout mice a Western-style diet containing lots of saturated fats and cholesterol makes them susceptible to a metabolic disorder (high fasting blood glucose, or overweight)

and after seven weeks, they exhibit features similar to human NASH which include liver-specific ballooning, fibrosis and hepatocellular steatosis, along with elevated levels of free cholesterol in the liver and hypertension at the portal vein.[143] A significant risk indicator for the onset of non-alcoholic fatty liver disease (NAFLD) among individuals is elevated cholesterol levels and cholesterol is becoming more widely acknowledged as the primary pathophysiological facilitator associated with the inflammatory alterations seen in NASH.[84]

5.8 Gankyrin (Gank)-Deficient Mice

Gankyrin has proven exceptionally well preserved throughout evolution and is found on the human chromosome Xq22.3. According to reports, gankyrin is involves in cells growth, cell proliferation, as well as the spreading of cancer cells.[144] A 26S proteasome subunit, gankyrin (Gank) is a tumor-associated oncogene which exhibits expression in a wide range of cancer varieties. Furthermore, it promotes liver improvement as well as is currently associated with the emergence of NAFLD.[55]

Gank liver-specific knockout mice are created when Gank is specifically abolishes from the livers of mice. These mice are then given an HFD for 24–28 weeks which contains 58% of calories in the form of fat and is supplemented with sucrose. These mice's livers show strong macro vesicular steatosis and no hepatocellular fibrosis which has been unexpectedly connected to the animals' better well-being. In contrast, the livers of WT mice nourishes with the same diet displays hepatocellular fibrosis but with a lesser degree of steatosis.[55,145]

5.9 microRNA deficiency-induced mouse model

The class of non-coding RNAs known as microRNAs (miRNAs) is crucial for controlling the expression of certain genes.[146] The role of microRNA as a vital regulator of hepatic metabolism as well as hepatocellular inflammation is becoming more widely accepted. For instance, microRNA 122 (miR-122) makes up 70% of all liver microRNA. While short-term decreases in miR-122a expression are advantageous but the suppression of miR-122a in the liver over a long period of time replicated human liver pathological conditions.[58]

The detected amount of miR-223 is up-regulated in an additional investigation that uses serum samples from NASH patients as well as the NASH mouse model.[146] Following a three-month period of HFD as well as MCD feeding, MiR-223-KO mice came up with NASH.[147] Chronic HFD-fed mice develop a full spectrum of NAFLD due to genetic

deletion of miR-223. MiR-223 works by suppressing the expression of Cxcl10 and Taz in the hepatocytes, therefore interrupting the development of steatosis into NASH.[148]

5.10 Sterol Regulatory Element-Binding Protein 1c Transgenic Mice

Sterol regulatory element-binding protein 1c, an insulin-regulated transcription element which primarily controls the production of lipids, is primarily expressed in lipid-producing tissues as well as in the hepatocytes.[149] A family of transcription factors known as "sterol regulatory element binding proteins" (SREBPs) controls the expression of multiple enzymes necessary for the formation of phospholipids, triacylglycerol, cholesterol and fatty acids, therefore controlling the biosynthesis of lipids as well as adipogenesis.[150]

In the adipose tissue, genetically engineered mice over express sterol regulatory element binding protein 1c, which results in Insulin Resistance and pronounced steatosis of the liver (age of eight day). In addition to steatoSsis, they additionally experience steatohepatitis on their own, which shows up as pericellular fibrosis, liver ballooning, as well as lobular inflammation after a period of 20 weeks.[151] This model has reducedbody fat, which is a stark contrast with the human condition, whilst it recapitulates the plenty of liver histological characteristics associated with human NASH. The aforementioned mice appear to be appropriate for the model of steatohepatitis linked to lipodystrophy.[47]

6. CONCLUSION

Animal models play a crucial role in understanding the pathophysiology of NAFLD and exploring potential therapeutic interventions. Various animal models, including rodents (mice and rats), non-human primates, and other species, have been developed to mimic different aspects of NAFLD/NASH progression observed in humans. These models replicate key features such as hepatic steatosis, inflammation, fibrosis, and hepatocellular carcinoma, providing valuable insights into disease mechanisms and allowing researchers to test novel therapeutic strategies. Rodent models, particularly high-fat diet-induced models and genetic models, are widely used due to their affordability, ease of handling, and ability to recapitulate many aspects of human NAFLD/NASH. However, no single animal model fully recapitulates the entire spectrum of human disease, necessitating the use of multiple models to comprehensively study NAFLD/NASH pathogenesis and therapeutic interventions. In conclusion, animal models of NAFLD have significantly advanced our understanding of the disease's pathogenesis and progression. They serve as invaluable tools for preclinical research, drug discovery, and the development of therapeutic strategies aimed at halting or

reversing NAFLD/NASH progression. Moving forward, continued refinement and utilization of these models will be essential for identifying novel targets and interventions to combat this growing global health burden.

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