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The Hidden Epidemic: Exploring Non-Alcoholic Fatty Liver Disease

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Abstract

The liver component of a group of illnesses linked to metabolic dysfunction is non-alcoholic fatty liver disease (NAFLD). While fatty liver hepatitis leading to cirrhosis was identified about two decades prior, Ludwig and associates initially used the term non-alcoholic steatohepatitis (NASH) in 1980. In addition to metabolic risk factors (obesity and type 2 diabetes, in particular), excessive alcohol consumption (≥30 g/day for men and ≥20 g/day for women), and other chronic liver diseases, NAFLD is defined as the presence of steatosis in more than 5% of hepatocytes. The clinicopathological condition known as non-alcoholic fatty liver disease encompasses a broad range of liver damage, including severe fibrosis, cirrhosis, steatohepatitis, and simple steatosis. Only one stage of non-alcoholic fatty liver disease, non-alcoholic steatohepatitis is characterized pathologically by the coexistence of steatosis and necro-inflammatory activity. Steatosis is not only a possible cause of progressive liver disease on its own, but it is also a key player in the pathophysiology of several other liver illnesses. Animal models of non-alcoholic fatty liver disease (NAFLD) may be broadly classified into two groups: those resulting from genetic mutations and those exhibiting an acquired phenotype due to pharmacological or nutritional modification. In industrialized nations, NAFLD is the most frequent liver disease in children and adolescents, with a prevalence estimate of 10–40% in adults globally. Other than dietary modifications, lifestyle adjustments, and maybe bariatric surgery, there are currently no viable medical procedures that can fully cure the illness. Currently being studied are a number of tactics that aim to address pathophysiological processes such inflammation, cell damage, and the overabundance of fatty acids in the liver. Many non-invasive techniques are being researched as alternatives to or additions to biopsies, particularly in the context of follow- up monitoring.

Keywords: Obesity; Hyperlipidemia; Diabetes Mellitus

Abbreviations

NAFLD: Non-Alcoholic Fatty Liver Disease; NASH: Non-Alcoholic Steatohepatitis; NAFL: Non- Alcoholic Fatty Liver; HCC: Hepatocellular Carcinoma; ELF: Enhanced Liver Fibrosis; NFS: NAFLD Fibrosis Score; FIB4: Fibrosis4; MRI: Magnetic Resonance Imaging; MRS: Magnetic Resonance Spectroscopy; NIDDK: National Institute of Diabetes and Digestive and Kidney Diseases; CRN: Clinical Research Network; GGT: Gamma-Glutamyl Transpeptidase; FLI: Fatty

Liver Index; LCHF: Low-Carbohydrate High-Fat Diet; FMT: Faecal Microbiota Transplantation; MCD: Methionine-and Choline-Deficient; LPS: Lipopolysaccharides; FOS: Fructo-Oligosaccharides; Hh: Hedgehog; NKT: Natural Killer T; HCD: High Cholesterol Diet; HFD: High Fat Diet.

Epidemiology

NAFLD is usually linked with comorbid diseases such as obesity, hyperlipidemia, and type 2 (non-insulin dependent) diabetes mellitus, either individually or in combination. The severity and frequency of non-alcoholic fatty liver disease (NAFLD) are directly correlated with obesity levels. Obese individuals have a 4.6-fold higher prevalence of NAFLD. Type 2 diabetes mellitus dramatically raises the incidence and severity of non-alcoholic fatty liver disease (NAFLD) in fat people of any weight [1]. In a Hong Kong research, Wong and colleagues used fibro scan to detect increased liver stiffness in 17.7% of diabetics. A subgroup of their cohort had liver biopsies, which revealed NASH in 50% of them [2]. According to magnetic resonance spectroscopy data, hepatic steatosis affects 31% of American adults and 33% of prospective live liver donors having a liver biopsy. 12.9% to 16.4% of people have fatty abnormalities in their livers that may be seen by ultrasound. Males and members of specific groups (such as up to 45% of the Hispanic population) are more likely to have steatosis than other groups [3]. The most common laboratory anomaly in NAFLD patients is mild to moderate increase of serum aminotransferases. The AST/ALT ratio can differentiate NAFLD from alcoholic liver disease. The majority of NAFLD patients have an AST/ALT ratio smaller than unity. However, when the disease advances to cirrhosis, the AST/ALT ratio rises and loses diagnostic accuracy [4]. It was determined that, accounting for 31% of cases in men and 16% in women, NAFLD is the most frequent cause of hypertransaminasemia in the United States. However, these estimations fail to account for the fact that liver enzyme values are normal in nearly half of NAFLD cases [5].

Risk Factors

- Older age
- Diabetes mellitus
- Elevated serum aminotransferases (>2 times normal in one study).
- Biopsy results show ballooning degeneration and Mallory hyaline or fibrosis.
- BMI ≥ 28 kg/m
- Increased visceral adiposity index, which considers waist circumference, BMI, triglycerides, and high-density lipoprotein levels [6].

Beneficial Factors

- Light or moderate alcohol use may benefit the liver.
- Coffee drinking has been linked to a reduced risk of advancement.

Prevalence

Globally, the pooled prevalence of NAFLD is 25.24%, with significant regional heterogeneity. The Middle East and South American nations have reported the highest prevalence rates, which are primarily ultrasound-based, at over 30%. In contrast, the few studies that have been conducted in Africa have revealed far lower prevalence rates, at around 13%. With a.7% prevalence rate for NAFLD, the USA and North America have been the focus of the majority of research on the disease's epidemiology [7]. In the USA, the prevalence of obesity among children aged 2 to 5 years grew from 8·4% in 2011–12 to 13·9% in 2015. Childhood obesity is a major risk factor for non-alcoholic fatty liver disease (NAFLD), and the number of cases is always rising. The combined mean prevalence of NAFLD in children is 34·2% in paediatric obesity clinics and 7·6% in the general population. A person's chance of experiencing liver-related events and other comorbidities is increased if their condition originated in infancy [8]. A prevalence rate of 17% was calculated for NAFLD in India based on the use of ultrasonography in the diagnostic process. A further research conducted at Brooke Army Medical Centre found an even greater prevalence of ultrasonographic non- alcoholic fast-fatigue syndrome (NAFLD), at 46%. Of the overall cohort, or 30% of ultrasound-positive patients, had a biopsy to confirm the diagnosis. Notwithstanding its less frequent application in extensive NAFLD screening investigations compared to ultrasonography, MRI has been employed to ascertain the prevalence of NAFLD. In the United States, 31% of a population-based, multi-ethnic sample had NAFLD, according to a widely reported MRI research from Dallas County, Texas [9]. Different regions of South Asia and the Far East have different rates: 12.5-38% on the Chinese Mainland, 23-26% in Japan, 27% in Korea, 12-51% in Taiwan, 28% in Hong Kong, 9-32% in India, and 5-30% in other places (Sri Lanka, Malaysia, and Indonesia). The so-called "lean NAFLD" or "Non-obese NAFLD," which accounts for about 10% of NAFLD cases and was first identified in Asian populations, is another facet of the epidemiology of NAFLD in Asia [7].

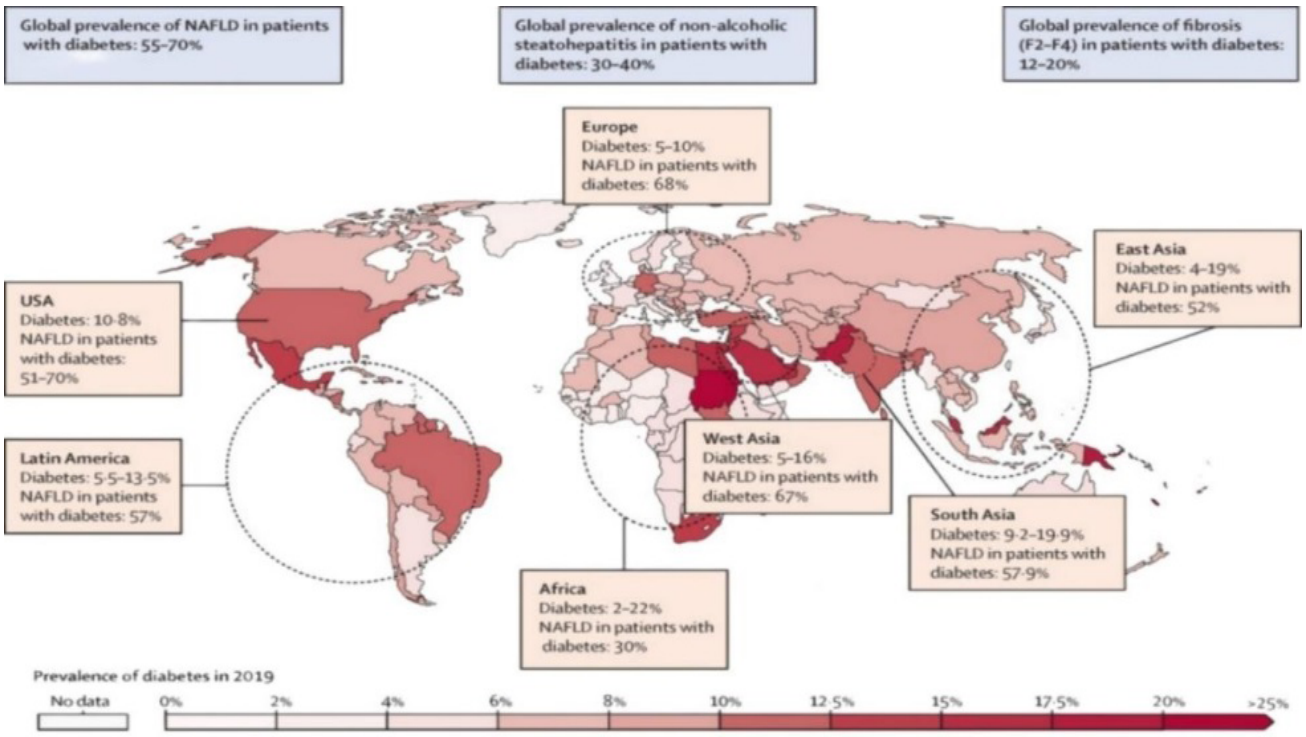

Figure 1: Global prevalence of NAFLD [10].

Pathogenesis

NAFLD is caused by a complicated and multifaceted mechanism. Various explanations have been proposed, culminating to the 'two hits hypothesis'. Sedentary lifestyle, high fat diet, obesity, and insulin resistance lead to hepatic lipid build-up, which serves as a 'first strike' and sensitizes the liver to additional insults as a 'second hit'. The 'second hit' triggers inflammatory responses and fibrogenesis. Animal models of obesity, such leptin-deficient ob/ob mice, show increased hepatic lipid build-up and require a second insult to trigger inflammation and fibrosis [11]. Such factors are:-

- Fat metabolism, Lipotoxicity and insulin resistance
- Adipose tissue dysfunction
- Genetic determinants
- Epigenetic factors
- Dietary factors [11]

Mechanisms for Fat Accumulation in the Liver

Hepatic steatosis occurs as a result of failure in various metabolic pathways. Increased circulating fatty acid levels are a significant factor in the development of fatty liver. Increased activation of transcription factors, adipokine, and alterations in hepatic fat oxidation and VLDL secretion are now identified as contributing causes. Fat accumulation reasons are:

- Increased adipose tissue-derived fatty acid pool
- Source of hepatic fatty acids in NAFLD
- Role of transcription factors, adipokine and novel metabolic mediators (like SREBP-1, LXR-alpha)
- Adiponectin
- Ghrelin
- Mitochondrial dysfunction [12].

Progression of NAFLD

NAFLD includes NAFL and NASH. NASH can proceed to cirrhosis, HCC, or liver failure. Patients without cirrhosis can also develop HCC. Cardiovascular disease is the most common cause of death in NASH. HCC stands for hepatocellular carcinoma, NAFL for non- alcoholic fatty liver, NAFLD for non-alcoholic fatty liver disease, and NASH for non-alcoholic steatohepatitis [13].

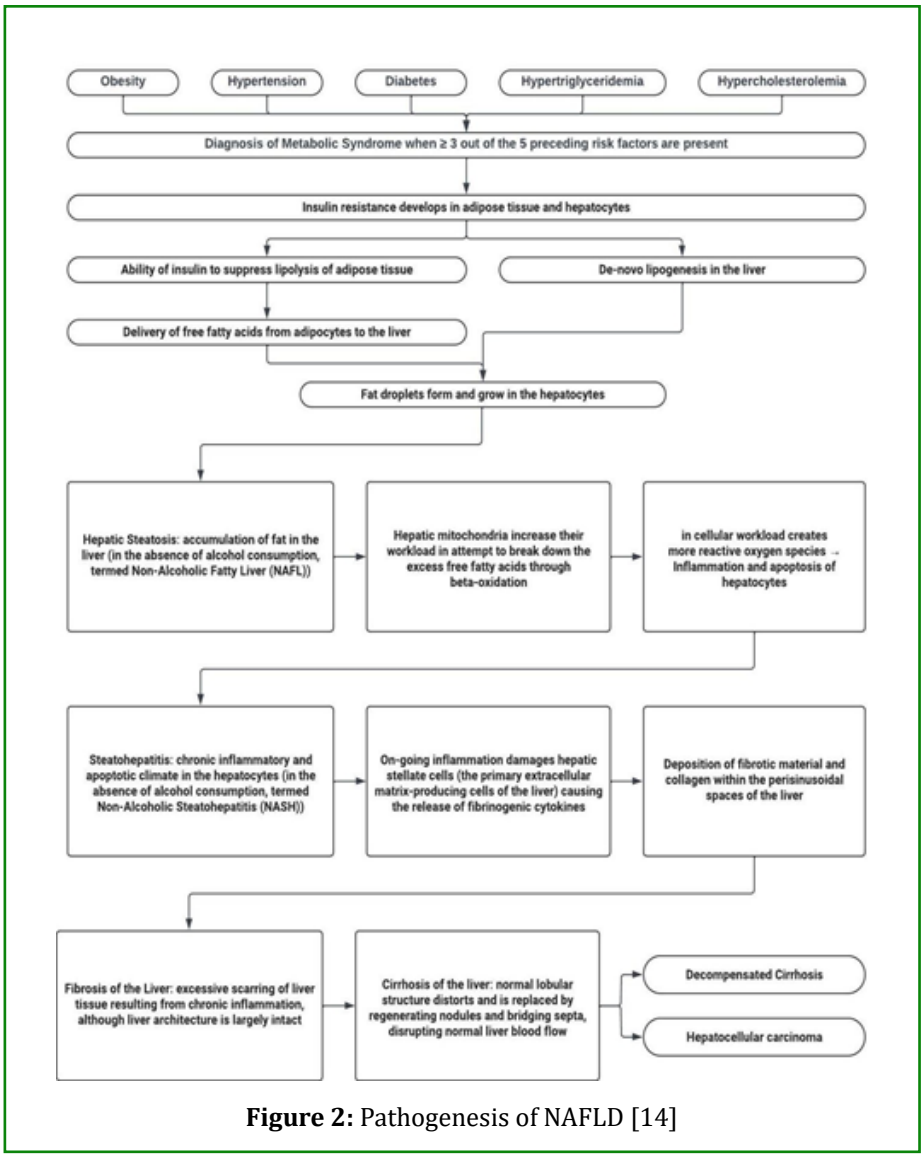

Figure 2: Pathogenesis of NAFLD [14]

Diagnosis

When transaminase levels are persistently somewhat elevated, there is hepatomegaly, and there are radiologic signs of fatty liver, NAFLD is suspected. Of course, a thorough medical history is necessary to rule out heavy alcohol consumption (more than 20–40 g/day). Alpha- 1-antitrypsin deficiency, hemochromatosis, primary biliary cirrhosis, autoimmune hepatitis, viral hepatitis, and Wilson's disease should all be ruled out in laboratory testing. The most reliable method for confirming a NAFLD diagnosis is still liver histology. There is little association between the histologic findings and the clinical presentation. Therefore, the only reliable method for diagnosing and staging the severity is a liver biopsy [15]. There is mounting evidence that the coexistence of non-alcoholic fatty liver disease (NAFLD) and another type of chronic liver disease, particularly HCV infection, can result in more severe hepatic impairment [16].

Scores and Biomarkers

Ultrasonography is typically used for individual patient screening for steatosis; however, for larger-scale screening, indicators or biomarkers are preferred. The NAFLD liver fat score, the SteatoTest, and the Fatty Liver Index are the well-validated biomarkers. While simple steatosis does not raise the risk of liver-related death, these indicators can predict mortality or the course of metabolic and cardiovascular diseases in a variety of ways149. Particularly the NAFLD Fibrosis Score (NFS), Fibrosis4 (FIB4), BARD, and commercially available panels like Fibro Test, Fibro Meter, and the Enhanced Liver Fibrosis (ELF) test150, serum markers of fibrosis appear to perform better. While the diagnostic accuracy of all these instruments is passable, only NFS and FIB4 have undergone substantial validation [17].

Imaging

Ultrasound has the benefits of being widely accessible, reasonably priced, and radiation- free. Hepatic and splenic attenuations are compared to estimate the amount of fat in the liver. A decrease in hepatic attenuation, which causes the liver to look darker than the spleen. When steatosis accounts for more than 33% of the liver parenchyma, it can more accurately predict moderate to severe degrees of steatosis. Ultrasound and CT are classified as "qualitative" tests; they are most useful in identifying hepatic steatosis. As a very sensitive and repeatable method of measuring liver fat, magnetic resonance imaging and spectroscopy (MRS) advances non-invasive measurement of hepatic fat. The same MRI technology used in clinical care magnets is also used in this procedure. Currently, MRS is only available at academic institutions, but this is probably going to change soon [18]. Although there are a number of drawbacks to abdominal ultrasonography, such as operator dependence, the difficulty to differentiate NASH from other subtypes of NAFLD, and the inability to precisely stage hepatic fibrosis, it is now the recommended technique for qualitative assessment of fatty infiltration. A number of studies have worked to reduce operator-dependent bias, enhance agreement across observers, and create ultrasonic grading systems. Furthermore, using contrast agents during ultrasonography could increase the precision of this radiologic method in NAFLD [19].

Liver Biopsy

The NAFLD activity score (NAS) was created by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)-sponsored NASH Clinical Research Network (CRN) in an effort to standardize the histological diagnostic criteria. The study found that not all samples with NAS ~5 fulfil diagnostic criteria for definite SH. Some cases of NAS – 4 do, showing that the threshold value of a NAS > 5 cannot consistently determine the presence or absence of NASH [20]. Almost all instances with a NAS of < 3 were regarded as "not NASH," while a NAS of >5 were nearly always linked to the diagnosis of NASH. Over time, a number of fibrosis grading methods have been used to NAFLD patients. Originally created for patients with chronic hepatitis C16, the METAVIR score (F0 ¼ no fibrosis, F1 ¼ stellate enlargement of portal tracts without septa, F2 ¼ stellate enlargement of portal tracts with few septa, F3 ¼ septal fibrosis without cirrhosis, F4 ¼ cirrhosis) has been used in multiple NAFLD studies [21].

Panels for Diagnostic Evaluation of Steatosis and NASH

Bedogni, et al. assessed a cohort from the Dionysos Nutrition & Liver Study in order to create the Fatty Liver Index (FLI). A total of 287 patients without known liver illness and 224 subjects with suspected liver disease (apart from hepatitis B and C) were matched. Following a series of analyses, a model was created using four predictors—waist circumference, gamma- glutamyl transpeptidase (GGT), BMI, and triglyceride to produce the FLI. According to the authors, hepatic steatosis may be ruled in or out using a FLI of less than 30 or greater than 60. The fact that ultrasonography was used to diagnose fatty liver is a restriction to this investigation. In order to determine whether steatosis and/or NASH are present, proprietary tests have been developed [22].

Treatment

Lifestyle Interventions: Including as nutrition, physical activity, and exercise, are the primary therapy options for NASH, as no drugs or surgeries are currently authorized. These treatments are primarily aimed at regulating body weight and metabolic problems.

Physical Activity and Exercise: Physical exercise is suggested for persons with NAFLD since it is a crucial factor in metabolic management. People with excessive obesity, metabolic syndrome, and type 2 diabetes (T2D) exhibit more sedentary behaviour. An increase in sedentary time may predispose to NAFLD. Interestingly, greater breaks from inactive time are shown to be advantageous for glucose and fatty acid metabolism, as well as obesity management. Several cross-sectional studies have revealed reduced levels of physical activity in persons [23].

Calorie Restriction: Consumption of a diet with fewer calories than required daily energy, such as the Mediterranean diet, can reduce body weight, hepatic lipid accumulation, and insulin resistance, as well as lower serum levels of saturated fatty acids and higher serum levels of monounsaturated and n-3 polyunsaturated fatty acids. In a controlled clinical trial, 74 patients with NAFLD were randomized in a 1:1:1 ratio to a 12-week treatment with either a 5:2 diet with an intermittent calorie restriction (500 kcal/day for women and 600 kcal/day for men) for two non-consecutive days per week, a low-carbohydrate high-fat diet (LCHF) with an average daily calorie intake of 1600 kcal/day for women and 1900 kcal/day for men, or general lifestyle advice from a hepatologist by choosing a healthy diet, The findings showed that the 5:2 diet and LCHF are more effective than general lifestyle changes in reducing hepatic steatosis and body weight [24].

Interventional Behavioural Studies: Three non-HCV patients who underwent a second liver biopsy after losing weight showed significantly less steatosis. Recently, the same group confirmed that comprehensive lifestyle intervention can effectively treat patients with liver disease, including NAFLD, through weight loss. At 6 months, an intensive lifestyle intervention resulted in weight loss in 83% of patients, compared to 24% for normal dietetic therapy. In a study of 15 biopsy-proven NASH patients, a 1-year intensive

nutritional counselling and moderately restricted diet led to improved steatosis and necroinflammation, but not fibrosis, on repeat liver biopsy in 60% of cases. The average weight loss was only 7% [25].

Modifications of Gut Microbiota

NAFLD is caused by dysbiosis of the gut microbiota, which results from changes in gut hormones, metabolites, and inflammatory factors. The development of liver disease is linked to the presence of specific bacterial species. For instance, in non-obese NAFLD patients, there is a negative association between the degree of liver fibrosis and the amount of Eubacterium. In persons who are not fat, the severity of liver fibrosis is strongly correlated with the abundance of Veillonellaceae and Ruminococcaceae. Liver disease can be improved by altering the gut microbiota using a variety of techniques, such as faecal microbiota transplantation (FMT), medication therapy (such as antibiotics), lifestyle adjustment (such as dietary changes), the previously mentioned BS, and others [25].

Liver Transplantation

In the majority of high-income nations, NASH-associated cirrhosis is currently one of the top three reasons for liver transplantation, and it is on a worrying trajectory to become the most common. Patients receiving a liver transplant for NASH-associated cirrhosis have outcomes at 1, 3, and 5 years that are essentially comparable to those for other reasons. However, the prevalence of obesity (defined as a BMI of ≥ 30 kg/m2), T2DM, post-transplant MetS, and recipient age (i.e., >60 years) appear to be more often linked with the overall mortality in patients with NASH-associated cirrhosis after liver transplantation. After liver transplantation, NAFLD recurrence is also frequent, occurring in 20% to 40% of cases, depending on the diagnostic methods employed [26].

Surgery for Bariatric Patients

Bariatric surgery aims not only to accomplish sufficient weight loss but also to address obesity-related comorbidities such as type 2 diabetes, obstructive sleep apnea syndrome, hyperlipidemia, and hypertension. Mummadi, et al. [27] found that the improvement or resolution rates of steatosis, steatohepatitis, and fibrosis after bariatric surgery were 91.6%, 81.3%, and 65.5%, respectively. NASH was completely resolved in 69.5% of patients. However, a recent Cochrane review stated that the lack of randomized clinical trials or quasi- randomised clinical research has hampered a comprehensive assessment of the benefits and drawbacks of bariatric surgery as a therapeutic option for individuals with NASH.

Role of Silybin

The primary constituent of the flavonoid silymarin is silybin. It functions as a radical scavenger, promotes hepatocyte RNA production, and inhibits collagen deposition and hepatic stargate cell growth in vitro. Silybin decreases lipid peroxidation and collagen build up in rats with fibrosis-induced fibrosis. When combined with vitamin E and a phytosome, silybin is quickly absorbed by humans. Based on preliminary evidence, patients with NAFLD may benefit from these substances in terms of improved liver steatosis, insulin resistance, and plasma markers of liver fibrosis [28].

Animal Models in NAFLD

Genetic Models: To compare rodent models to human NAFLD, we analysed their metabolic phenotype, histology, and gene expression. Our findings revealed that 44% of the models had insulin resistance, 47% were obese, and 51% had dyslipidemia. Additionally, 47% (1,839/3,920) of the models had at least two features of the metabolic syndrome. Chemically induced models were less likely to exhibit NAFLD metabolic features [29].

Db/db Mice: The model adipokine, leptin, regulates feeding behaviour by, among other things, lowering food intake by stimulating satiety at the hypothalamic level. The autosomal recessive diabetes gene (db), which codes for a point mutation that prevents the long isoform of the leptin receptor (Ob-Rb) from functioning, is homozygous in db/db mice. This results in impaired leptin signalling. These mice exhibit severe hyperglycaemia, hyperinsulinemia, insulin resistance, elevated serum leptin levels, and the development of macrovesicular hepatic steatosis. As a result, they have normal or elevated levels of leptin but are resistant to its effects, resulting in persistent hyperphagia and obesity and diabetes. Interestingly, when given a typical control diet, db/db mice do not naturally develop inflammation [30].

Ob/ob Mice: To gain a better understanding of NAFLD, leptin-deficient genetically engineered mice have been created. The peptide leptin, sometimes referred to as the "satiety hormone". The satiety signal is transmitted by its hypothalamus receptor, which also has anorexic properties. A homozygous point mutation in the leptin gene is present in ob/ob mice. They acquire extreme obesity, hyperlipidemia, hyperglycaemia, hyperinsulinemia, and insulin resistance in addition to being hyperphagia and sedentary. Despite the fact that leptin insufficiency is not a major cause of NAFLD, hyperphagia is a contributing factor to obesity. In fact, NAFLD patients have normal or high serum leptin levels [31].

Fat Aussie Mice (Alms1 foz/foz): Alstrom syndrome is an autosomal recessive disorder. Mutations in the human ALMS1 gene lead to this condition. Alms1 foz/foz mice are initially normal in weight, but develop hyperphagia at 2

months and obesity, hyperinsulinemia, and diabetes by 4 months. Female foz/foz mice are fertile until they experience metabolic problems, but males are infertile. HFD causes NASH after 3 months and pericellular fibrosis after 6 months. HFD-fed foz/foz mice exhibit considerably worse liver injury compared to wild-type mice fed the same diet [32].

Dietary Rodent Models of NAFLD

MCD Diet: The methionine-and choline-deficient (MCD) diet is high in sucrose (40%) and fat (10%) but low in methionine and choline. In contrast to human NASH, mice fed an MCD diet had lower FA plasma levels and lose weight. However, by altering the diet, the relevance to human disease can be improved. Administering lipopolysaccharides (LPS) to MCD-diet-fed mice increased TNF-α production by Kupffer cells and fibrogenesis by hepatic stellate cells. Dietary fructo-oligosaccharides (FOS) enhanced the gastrointestinal microbiome and intestinal barrier, resulting in a decrease in CD 14-positive Kupffer cells and reduced liver damage. Furthermore, stimulation of the hedgehog (Hh) pathway increased natural killer T (NKT) cell enrichment in the liver, which was followed by hepatic stellate cell activation and fibrosis [33]. In steatotic db/db mice, the MCD diet leads to increased fibroins steatohepatitis. In this scenario, db/db mice had a 10-fold increase in hepatic pro-collagen type 1 mRNA levels, compared to non-steatotic db/m mice who had a 4-fold rise. Ob/ob mice showed no increase in fibrogenesis with MCD feeding. The scientists found that an obese/diabetic experimental model of developing NASH and the short-form leptin receptor play a significant impact in steatohepatitis [34].

High Cholesterol Diet (HCD): Humans eat a wide variety of foods that are rich in cholesterol. According to recent research, dietary cholesterol may play a significant role in the development of hepatic inflammation and steatohepatitis in humans as well as animal models. Serum insulin levels in mice given an HCD (1%) alone rise noticeably, although liver weight, triglyceride levels, FFA levels, and serum ALT levels only slightly increase. However, when a high cholesterol dose is combined with a high fat or cholate content, the characteristics of non-alcoholic steatohepatitis (NASH) are exacerbated. Mice given a high-fat (15%), high-cholesterol (1%), or HFHC diet displayed increased hepatic lipid accumulation, increased weight gain, 10-fold increases in blood ALT levels, and decreased adiponectin levels [35].

High Fat Diet (HFD): A high-fat diet consisting of 71% fat, 11% carbs, and 18% proteins offered to rats for three weeks has been shown to cause insulin resistance, significant pan lobular steatosis, inflammation, and fibrosis. Around 16 weeks, mice fed a high-fat diet demonstrated comparable outcomes. Thus, the major aspect of this model is that the results change depending on the rodent strain and diet composition. The model has NAFLD traits that are similar to human NAFLD, however the pathological result is less severe, limiting its applicability in the study. C57Bl6/J mice are more insulin resistant, making them more likely to be relevant than C57Bl6/N mice [36].

Model	Summary of Diet Composition	Obese	Steatosis	NASH	Fibrosis	HCC
High fat diet	45-75% of the animals total calorie intake is derived from fat. The classic reported HFD model comprised 71% fat, 11% carbohydrates,18% protein	Yes	Yes	Yes (Mild)	Yes	No
Ob/ob mice	NA	Yes	Yes	No	No	No
Db/db mice	NA	Yes	Yes	No	No	No
Methionine and choline- deficient diet	Diet usually consists of sucrose(40%) and fat (10%); however it is deficient in methionine and choline.	No	Yes	Yes	Yes	No
High- cholesterol diet	Approx. 1% of animals' total calorie intake is from cholesterol. Often fed in conjunction with high fat(15%) pr high cholate (0.5%)	Yes	Yes	Yes	Yes	No
Foz/foz mice	NA	Yes	Yes	Yes	Yes	No
Choline- deficient high-fat diet	20% protein, 35%, carbohydrate, and 45% fat without choline added	Yes	Yes	Yes	Yes	Yes
Choline- deficient L-amino acid defined diet	28.9kcal/ g L- glutamic acid,12.7kcal/g L-arginine 15.8kcal/g aspartic acid.	Yes	Yes	Yes	Yes	Yes

Table 1: Animal Models for NAFLD.

In Vitro Models for NAFLD

Recent research use in vitro methods to understand the molecular pathways behind illness progression. *In vitro* models are a valuable study tool for various liver diseases, but few studies have been conducted on NAFLD. Primary cell cultures and immortalized cell lines are commonly utilized to create in vitro models for study. Although primary human hepatocytes, Kupffer cells, stellate cells, or sinusoidal endothelial cells appear to be a physiologically relevant model for clinical problems, ethical concerns and a limited number of human liver samples make its usage challenging. The other possibility would be primary rodent cells, which, depending on the model employed, may more or less replicate the scenario found. Immortalized cell lines are a viable alternative to primary cell cultures due to their high replication capability, stable phenotype, and ability to maintain consistent cells throughout a research effort. Furthermore, cultivating immortalized cell lines is simpler and more standardized. Table 2 lists cell lines and model systems suitable for studying NAFLD *in vitro* [37].

In vitro Models	Cell Lines	Pros	Cons
Primary cell culture	Hepatocytes from NAFLD patients/ rodents/Kupffer cells/stellate cells/ iNKT cells from human patients/ rodents.	Mimics *in vivo* settings	Isolation problem, Ethical issues Limited, culture time
Immortalized Cell lines	RAW264.7, AML-12, J774A, HepG2, HuH7, H4IIE, LX2	Continuous growth. Easy to culture Stable phenotype	Expression of several enzymes and nuclear factors alter according to immortalization method.
Co-culture Models	RAW264.7 and AML-12 human hepatocytes and adipocytes.	Mimics in vivo liver architecture Important tools in cellular cross talk studies.	Difficult to cultivate
3D cultures	H35 rat hepatoma cell line	Mimics *in vivo* liver architecture Liver specific differentiation and function	Difficult to cultivate

Table 2: Summary of Available Cell Lines and Cell Culture Models.

Conclusion

Over the last decade, a wealth of new understanding about the physiopathology of NAFLD has emerged, exposing the complexities of the processes involved in its genesis and progression. The most recent guidelines/expert opinions for NAFLD care suggest a novel "systems medicine" approach to the interactions between the brain and neurological system, endocrine system, digestive system (gut, liver, and microbiome), and immune system. In the complicated pathogenetic riddle of NAFLD, genes definitely play a crucial role in determining NAFLD prevalence and severity, as well as cardiovascular risk. The PNPLA3 gene mutation is now the most proven susceptibility factor for steatosis, NASH, fibrosis, and HCC, despite the fact that a variety of other genetic variants contribute to liver disease. However, while the discovery of these variations has helped us better understand NAFLD in terms of both clinical phenotypes and pathogenetic pathways, their value in clinical practice and in individual patients is limited. The prevalence of NAFLD has reached worrying levels in many regions of the world, and the disease's socioeconomic effect is expected to be severe over the next several decades. Because no authorized pharmaceutical therapy for NAFLD is currently available, there is an urgent need to find and confirm potential therapeutic targets in preclinical trials. These preclinical research necessitate the use of animal models that closely approximate the pathophysiology of the human illness. In addition to these critical and key criteria, a viable NAFLD model should include the utilization of widely available genetic backgrounds and appropriate timelines. The primary line of therapy for NAFLD and NASH is lifestyle improvement, which includes dietary changes and increased physical activity. Weight loss is the most proven therapy for NAFLD and NASH, with a strong dose-response relationship. In general, any healthy diet (low fat, low carb, or Mediterranean diet) that reduces calorie intake while remaining tolerable to the patient should be promoted. For people who find calorie restriction challenging, modifying their diet.

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