Pathogenesis of Non-Alcoholic Fatty Liver Disease and Non-Alcoholic Steatohepatitis

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ABSTRACT
Non-alcoholic fatty liver disease occurs when excess fat builds up in the liver of individuals who do not consume excessive amounts of alcohol and is often associated with metabolic syndrome. It affects approximately 20–30% of people worldwide, with higher rates observed in underdeveloped countries. Patients with NAFLD often experience symptoms of the metabolic syndrome, such as hypertension, dyslipidemia, insulin resistance, and abdominal obesity. NAFLD can manifest as a range of diseases, from simple fat accumulation (steatosis) to more severe stages such as steatohepatitis, fibrosis, and cirrhosis. A disruption in lipid metabolism caused by factors like insulin resistance and an excessive intake of fatty acids leads to the abnormal accumulation of fat in the liver. This fat accumulation can cause damage to liver cells due to lipotoxicity and cellular stress from oxidative stress and endoplasmic reticulum stress. Proinflammatory cytokines and chemokines generated by injured liver cells and activated Kupffer cells can promote inflammation and fibrosis. While significant progress has been made in understanding the causes of NASH, its complete mechanism is still not fully understood. The goal of this review is to describe the current understanding of NAFLD pathogenesis with a focus on the factors that contribute to steatosis, hepatocyte damage, inflammation, and fibrosis.

Key words: *steatohepatitis, steatosis, lipotoxicity, lipid metabolism, metabolic syndrome*

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a widespread liver disease that has become a global pandemic in the twenty-first century. The incidence rates of NAFLD range from 23% to 32% depending on the location, and these figures are projected to increase worldwide. NAFLD occurs when more than 5% of hepatocytes accumulate fat, as identified by radiological or histopathological examination, and there is no underlying chronic liver disease or secondary source of steatosis, such as drugs, excessive alcohol intake, or acquired or inherited metabolic conditions [1]. Non-alcoholic steatohepatitis (NASH) is a disease characterized by liver steatosis associated with inflammation and hepatocyte ballooning that can eventually progress to advanced fibrosis, cirrhosis, and hepatocellular carcinoma. NASH ranges from a simple accumulation of fat in hepatocytes without any inflammation to both [2]. The pathogenesis of fatty liver disease is not entirely understood, but according to the two-hit theory, insulin resistance and an excess of fatty acids cause simple steatosis, which is the first "hit" that makes the liver more sensitive to a second "hit." [3] Inflammation, hepatocyte damage, and fibrosis result from the second "hit," advancing the disease from steatosis to NASH. The second "hit" is believed to be caused by reactive stress, lipid peroxidation, and mitochondrial dysfunction. Increased fatty acid inflow to the liver and insulin insensitivity are the main causes of steatosis. Oxidative and endoplasmic reticulum stress, lipotoxicity, and hepatocyte damage are likely causes of excessive fat deposits in the liver, triggering inflammatory and wound healing responses that accelerate disease progression from steatosis to NASH [4–7]. NAFLD is considered a component of the metabolic syndrome because it is associated with risk factors such as obesity, insulin intolerance, and hypertension. Its incidence increases to around 70% in type 2 diabetes patients and 90% in morbidly obese individuals. Furthermore, NASH is a complex disease with extrahepatic components such as dysfunctional adipose tissue, altered gut flora, and genetic susceptibility. The purpose of this study is to provide the most up-to-date understanding of NAFLD pathogenesis, with a focus on factors that cause steatosis, hepatocyte injury, inflammation, and fibrosis [8–10].

Steatosis

Hepatic steatosis is a medical condition that occurs when lipid droplets, which are composed mainly of triglycerides, accumulate in liver cells. This histological characteristic is often used to diagnose NASH. An imbalance in lipid metabolism, with an excess of lipid inputs over lipids consumed, causes steatosis in hepatocytes [11-13]. The liver acquires lipids from three primary sources: dietary fats, circulating free fatty acids (FFAs), and de novo lipogenesis (DNL). High-fat meals are commonly used in rodent models to induce hepatic steatosis. FFAs are absorbed by the liver from fatty tissues, which is dependent on the FFA concentration in the blood and transport proteins. DNL is a metabolic route that synthesizes fatty acids from basic metabolic precursors, and it is mainly controlled by insulin and glucose [14,15]. NAFLD patients have a higher influx of fatty acids to the liver and higher hepatic expression of FFA transport proteins than healthy individuals. FFAs provide energy to the liver through oxidation, and when these processes cannot use the extra FFAs, they are esterified into TGs and deposited in lipid droplets, causing hepatic steatosis. Hepatic steatosis can result from increased FFA flow to the liver, increased DNL, decreased FFA oxidation, and decreased VLDL production [16-19].

Insulin Resistance

The activation of phosphoinositol-3-kinase and AKT/PKB kinase is triggered when insulin binds to its receptor, resulting in several effects on the liver. Insulin normally reduces glucose synthesis, increases glucose uptake, and has a positive impact on postprandial de novo lipogenesis (DNL) in healthy individuals [20-22]. However, in cases of insulin resistance (IR), the inhibitory effect on glucose synthesis is diminished, while the stimulatory effect on DNL persists, leading to hepatic steatosis. All NAFLD patients have IR, which affects insulin signalling in hepatocellular and disturbs lipid metabolism. Skeletal muscle and adipose tissue IR also contribute to hepatic steatosis [23-25]. In addition, the increase in DAG level, mediated by traditional (b) and new (d and e) PKC activation, has been found to link steatosis to IR. However, it is possible for genes that cause hepatic steatosis in humans not to cause IR, as observed in individuals with familial hypobetalipoproteinemia who have hepatic steatosis due to a genetic defect in the synthesis of hepatic apolipoprotein B but do not show insulin resistance [26-28].

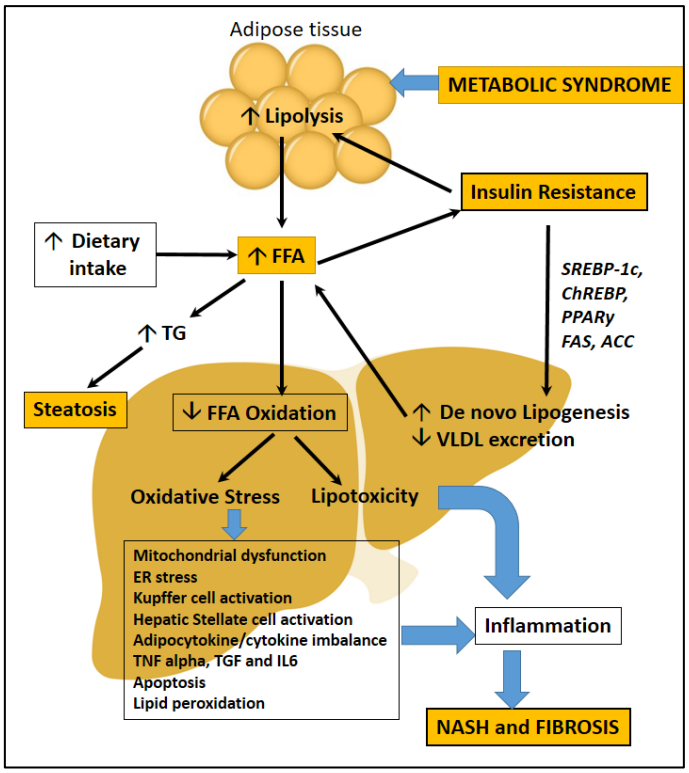

Figure 1: The multiple-hit pathogenesis of NAFLD and NASH

Autophagy

Autophagy is a lysosome-dependent process that helps to maintain cellular energy levels by breaking down cellular components. Recently, it has been associated with fat metabolism in hepatocytes, where it is known as lipophagy. Studies have shown that dysfunctional autophagy can exacerbate non-alcoholic fatty liver disease (NAFLD), with blockage of autophagy resulting in increased lipid droplet storage in hepatocytes, while activation of autophagy decreases it [29-31]. Additionally, extreme cases of steatosis have been found to have reduced levels of autophagy markers. Insulin resistance, which is frequently linked with NAFLD, may

also impact autophagy. While the exact role of autophagy in NAFLD is unclear, there appears to be a link between autophagy, lipolysis, and regulation of lipid levels in the liver [32-34].

Adipose Tissue Dysfunction

Although hepatic steatosis mainly affects hepatocytes, adipose tissue, among other extrahepatic factors, can have a significant impact on NAFLD. In NAFLD patients, dysfunctional adipose tissue is common and has various roles in the etiology of NASH, such as adipose hypertrophy, insulin resistance, and altered adipokine production. The ability of adipose tissue to store lipids is compromised due to excessive lipid accumulation and hypertrophy, which impairs its normal function. Excessive cholesterol buildup in the liver and fatty tissue can lead to insulin resistance [35-37].

Insulin resistance is often caused by the inhibition of hormone-sensitive lipase (HSL), adipose triglyceride lipase (ATGL), and monoglyceride lipase (MGL)-induced degradation of lipids in adipose tissue. Insulin inhibits lipolysis by reducing the production of ATGL and cAMP, which in turn results in less PKA activation and less PKA phosphorylation of HSL. As a result of increased lipolysis rates and elevated FFA release into circulation, which is then absorbed by the liver, individuals who are obese and insulin-resistant tend to have elevated ATGL expression in their adipose tissue [38-40].

Microbiota

Apart from adipose tissue, the gut microbiota can also impact lipid metabolism in hepatocytes and contribute to the progression of steatosis. A high Firmicutes/Bacteroidetes ratio, which is commonly associated with obesity and NAFLD, may exacerbate the condition. However, studies have shown that Firmicutes are reduced in NASH. Modulating the gut microbiota can affect the development of steatosis, as demonstrated by research showing that antibiotics can decrease hepatic TG accumulation in mice fed a high-fat diet [41-44]. Short-chain fatty acids (SCFAs) produced by gut microbiota can provide energy and promote their transport to the liver, leading to the development of steatosis. Alcohol generated by bacterial enzymes can also cause hepatic steatosis, and changed gut microbiota can lead to choline deficiency, exacerbating NAFLD. Changes in the gut microbiota's composition in NAFLD can have significant effects on host energy metabolism and contribute to the progression of steatosis [45-48].

Genetic Factors

SCFAs produced by gut microbiota have been implicated in providing energy and promoting their transportation to the liver, thus contributing to the development of steatosis. The production of alcohol by bacterial enzymes can also lead to hepatic steatosis, and NAFLD may result in choline deficiency caused by changes in the gut microbiota. Alterations in the composition of gut microbiota in NAFLD can significantly affect the host's energy metabolism and contribute to the development of steatosis [45-48]. The PNPLA3 gene is expressed in hepatocytes, hepatic stellate cells, and adipocytes, and it is located in the endoplasmic reticulum and on lipid droplets [52,53]. The PNPLA3 I148M mutation has been found to impair lipid droplet remodeling and VLDL secretion, thereby increasing the risk of hepatic steatosis.

The TM6SF2 E167K variant, which is associated with decreased VLDL production, is also linked to NAFLD and its more severe variants. Although several genetic modifiers have been investigated, further research is needed to confirm and understand their mechanisms [54-56].

Oxidative Stress

Rewritten: The duration of cellular stressors can cause severe injury, leading to cell death through necrosis or apoptosis. Hepatocyte injury is a common finding in nonalcoholic steatohepatitis (NASH), with hepatocyte ballooning observed under microscopy as an indication of damage. The enlargement of hepatocytes is due to oxidative stress-induced changes in the distribution of intermediate filaments, which can cause damage such as mitochondrial dysfunction. Mitochondria are especially vulnerable to reactive oxygen species (ROS) because they produce ROS [57-59]. Polyunsaturated fatty acids (PUFA), the major component of phospholipids in mitochondrial membranes, are targeted and destroyed by ROS. ROS also deactivates antioxidant enzymes like superoxide dismutase, reducing antioxidant capacity, and the products of lipid peroxidation can impede the electron-transport chain, aggravating ROS generation. ROS can damage mitochondrial DNA, causing mitochondrial malfunction. Dysfunctional mitochondria can result in more ROS generation, lowering the capacity for oxidising free fatty acids (FFA) and initiating a self-perpetuating loop of increased ROS production. Deficiencies in ATP homeostasis, an impeded electron transport chain, paracristalline inclusions, and cristae loss are all symptoms of mitochondrial dysfunction, which is frequent in nonalcoholic fatty liver disease [60-62]. Normally, intracellular organelles that become damaged are removed and repurposed through mechanisms like autophagy. However, if stress persists, despite robust antioxidant defenses, hepatocytes may suffer irreparable damage, leading to cell death via necrosis or apoptosis. Apoptosis is the primary cause of cell death in NASH, with its severity corresponding with the extent of the disease. Both intrinsic and extrinsic apoptosis mechanisms contribute to the etiology of NASH, with defective mitochondria causing apoptosis via cytochrome c release. Prolonged endoplasmic reticulum stress increases calcium release from the ER, which can cause apoptosis. FFAs are involved in apoptosis, with hepatocyte apoptosis levels corresponding with serum FFA levels. FFAs can cause cellular damage and death by activating the Fas receptor and upregulating Fas ligands, lysosomal subsequent activation and release of the lysosomal protease, cathepsin B, and activating the TLR4 receptor. In conclusion, increasing cellular stressors can cause cell damage and apoptosis, which are the primary causes of fibrosis and inflammation in liver diseases [63-66].

Apoptosis

Prolonged cellular stress can result in significant harm, leading to necrosis or apoptosis. In NASH, hepatocytes exhibit damage, characterised by an enlarged hyperchromatic nucleus and foamy, pale cytoplasm due to oxidative stress-induced changes in the distribution of intermediate filaments [67-68]. Oxidative stress damages mitochondrial membranes, inhibiting the electron-transport chain and disrupting ATP homeostasis, leading to dysfunctional mitochondria. This can lead to apoptosis via cytochrome c release, while chronic endoplasmic reticulum stress also leads to apoptosis. Excess FFAs activate Fas receptors, promote lysosomal

permeabilization, activate TLR4 receptor, and trigger apoptosis. Increased cellular stress can cause inflammation and fibrosis in liver disorders [69,70].

Inflammation

TNFa, IL6, and CCL2 are examples of proinflammatory cytokines and chemokines that play a role in inflammation in NASH. These mediators can be secreted by injured hepatocytes and adipose tissue, stimulating the NF-jB pathway and increasing proinflammatory cytokines. In NASH inflammation, Kupffer cells, which are the liver's resident macrophages, are crucial [71-73]. The balance of proinflammatory M1 and anti-inflammatory M2 Kupffer cells is critical, and an uneven M1/M2 Kupffer cell phenotype can cause steatohepatitis. The chemokine CCL2 is responsible for increasing the infiltration of macrophages, monocytes, and neutrophils into the liver. Kupffer cells are equipped with pattern recognition receptors that recognize and eliminate infections and harmful chemicals through Toll-like and NOD-like receptors. The upregulation of Kupffer cells is influenced by TLR and NLR receptors [74-77].

Fibrosis

Persistent liver damage, as seen in nonalcoholic steatohepatitis (NASH) and other chronic liver diseases, leads to hepatic fibrogenesis and eventual liver cirrhosis. This fibrosis occurs due to the accumulation of high-density extracellular matrix proteins in response to a wound-healing reaction. Resident mesenchymal cells that accumulate vitamin A, known as hepatic stellate cells (HSCs), play a key role in the fibrogenic response. HSC activation is complex and involves paracrine stimulation from neighboring cells and cytokines. The metabolic environment of NAFLD, including hyperinsulinemia, dysglycemia, and type 2 diabetes, may impact fibrogenesis [78-83]. Adipokines, such as leptin and adiponectin, are also involved in the development of hepatic fibrosis. Other factors that may contribute to fibrosis in NAFLD/NASH include free cholesterol accumulation and changes in gut bacteria. Dysbiosis, which promotes inflammation and HSC activation, can contribute to fibrogenesis in NAFLD, and gut-derived bacterial metabolites can activate HSCs in NASH. Inflammasome activation in the liver and gut has also been linked to NASH-related liver damage and fibrosis [84-89].

Conclusion

The accumulation of excess fat in the liver, known as hepatic steatosis, is a consequence of abnormal lipid metabolism caused by several factors such as insulin resistance, increased fatty acid influx into the liver, genetic predisposition, and imbalanced gut flora. Although previously considered a benign condition, a significant proportion of individuals with hepatic steatosis may progress to nonalcoholic steatohepatitis (NASH), although the exact mechanisms underlying this are not well understood. Oxidative and endoplasmic reticulum stress are thought to be the primary drivers of hepatocyte damage and apoptosis, leading to inflammation and fibrogenesis. NASH is associated with an increased risk of cirrhosis and liver cancer, emphasizing the urgent need for effective therapies. A better understanding of the underlying causes of NASH may pave the way for the development of novel treatment options for this condition.

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