Non-alcoholic Fatty Liver Disease and Steatohepatitis: Risk Factors and Pathophysiology

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) and its progressive subtype non-alcoholic steatohepatitis (NASH) are the most prevalent liver diseases, often leading to hepatocellular carcinoma (HCC). This review aims to describe the present knowledge of the risk factors responsible for the development of NAFLD and NASH. I performed a literature review identifying studies focusing on the complex pathogenic pathway and risk factors of NAFLD and steatohepatitis. The relationship between NAFLD and metabolic syndrome is well established and widely recognized. Obesity, dyslipidemia, type 2 diabetes, hypertension, and insulin resistance are the most common risk factors associated with NAFLD. Among the components of metabolic syndrome, current evidence strongly suggests obesity and type 2 diabetes as risk factors of NASH and HCC. However, other elements, namely gender divergences, ethnicity, genetic factors, participation of innate immune system, oxidative stress, apoptotic pathways, and adipocytokines, take a leading role in the onset and promotion of NAFLD. Pathophysiological mechanisms that are responsible for NAFLD development and subsequent progression to NASH are insulin resistance and hyperinsulinemia, oxidative stress, hepatic stellate cell (HSC) activation, cytokine/adipokine signaling pathways, and genetic and environmental factors. Major pathophysiological findings of NAFLD are dysfunction of adipose tissue through the enhanced flow of free fatty acids (FFAs) and release of adipokines, and altered gut microbiome that generate proinflammatory signals and cause NASH progression. Understanding the pathophysiology and risk factors of NAFLD and NASH; this review could provide insight into the development of therapeutic strategies and useful diagnostic tools.

KEYWORDS:

Non-alcoholic fatty liver disease; Hepatocellular carcinoma; Metabolic syndrome; Insulin resistance; Obesity; Type 2 diabetes

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INTRODUCTION

Liver disease is a major cause of illness worldwide, and surgical intervention is needed in most cases, which is followed by severe post-surgical complications.[1-3] In China alone, liver diseases, primarily viral hepatitis (predominantly hepatitis B virus [HBV]), non-alcoholic fatty liver disease (NAFLD), and alcoholic liver disease, affect approximately 300 million people.[4] NAFLD is defined as the uptake of triglycerides in hepatocytes so that the liver weight goes above 5%.[5] NAFLD and its progressive subtype, non-alcoholic steatohepatitis (NASH), have no approved drug treatments to date.[6] Therefore, it is an urgent need to identify the causative factors, pathophysiology, and molecular mechanisms

responsible for the development of NAFLD/NASH. NASH was initially identified more than two decades ago and is currently treated as a wing of NAFLD. NASH coincides with alcoholic steatohepatitis (ASH), irrespective of excessive consumption of alcohol. From clinical and experimental data, NAFLD has been measured as the most frequent cause of chronic liver disease and the most recurrent cause of increased aminotransferase and cryptogenic cirrhosis.[7-9] The estimated prevalence of NAFLD is 5-18% in Asia and 20-30% in Western countries, while for NASH, it is estimated at 3.5–5%.[7] NAFLD is associated with high cardiovascular morbidity and mortality, independent of the diagnostic methods. Researchers who used alanine aminotransferase (ALT) or gamma-glutamyl transferase (GGT) elevation as a marker of NAFLD found that patients with elevated liver enzymes had a higher incidence of cardiovascular disease-related mortality.[8] On the other hand, NAFLD is associated with asymptomatic brain lesions, alterations in cerebral perfusion and activity, cognitive disorders, and brain aging with increased risk and severity of both ischemic and hemorrhagic stroke.[9,10]

This review aims to describe the present knowledge of the risk factors of NAFLD. NAFLD occurs in both male and female patients, all age groups and in all ethnicities, as well as in children.[11-13] However, NAFLD subsets vary in their ability for advancement. A number of people having only steatosis do not develop steatohepatitis. 10–15% with histologically confirmed NASH can develop cirrhosis, liver failure, and hepatocellular carcinoma (HCC).[14-16] The mechanisms of NAFLD are complex and multifactorial. It is likely that several factors are associated with non-alcoholic fatty liver injury. Major risk factors that lead to NAFLD are obesity, insulin resistance, hepatitis B viral infection,[17,18] oxidative stress, cytokines, dyslipidemia, and type 2 diabetes.[14,19] All those elements can enhance intra-hepatic fat deposition and lipotoxicity, progression of an inflammatory condition, and oxidative stress that regulate disease promotion.[20] The pathogenesis of NAFLD is still unclear; however, infiltration of triglycerides within hepatocytes causes insulin resistance, which is treated as the primary and widely accepted causal agent so far.[21] Oxidative stress and expression of proinflammatory cytokines have been reported as additional etiological agents for NAFLD and steatohepatitis.[22] Histopathological examination is considered one of the important methods to distinguish NASH from NAFLD. The most important lesions of NAFLD are lobular to portal inflammation, steatosis, ballooning degeneration of hepatocytes, and finally apoptosis.[23] Further issues are increased level of aminotransferase[24] associated with mitochondrial beta-oxidation of fatty acids, and producing reactive oxygen species (ROS) with increased generation of inflammatory cytokines. However, risk factors for the development of NAFLD have not been completely understood. The current review will focus on identifying the important causative agents involved in the development of NAFLD, from steatosis to NASH.

MATERIALS AND METHODS

A PubMed, MEDLINE, and Google Scholar literature search was done to find out the information explaining the pathophysiology and risk factors for NAFLD and NASH from 2000 to 2021. The keywords of MeSH terms used for the strategy were “Non-alcoholic fatty liver disease or NAFLD, non-alcoholic steatohepatitis or NASH, and pathophysiology and risk factors”. Other searches were also made, such as dyslipidemia, obesity, type 2 diabetes, hypertension, smoking, genetic factors, etc. Also, we identified literature cited by articles retrieved from the database. Both research and review articles were considered for writing this manuscript. Studies published in only English language were included.

Risk Factors Associated with NAFLD and NASH

Obesity

Adiposity or obesity is an utmost public health hazard and one of the well-studied risk factors for NAFLD.[25] Obesity/adiposity is endemic worldwide, with more than 1 billion fatty aged people and at a minimum 300 million obese people all over the world.[26] Theprevalenceofprimary NAFLDis 80-90% in obese adults, 30-50% inpeople who havediabetes,and up to 90% inpeople who have hyperlipidemia.[27] Obesity has been considered as an increasing epidemic situation. The prevalence and consequences of NAFLD are continuously increasing, making NASH possibly the most frequent cause of progressive liver disease

in forthcoming years.[28] It has been suggested that abdominal adiposity is related to particularly NAFLD, a hepatic manifestation of metabolic syndrome.[29] Also, obese women are more likely to develop subacute liver failure, where there is evidence of a possible underlying NASH in some cases.[30] In an American school-based study, obese young boys had a higher rate of the fatty liver compared with obese young girls.[31] NASH can be found in 40-100% of obese adults[32] and 15-25% of children.[33]

Diets Rich in Saturated Fat

Generally, high-fat diets, diets high in saturated fats, have been shown to cause obesity, hypercholesterolemia, hypertriglyceridemia, and NAFLD.[34] Diets rich in saturated fat cause triglyceride accumulation in the liver. When diets are enriched in monounsaturated fatty acids (MUFA) or polyunsaturated fatty acids (PUFA), they tend to reduce liver triglycerides (TG). The potential mechanisms that may play a role are the lipotoxic effects of saturated fatty acids, endoplasmic reticular (ER) stress, oxidative stress, and mitochondrial dysfunction. The mechanism by which saturated fatty acid induces ER stress is still unknown, but current studies suggest defective phospholipid metabolism is probably a key element. Whereas growing evidence implies ER stress coupled with a saturated fatty acid induced cellular malfunction, patients with NAFLD have been shown to have higher levels of ER stress markers.[35] We studied that mice fed a diet enriched with saturated fat had a substantial rise in total cholesterol and low-density lipoprotein cholesterol, where they developed atherosclerotic and fatty liver lesions.[15] High-fat diets are also associated with insulin resistance and hepatic inflammation.[36] The presence of dyslipidemia has been found in 20-80% of events related to NAFLD.[21]

Carbohydrates Consumption

The increased amount of carbohydrate intake is also associated with hepatic inflammation. Among carbohydrates, fructose intake is an important dietary contributor to NAFLD pathogenesis. Fructose cannot be easily absorbed through the gastrointestinal tract due to the lack of expression of a sufficient amount of glucose transporter-5 in the gut cells.[37] Fructose is absorbed from the intestine via the portal vein and delivered to the liver. In the liver, fructose enhances its own metabolism by upregulating ketohexokinase. Furthermore, fructose upregulates the expression of lipogenic enzymes involved in *de novo* lipogenesis(DNL), which helps to increase hepatic lipogenesis, hepatic IR and therefore potentiating hepatic steatosis.[38] Chronic fructose intake causes disruption of the insulin-signaling pathway resulting in a type of hyperglycemia together with compensating hyperinsulinemia. The continuing release of acetyl-CoA due to fructose metabolism outdoes the mitochondrial capacity for its metabolism (Krebs cycle), and acetyl-CoA is then transformed into citrate. Citrate is the fuel for the action known as DNL, which leads to enhanced hepatic lipogenesis.[38] DNL is increased in people with NAFLD compared with healthy individuals. In the liver, fructose inhibits the free fatty acids (FFAs) oxidation, thus favoring re-esterification with glycerol to produce triglycerides, very low-density lipoprotein (VLDL), and fat stock intrahepatic, which leads to NAFLD.[39] Thus high fructose consumption has been associated with elevated hepatic fat, inflammation, and potentially fibrosis.[40]

Diabetes

Insulin resistance and hyperinsulinemia are coupled with NASH or NASH-related fibrosis in a number of patients. Type 2 diabetes mellitus (T2DM), insulin resistance (IR), and NAFLD have a close relationship,[41] due to the direct delivery of insulin to the portal vein after its secretion, taking the same route as the absorbed glucose.[42] Leite and colleagues[43] found that the prevalence of NAFLD confirmed by ultrasonography was 69.4% in 180 patients with T2DM. IR and T2DM are common phenomena of metabolic syndrome coupled with obesity. Several recent studies show an increased prevalence and severity of liver disease in patients with diabetes.[44] In a prospective histologic study of nearly 100 patients with T2DM, the prevalence of non-alcoholic hepatic steatosis was estimated to be 78%, confirming this strong independent risk factor for NAFLD.[45] Although IR is closely related to NASH, a straightforward association between disease pathogenesis and hyperinsulinemia has not been settled.

Sleep Apnea

Obstructive sleep apnea (OSA), a chronic intermittent breathing cessation, may be a risk factor of NAFLD without severe obesity, which is connected with the severity of liver damage independently of body mass and other associated factors.[46] Tanné et al reported that the prevalence and severity of NASH were more in people with pronounced OSA, proposing that the intermittent hypoxia due to OSA may take part in NASH pathogenesis.[47] While intermittent hypoxia did not directly cause liver injury in non-obese animals, exacerbated macro-inflammation and fibrosis were observed in the livers that previously possessed steatosis, implying that hypoxia may serve as an additional insult that helps in the development from simple steatosis to progressive disease stages.[48,49]

Sex and Ethnicity

It is widely known that NAFLD and NASH exhibit age and sex divergences in both prevalence and severity. Men usually tend to preferentially store fat in their upper body, particularly around the organs in the abdominal cavity: the visceral fat.[50] On the other hand, women with less body fat tend to deposit in the subcutaneous tissues.[30] In accordance with the annual health check findings in Japan, the prevalence of NAFLD in men was about 27% for all ages more than 30 years.[51] While in women, it progressively increased from 7% in the 30s to 23% more than 60 years of age. It was also found that among 492 biopsy-confirmed patients with NASH, male patients were considerably younger; however, the number of NASH cases in women was more than that in men aged above 50 years.[52] Lipid metabolism plays an important role in the sex difference in fat accumulation.[20] In a study in Japan, it was revealed that the specific size of triglyceride and cholesterol were larger in men than women, with signs of metabolic syndrome.[53] Metabolic risk factors and sociodemographicfeaturesrelated to NASH differ by ethnicity. The definition of obesity varies among ethnic groups; like Asian people have been reported to have higher visceral fat than Caucasians; the definition of obesity for Japanese is a BMI more than 25, instead of more than 30 as it is for Caucasians.[52] According to annual health checks, 9-30% of adult Japanese have NAFLD by ultrasonography (US), and the prevalence of NASH is 1-3%.[54] In the USA, it was stated that the frequency of hepatic steatosis differed greatly with ethnicity.[55] Solga and colleagues[56] performed a study showing that there was no steatohepatitis in obese African-Americans.

Dyslipidemia

NAFLD and NASH-affected patients often have dyslipidemia, which is related to higher serum triglycerides and higher small dense low-density lipoprotein cholesterol. Dyslipidemia in NAFLD is associated with excessive hepatic production of the VLDL 1 and reduced elimination of lipoproteins from the circulation.[57] The existence of dyslipidemia (hypercholesterolemia, hypertriglyceridemia, or both) has been found in 20-80% of cases related to NAFLD.[58] The development of NAFLD arises from the disparity between the entry and development of fatty acids and the utilization of fatty acids for oxidation or secretion as VLDL triglycerides. In people with excess liver fat, insulin is not able to regulate VLDL generation resulting in a higher amount of VLDL particles in the blood. Besides, alterations are observed in the metabolism of other lipoproteins that bind with VLDL particles, resulting in decreased HDL cholesterol and increased small, dense, low-density lipoprotein.[59]

Hypertension

Hypertension is associated with liver illness like other metabolic syndromes such as diabetes mellitus, obesity, and dyslipidemia.[60] Hypertension, along with high LDL-cholesterol, insulin resistance, inflammation, and abnormalities in adipocytokines release, causes vascular dysfunction, which leads to the development of atherosclerosis.[61] And 64% prevalence of hypertension was reported among NAFLD patients with steatohepatitis.[62] The severity of fatty liver coupled with the frequency and extent of hypertension, abnormal glucose, and triglyceride metabolism.[63]

Vitamin D Deficiency

Vitamin D deficiency is more often identified as a global health hazard. The involvement of vitamin D deficiency in the progression of NAFLD and NASH remains unclear. Some recent reports have revealed that vitamin D shows an immune-regulating role on adipose

tissue, and the growing wealth of epidemiological data is showing that vitamin D deficiency is correlated with obesity and NAFLD.[64-66] It was also reported that vitamin D supplementation might cause a decrease in glycemia and reversing of prediabetes to normal glucose regulation, together with improvement in insulin sensitivity and reduced risk of NAFLD.[67]

Associated Conditions

The association between NAFLD and urolithiasis has also drawn scientific attention. There is growing evidence that NAFLD is linked with a greater risk of urolithiasis. Currently, the increasing importance of NAFLD and its strong correlation with metabolic syndrome has arisen an interest in the important role of NAFLD in the development and progression of extrahepatic diseases, including urolithiasis.[68,69] Again, a meta-analysis including seven observational studies and 226 541 individuals exhibited a 1.73-fold increased risk of urolithiasis among patients with NAFLD compared with healthy controls.[68,70] On the other hand, fat deposition, along with obesity and metabolic syndrome, has been defined as "fatty infiltration" or NAFPD.[71] Non-alcoholic fatty pancreatic disease (NAFPD) has been considered as another associated factor of NAFLD. Growing evidence confirm the contribution of pancreatic fat in the progression of T2DM, NAFLD, atherosclerosis, severe acute pancreatitis, and even pancreatic cancer.[72,73] Reports show that fatty pancreas could be used as the initial indicator of "ectopic fat deposition", which is a major contributing factor of NAFLD.[74]

Pathophysiology Of NAFLD and NASH

The pathophysiology of NAFLD and NASH is very complex, and several intermediaries are involved. Even though NAFLD progresses to NASH in many patients, the underlying mechanisms are not completely understood. However, here we reviewed some important pathophysiologic concepts related to NAFLD and NASH and also enlisted some recent studies associated with risk factors of NAFLD and NASH (Table 1).

Increased Lipid Accumulation to the Liver

The pathogenesis of NAFLD seems to be a complex and multifactorial process. In NAFLD, the initial insult is the accumulation of fat in the liver[84] due to the increased delivery of FFAs to the liver.[85] The elevated level of hepatic FFAs promotes increased lipid synthesis and gluconeogenesis.[85] Lipolysis within adipose tissue, dietary sources, and DNL may result in increased FFAs in the liver.[86] DNL due to hyperinsulinemia coupled with insulin resistance aggravates the production and storage of triglycerides (Figure 1).

The feature of NAFLD is the accumulation of triglyceride in the hepatocytes as a result of the increased influx of FFAs. An increase in FFAs influx derived from the circulation due to increased lipolysis from visceral adipose tissue and/or from a diet rich in saturated fat and carbohydrate. An increase in glucose level in response to carbohydrate intake promotes DNL, and FFA oxidation leads to the development of steatosis. The imbalance of adipokines (adiponectin, leptin, and ghrelin) and proinflammatory cytokines (TNF-α, IL-6, and IL) secreted by adipose tissue may profoundly cause liver injury and steatohepatitis.

In NAFLD, around 60% of liver triglyceride comes from FFA deposition from adipose tissue, while DNL adds 26%.[20,84] This FFA either undergoes beta-oxidation or is esterified with glycerol aiming to generate triglycerides, generating hepatic fat influx.[85] Energy received from circulating glucose, fructose, and lipids is stored in the hepatocytes as glycogen. Increased lipids are redistributed to adipose tissue, where there is little capacity for storage of lipids. In adipose tissue, excessive accumulation of lipids yields metabolic incompetence and subsequent macrophage infiltration. Adipose tissue dysfunction has been considered to play an important role in the progression of metabolic disorders, such as insulin resistance and NAFLD.[87] In NAFLD, the processes of lipid trafficking are dysregulated with resultant elevated hepatic lipid accumulation.[88]

Insulin Resistance

IR, is defined as the failure of insulin to stimulate glucose uptake, a major contributing agent for the development of NAFLD.[89] Normally insulin causes phosphorylation of insulin receptor substrates (IRS)-1, -2, -3, and -4, which generate the insulin signal.[86] Abnormalities related to NAFLD hamper the insulin signaling cascade

Table 1: Some recent studies associated with risk factors of NAFLD and NASH

Study design	Assessment of NAFLD and NASH	Conclusion	Reference
MEDLINE via PubMed, Embase, Scopus and CINAHL were searched forstudiesfrom 2000-2020.	Post liver-transplant BMI and hyperlipidemia were the predictors of NAFLD and NASH	NAFLD and NASH after liver transplant are associated with metabolic risk factors	Saeed et al[75]
Retrospective analysis of 144 patients diagnosed with NASH between 2015 and 2017.	Low free tri-iodothyronine is associated with higher NAFLD and NASH	A low-normal thyroid hormone function may have a pathogenic role in modulating NAFLD and NASH.	Manka et al[76]
Systemic review and meta-analysis of publication between 2000 to 2018	NAFLD was diagnosed either by imaging or by histopathology	The presence and severity of NAFLD are linked with reduced whole-body bone marrow density Z score in children and adolescents.	Mantovani et al[41]
Cross-sectional study on 17 patients with simple steatosis, 15 with NASH, and 22 with living liver donors.	NASH was associated with a high level of plasma retinol level and overexpression of AKR1B10.	An altered retinol metabolism is involved in the process of hepatic fibrosis.	Pettinelli et al[77]
A narrative review and literature search from PubMed, Ovid Medline, and the Cochrane Library database until 2018	Sarcopenia is coupled with NAFLD independent of obesity, IR, or metabolic syndrome.	Management of sarcopenia has become an important issue in the management of patients with chronic liver disease.	Hsu et al[78]
A critical review on the relationship between vitamin D deficiency and NAFLD/NASH.	Liver biopsy, imaging techniques, and liver ultrasound have been considered as the most widely used techniques to identify NASH.	The deficiency of vitamin D has been linked to the pathogenesis and severity of NAFLD because of vitamin D pleiotropic functions.	Pacifico et al[79]
A retrospective study	Extensive NAF-P is predictive of advance fibrosis	NAF-P is strongly linked with NAFLD.	Rosenblatt et al[80]
A systemic review and meta-analysis	NAFLD and urolithiasis were diagnosed by either ultrasonography or computerized tomography	NAFLD is associated with an increased risk of urolithiasis.	Qin et al[81]
Patients with NAFLD who had undergone liver biopsy were specified from a prospectively maintained database.	The diagnosis of NAFLD is defined by the presence of ^{3}	Modest (1-70 g per week) alcohol consumption, particularly wine in a non-binge pattern, is related with lower fibrosis in patient with NAFLD.	Mitchell et al[82]
A cross-sectional pilot study consisting of biopsy-proven patients with NASH	Liver fibrosis ^{3}	Lean patients with NASH showed a lack of Lactobacillus compared with overweight and obese patients with NASH.	Duarte et al[83]

Abbreviations: NAFLD, Non-alcoholic fatty liver disease; NASH, Non-alcoholic steatohepatitis; HCC, Hepatocellular carcinoma; HBV, Hepatitis B virus; ASH, Alcoholic steatohepatitis; ALT, Alanine aminotransferase; GGT, Gamma-glutamyl transferase; ROS, Reactive oxygen species; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids; TG, Triglycerides; ER, Endoplasmic reticular; DNL, *De novo lipogenesis*; FFA, Free fatty acid; IR, Insulin resistance; VLDL, Very low-density lipoprotein; T2DM, Type 2 diabetes mellitus; OSA, Obstructive sleep apnea; BMI, Body mass index; US, Ultrasonography; HDL, High-density lipoprotein; LDL, Low-density lipoprotein; NAFPD, Non-alcoholic fatty pancreatic disease; NAF-P, Non-alcoholic fatty pancreas disease; TNF-α, Tumor necrosis factor-α; IL, Interleukin; IRS, Insulin receptor substrates; HSC, Hepatic stellate cell; ETC, Electron transport chain; TLR4, Toll-like receptor 4; TE, Transient elastography; MR, Magnetic resonance; MRI, Magnetic resonance imaging.

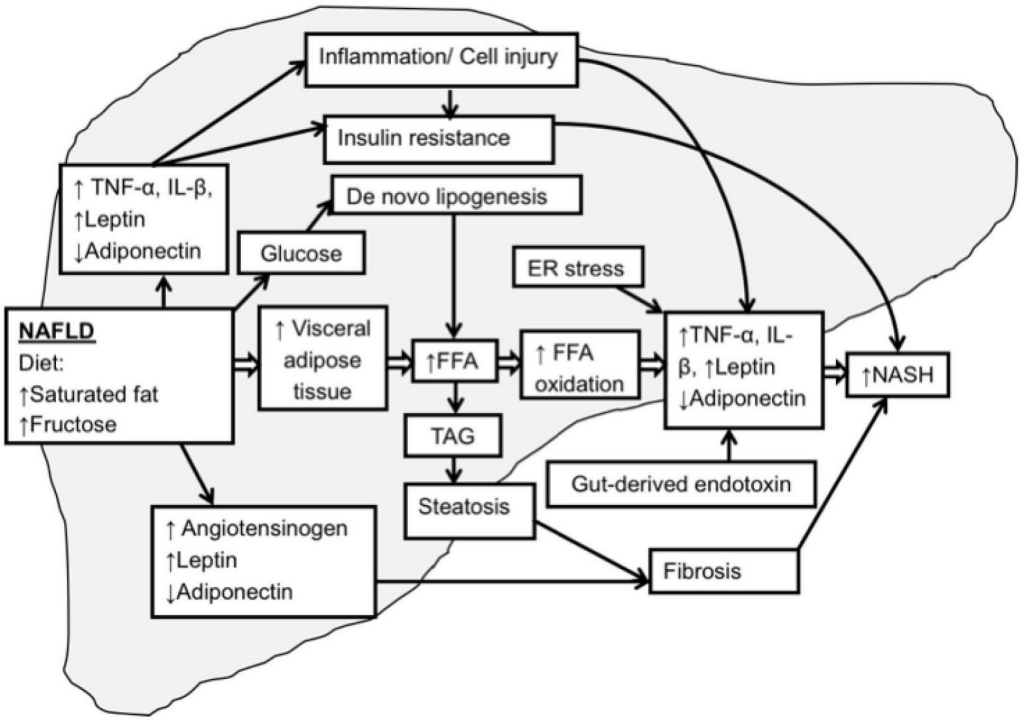

Fig. 1: Proposed mechanism describing the onset and progression of non-alcoholic fatty liver disease (NAFLD) and steatohepatitis.

lead to IR. According to the two-hit hypothesis, IR causes an elevated intrahepatic triglyceride known as the first hit, followed by the second hit. The second hit likely involves stimulation of cytochrome P450, oxidative stress, increased inflammatory cytokines, lipid peroxidation, stimulation of hepatic stellate cells (HSCs), and apoptosis.[90] People with NASH have an elevated amount of TNF-α, which promotes IR.[91] However, in the situation of IR enhance peripheral lipolysis from adipose tissue, increases triglyceride synthesis, develops fatty liver, increases the influx of fatty acids, and causes an overall increase in hepatic FFA accumulation.[92,93] The FFA, which results from increased fat accumulation in the liver, can cause lipid peroxidation in the hepatocyte membrane, resulting in the release of proinflammatory cytokines and stellate cell activation, which cause fibrosis.[94,95] Therefore, IR characterized by hyperinsulinemia in patients with NAFLD enhances DNL, which further accelerates hepatic fat accumulation and the progression of NASH.[90]

Adipokines/Proinflammatory Cytokines

Adipokines and proinflammatory cytokines have been shown to take part in the progression of NAFLD. The most extensively investigated adipokines in NAFLD/NASH are adiponectin, leptin, ghrelin, resistin, and visfatin,and proinflammatory cytokines are TNF-α, IL-6, and IL-1. Adiponectin enhances NASH and hepatic fibrosis by repressing the function of Kupffer cells and HSCs. The inflammatory cytokines are the main contributors of hepatic inflammation, cell death, and fibrosis, together with regeneration after massive or focal liver injury.[96] Besides, adipocytokines are coupled with an increase in insulin sensitivity and are also related to visceral obesity.[97] However, in NAFLD serum leptin levels are increased, and the liver turns refractory to the "anti-steatotic" effects of leptin.[98] A proinflammatory adipokine, TNF-α, hinders insulin signaling and facilitates steatosis, and may play a pivotal role in the progression of NASH.[99] Hepatic cytokines can help in the progression of steatosis to NASH by replicating the histological lesions accompanied with NASH, such as neutrophil chemotaxis, hepatocyte apoptosis/necrosis, formation of Mallory body, and activation of stellate cell.[100] Obesity-related hepatic steatosis is directly related to a higher amount of inflammatory cytokines and a lower amount of anti-

inflammatory cytokines.[101] In obesity, the amount of adiponectin is decreased, resulting in FFA influx, and oxidation in the mitochondria is thereafter reduced, facilitating FFA to store in the cytoplasm (Figure 1).[102]

Mitochondrial Dysfunction

Structural or functional abnormalities of mitochondria have been reported to accelerate the progression of NAFLD.[103] Mitochondria cause lipid influx in hepatocytes by enhancing beta-oxidation, but in case of NAFLD, this process can become overwhelmed due to increased FFA load, resulting in ROS generation.[104] Increased ROS production eventually leads to electron transport chain ETCdysfunction.[104] Furthermore, ROS induces oxidative stress, activates inflammatory pathways,[105] and finally leads to mitochondrial damage. Peroxidation of cardiolipin, a phospholipid positioned at the inner mitochondrial membrane, has been connected with mitochondrial dysfunction in several physio-pathological conditions, including NAFLD.[106] Structural mitochondrial abnormalities,[103] and a decrease in mitochondrial respiratory chain activity have been found in human studies of NASH.[107,108]

Gut Microbiota

Recently, new evidence showed that gut microbiota plays a major role in the pathogenesis of NASH. Dysbiosis (an imbalance of microbiota or enrichment of specific bacterial strains in the intestinal microbiota), impairment of intestinal barrier, and altered immune condition have been linked with a proinflammatory response, IR, obesity, NAFLD, and NASH through multiple interactions with the host's innate immune system.[109] The gut microbiota has also been linked with ethanol production, which is hepatotoxic and alters gut permeability, inducing endotoxemia.[110] An imbalance of microbiota or enrichment of specific bacterial strains in the intestinal microbiota has been linked with NAFLD or NASH.[109] Bäckhed and colleagues[111] firstly reported that intestinal flora enhanced monosaccharide absorption from the lumen of the intestine acceleratedDNL and triglyceride production, as approved by increased activity of acetyl-CoA carboxylase and fatty acid synthase. Intestinal microbiota may also stimulate liver steatosis by induction of obesity from indigestible dietary polysaccharides, control of gut permeability and acceleration of low-grade inflammation, modulation of dietary choline metabolism, regulation of bile acid metabolism, and stimulation of endogenous ethanol production by enteric bacteria.[112,113] Endotoxin, a component of the outer wall of gram-negative bacteria, is generated by the microbiota in the gut and is directly introduced into the liver via the portal blood, contributing to inflammation *via* cytokines by increasing intestinal permeability resulting in endotoxemia. Endotoxin modulates the innate immune system via Toll-like receptor 4 (TLR4), activates an inflammatory response, including higher levels of TNF-α. Kupffer cells (the first line of defense) are activated by endotoxin, and marked activation of Kupffer cells in human NASH has been observed.[114] NAFLD is often diagnosed in patients with *Helicobacter pylori*. *H. pylori* infection is considered as one of the independent risk factors for the development of NAFLD.[115] However, the pathogenic mechanism of this phenomenon is unclear. The effect of *H. pylori* on liver damage has not been studied sufficiently. Helicobacter species may cause liver injury via the release of specific toxins. Invasion of Helicobacter in the small bowel mucosa might increase gut permeability and facilitate the passage of bacterial endotoxins through the portal vein to the liver.[116]

Development of NASH from NAFLD

NAFLD is presented by the influx of triglycerides within the hepatocytes, which are produced from the esterification of FFA and glycerol. Therefore, hepatic fat infiltration can occur as a consequence of higher fat synthesis, delivery, and decreased fat excretion or oxidation.[86] The most commonly accepted mechanism regarding the progression of NASH is that NASH, develops in the presence of hepatic fat accumulation and oxidative stress, the so-called "two-hit" mechanism. The 'first hit', which is steatosis or hepatic TG accumulation, induces liver injury, and the 'second hit' is derived from various sources such as adipokines or inflammatory cytokines and oxidative stress, which ultimately leads to fibrosis (Figure 2).[22]

IR is considered as a key pathophysiological element associated with the development of NAFLD. IR enhances peripheral lipolysis, excessive triglyceride

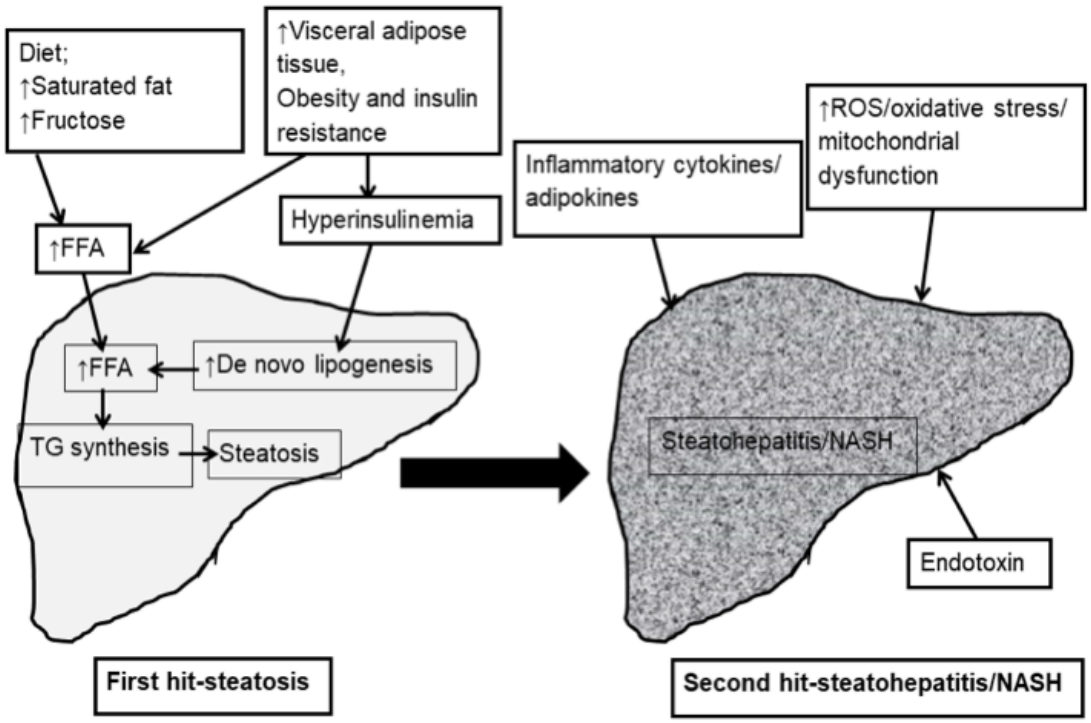

Fig. 2: The two-hit hypothesis for the development of NASH, which theorizes that dietary fat and insulin resistance, the 'first hit', contributes to the development of steatosis. The 'second hit' in the form of oxidative stress derived from various sources such as inflammatory cytokines, adipokines, ER stress, endotoxin, and mitochondrial dysfunction leads to steatohepatitis and fibrosis.

synthesis, and in turn increases hepatic uptake of fatty acids resulting in an elevation of hepatic free fatty acid infiltration. Increased synthesis and delivery of FFA to the liver and reduced metabolism and removal of hepatic FFA result in infiltration of excessive hepatic triglyceride. Dietary fat, obesity, DNL, increased conversion of carbohydrates and proteins to triglycerides lead to accumulation of hepatic FFA. Therefore, increased amounts of hepatic FFA contributes to the development of hepatotoxicity by down-regulating beta-oxidation and activating critical pathways that signals inflammation, apoptosis, and fibrosis, which characterize NASH. Adult and pediatric patterns of NAFLD/NASH represent substantial variability in the pattern of fat, inflammation, and fibrosis. The pediatric pattern of NASH has two distinct subtypes: type 1 NASH involves steatosis with ballooning degeneration of hepatocytes and perisinusoidal fibrosis, whereas type 2 pattern includes steatosis with portal inflammation or fibrosis without evidence of ballooning degeneration. The histologic criteria for the diagnosis of adult NASH involve steatosis, hepatocellular ballooning, and lobular inflammation.

Diagnostic Tools and Therapeutic Strategies

NAFLD resides asymptomatic in many people. Therefore, the diagnosis is often assumed by abnormal liver functions on biochemical testing or hepatic imaging (ultrasonography, computed tomography, or magnetic resonance imaging of liver), which indicate fatty liver when carried out for other illnesses. Liver biopsy is considered as the gold standard for diagnostic evaluation of NAFLD. Recent studies also show that the ultrasound-based controlled attenuation parameter value used in the TE (transient elastography) technique can detect the extent of steatosis in patients with NAFLD.[117] The gold standard for the non-invasive evaluation of hepatic steatosis is the application of MRI protein density fat fraction. Modern MRI techniques, such as MR elastography, can evaluate the extent of fibrosis non-invasively to diagnose and evaluate the prognosis of patients with NAFLD.[118]

Early evidence from clinical trials demonstrates that features of NASH are pharmacologically responsive.

However, no more than ~40% of patients in these trials have exhibited benefit from a single therapy, which may not be much effective in evaluating regulatory approval for long-term monotherapy.[119] Therefore, the field is quickly moving toward combined therapies. Combinations may also include conjugate therapies-for example, a fatty acid–bile acid conjugate, aramchol, that is under clinical trials for NASH.[120] No drug–antibody conjugates has been approved so far. Similarly, single drugs with multiple targets and modes of action have the possibility to be efficacious, as NASH pathogenesis involves many disease drivers. Lifestyle modification, consisting of diet and exercise, is essential for NAFLD therapy and has been proven by many studies to improve liver histology. However, lifestyle modification is difficult to attain and sustain. The hallmark progress that has been made in previous years in understanding disease pathogenesis has led to an explosion of medical therapies targeting various aspects of fat accumulation and injury pathways.

CONCLUSION

NAFLD is regarded as an important and emerging health hazard. The pathogenesis ofNAFLDand its advancement to fibrosis and chronicliverdiseaseis still obscure. Many studies show that NAFLD can be associated with an increased risk of IR. Dyslipidemia, IR, obesity, low adiponectin, postprandial dyslipidemia, and hyperglycemia are the main factors that give rise to NAFLD and further accelerate the course of NAFLD along with accelerating the development of NASH. NAFLD may affect any age of people and seems to be different among different ethnic groups. Environmental and lifestyle-related factors such as reduced physical activity and high-fat diets are well-studied factors for the development of insulin resistance-associated comorbidities and NAFLD. Recent studies have advanced in the field of genetic and immune response in NASH pathogenesis, even though family studies and studies explicitly addressing the genetic predisposition for the development of NAFLD are still missing. Lifestyle change in the form of modification of diet and physical activity is an important mode of action. However, alteration of any of the multiple mechanisms involved in the progression of NASH could provide useful insights to prevent the development of fibrosis and its associated complications.

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ETHICAL APPROVAL

There is nothing to be declared.

CONFLICT OF INTEREST

The author declares no conflict of interest related to this work.

REFERENCES

1. Akter S, Kawauchi S, Sato S, Aosasa S, Yamamoto J, Nishidate I. In vivo imaging of hepatic hemodynamics and light scattering property during ischemia-reperfusion in rats based on spectrocolorimetry. *Biomed Opt Express* 2017;8(2):974-92. doi: 10.1364/boe.8.000974
2. Akter S, Tanabe T, Maejima S, Kawauchi S, Sato S, Hinoki A, et al. In vivo estimation of optical properties of rat liver using single-reflectance fiber probe during ischemia and reperfusion. *Opt Rev* 2016;23(2):354-9. doi: 10.1007/s10043-015-0171-9
3. Akter S, Maejima S, Kawauchi S, Sato S, Hinoki A, Aosasa S, et al. Evaluation of light scattering and absorption properties of in vivo rat liver using a single-reflectance fiber probe during preischemia, ischemia-reperfusion, and postmortem. *J Biomed Opt* 2015;20(7):076010. doi: 10.1117/1.jbo.20.7.076010
4. Wang FS, Fan JG, Zhang Z, Gao B, Wang HY. The global burden of liver disease: the major impact of China. *Hepatology* 2014;60(6):2099-108. doi: 10.1002/hep.27406
5. Angulo P. GI epidemiology: nonalcoholic fatty liver disease. Aliment Pharmacol Ther 2007;25(8):883-9. doi: 10.1111/j.1365-2036.2007.03246.x
6. Kostrzewski T, Maraver P, Ouro-Gnao L, Levi A, Snow S, Miedzik A, et al. A microphysiological system for studying nonalcoholic steatohepatitis. *Hepatol Commun* 2020;4(1):77-91. doi: 10.1002/hep4.1450
7. Masarone M, Federico A, Abenavoli L, Loguercio C, Persico M. Non alcoholic fatty liver: epidemiology and natural history. *Rev Recent Clin Trials* 2014;9(3):126-33. doi: 10.2174/1574887109666141216111143
8. Brea A, Puzo J. Non-alcoholic fatty liver disease and cardiovascular risk. *Int J Cardiol* 2013;167(4):1109-17. doi: 10.1016/j.ijcard.2012.09.085
9. Lombardi R, Fargion S, Fracanzani AL. Brain involvement in non-alcoholic fatty liver disease (NAFLD): a systematic review. *Dig Liver Dis* 2019;51(9):1214-22. doi: 10.1016/j.dld.2019.05.015
10. Hadjihambi A. Cerebrovascular alterations in NAFLD: is it increasing our risk of Alzheimer's disease?

Anal Biochem 2022;636:114387. doi: 10.1016/j.ab.2021.114387
11. Clark JM, Brancati FL, Diehl AM. The prevalence and etiology of elevated aminotransferase levels in the United States. *Am J Gastroenterol* 2003;98(5):960-7. doi: 10.1111/j.1572-0241.2003.07486.x
12. Oda K, Uto H, Mawatari S, Ido A. Clinical features of hepatocellular carcinoma associated with nonalcoholic fatty liver disease: a review of human studies. *Clin J Gastroenterol* 2015;8(1):1-9. doi: 10.1007/s12328-014-0548-5
13. Weston SR, Leyden W, Murphy R, Bass NM, Bell BP, Manos MM, et al. Racial and ethnic distribution of nonalcoholic fatty liver in persons with newly diagnosed chronic liver disease. *Hepatology* 2005;41(2):372-9. doi: 10.1002/hep.20554
14. Younossi ZM. Review article: current management of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 2008;28(1):2-12. doi: 10.1111/j.1365-2036.2008.03710.x
15. Akter S, Miah MA, Khan MA, Islam MK. Effects of estrogen and folic acid on high fat induced hypercholesterolemic mice. *Br Biotechnol J* 2013;3(1):39-53. doi: 10.9734/bbj/2013/2003
16. Akter S, Miah A, Islam K, Khan AH. Comparative effects of animal and vegetable fats on lipid profile and patho-physiological changes in mice. *J Sci Res* 2013;5(2):353-61. doi: 10.3329/jsr.v5i2.11909
17. Hossain MG, Mahmud MM, Rahman MA, Akter S, Nazir K, Saha S, et al. Complete genome sequence of a precore-defective hepatitis B virus genotype D2 strain isolated in Bangladesh. *Microbiol Resour Announc* 2020;9(11):e00083-20. doi: 10.1128/mra.00083-20
18. Hossain MG, Akter S, Ohsaki E, Ueda K. Impact of the interaction of hepatitis B virus with mitochondria and associated proteins. *Viruses* 2020;12(2):175. doi: 10.3390/v12020175
19. Kawai T, Kayama K, Tatsumi S, Akter S, Miyawaki N, Okochi Y, et al. Regulation of hepatic oxidative stress by voltage-gated proton channels (Hv1/VSOP) in Kupffer cells and its potential relationship with glucose metabolism. *FASEB J* 2020;34(12):15805-21. doi: 10.1096/fj.202001056RRR
20. Petta S, Muratore C, Craxì A. Non-alcoholic fatty liver disease pathogenesis: the present and the future. *Dig Liver Dis* 2009;41(9):615-25. doi: 10.1016/j.dld.2009.01.004
21. de Araújo Souza MR, de Fátima Formiga de Melo Diniz M, de Medeiros-Filho JE, de Araújo MS. Metabolic syndrome and risk factors for non-alcoholic fatty liver disease. *Arq Gastroenterol* 2012;49(1):89-96. doi: 10.1590/s0004-28032012000100015
22. Basaranoglu M, Basaranoglu G, Sentürk H. From fatty liver to fibrosis: a tale of "second hit". *World J Gastroenterol* 2013;19(8):1158-65. doi: 10.3748/wjg.v19.i8.1158
23. Brown GT, Kleiner DE. Histopathology of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *Metabolism* 2016;65(8):1080-6. doi: 10.1016/j.metabol.2015.11.008
24. Molleston JP, Schwimmer JB, Yates KP, Murray KF, Cummings OW, Lavine JE, et al. Histological abnormalities in children with nonalcoholic fatty liver disease and normal or mildly elevated alanine aminotransferase levels. *J Pediatr* 2014;164(4):707-13.e3. doi: 10.1016/j.jpeds.2013.10.071
25. Reha JL, Lee S, Hofmann LJ. Prevalence and predictors of nonalcoholic steatohepatitis in obese patients undergoing bariatric surgery: a Department of Defense experience. *Am Surg* 2014;80(6):595-9.
26. Chiang DJ, Pritchard MT, Nagy LE. Obesity, diabetes mellitus, and liver fibrosis. *Am J Physiol Gastrointest Liver Physiol* 2011;300(5):G697-702. doi: 10.1152/ajpgi.00426.2010
27. Bellentani S, Scaglioni F, Marino M, Bedogni G. Epidemiology of non-alcoholic fatty liver disease. *Dig Dis* 2010;28(1):155-61. doi: 10.1159/000282080
28. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther* 2011;34(3):274-85. doi: 10.1111/j.1365-2036.2011.04724.x
29. Milić S, Lulić D, Štimac D. Non-alcoholic fatty liver disease and obesity: biochemical, metabolic and clinical presentations. *World J Gastroenterol* 2014;20(28):9330-7. doi: 10.3748/wjg.v20.i28.9330
30. Caldwell SH, Hespenheide EE. Subacute liver failure in obese women. *Am J Gastroenterol* 2002;97(8):2058-62. doi: 10.1111/j.1572-0241.2002.05922.x
31. Schwimmer JB, McGreal N, Deutsch R, Finegold MJ, Lavine JE. Influence of gender, race, and ethnicity on suspected fatty liver in obese adolescents. *Pediatrics* 2005;115(5):e561-5. doi: 10.1542/peds.2004-1832
32. Tarantino G, Saldalamacchia G, Conca P, Arena A. Non-alcoholic fatty liver disease: further expression of the metabolic syndrome. *J Gastroenterol Hepatol* 2007;22(3):293-303. doi: 10.1111/j.1440-1746.2007.04824.x
33. Povel CM, Boer JM, Reiling E, Feskens EJ. Genetic variants and the metabolic syndrome: a systematic review. *Obes Rev* 2011;12(11):952-67. doi: 10.1111/j.1467-789X.2011.00907.x
34. Koteish A, Diehl AM. Animal models of steatosis. *Semin Liver Dis* 2001;21(1):89-104. doi: 10.1055/s-2001-12932
35. Leamy AK, Egnatchik RA, Young JD. Molecular mechanisms and the role of saturated fatty acids in the progression of non-alcoholic fatty liver disease.

Prog Lipid Res 2013;52(1):165-74. doi: 10.1016/j.plipres.2012.10.004

36. Chapman MJ, Ginsberg HN, Amarenco P, Andreotti F, Borén J, Catapano AL, et al. Triglyceride-rich lipoproteins and high-density lipoprotein cholesterol in patients at high risk of cardiovascular disease: evidence and guidance for management. *Eur Heart J* 2011;32(11):1345-61. doi: 10.1093/eurheartj/ehr112
37. Basaranoglu M, Basaranoglu G, Bugianesi E. Carbohydrate intake and nonalcoholic fatty liver disease: fructose as a weapon of mass destruction. *Hepatobiliary Surg Nutr* 2015;4(2):109-16. doi: 10.3978/j.issn.2304-3881.2014.11.05
38. Schultz A, Neil D, Aguila MB, Mandarim-de-Lacerda CA. Hepatic adverse effects of fructose consumption independent of overweight/obesity. *Int J Mol Sci* 2013;14(11):21873-86. doi: 10.3390/ijms141121873
39. Lim JS, Mietus-Snyder M, Valente A, Schwarz JM, Lustig RH. The role of fructose in the pathogenesis of NAFLD and the metabolic syndrome. *Nat Rev Gastroenterol Hepatol* 2010;7(5):251-64. doi: 10.1038/nrgastro.2010.41
40. Pollock NK, Bundy V, Kanto W, Davis CL, Bernard PJ, Zhu H, et al. Greater fructose consumption is associated with cardiometabolic risk markers and visceral adiposity in adolescents. *J Nutr* 2012;142(2):251-7. doi: 10.3945/jn.111.150219
41. Mantovani A, Gatti D, Zoppini G, Lippi G, Bonora E, Byrne CD, et al. Association between nonalcoholic fatty liver disease and reduced bone mineral density in children: a meta-analysis. *Hepatology* 2019;70(3):812-23. doi: 10.1002/hep.30538
42. Firneisz G. Non-alcoholic fatty liver disease and type 2 diabetes mellitus: the liver disease of our age? *World J Gastroenterol* 2014;20(27):9072-89. doi: 10.3748/wjg.v20.i27.9072
43. Leite NC, Salles GF, Araujo AL, Villela-Nogueira CA, Cardoso CR. Prevalence and associated factors of non-alcoholic fatty liver disease in patients with type-2 diabetes mellitus. *Liver Int* 2009;29(1):113-9. doi: 10.1111/j.1478-3231.2008.01718.x
44. Bell DS, Allbright E. The multifaceted associations of hepatobiliary disease and diabetes. *Endocr Pract* 2007;13(3):300-12. doi: 10.4158/ep.13.3.300
45. Leite NC, Villela-Nogueira CA, Pannain VL, Bottino AC, Rezende GF, Cardoso CR, et al. Histopathological stages of nonalcoholic fatty liver disease in type 2 diabetes: prevalences and correlated factors. *Liver Int* 2011;31(5):700-6. doi: 10.1111/j.1478-3231.2011.02482.x
46. Pulixi EA, Tobaldini E, Battezzati PM, D'Ingianna P, Borroni V, Fracanzani AL, et al. Risk of obstructive sleep apnea with daytime sleepiness is associated with liver damage in non-morbidly obese patients with nonalcoholic fatty liver disease. *PLoS One* 2014;9(4):e96349. doi: 10.1371/journal.pone.0096349
47. Tanné F, Gagnadoux F, Chazouillères O, Fleury B, Wendum D, Lasnier E, et al. Chronic liver injury during obstructive sleep apnea. *Hepatology* 2005;41(6):1290-6. doi: 10.1002/hep.20725
48. Savransky V, Bevans S, Nanayakkara A, Li J, Smith PL, Torbenson MS, et al. Chronic intermittent hypoxia causes hepatitis in a mouse model of diet-induced fatty liver. *Am J Physiol Gastrointest Liver Physiol* 2007;293(4):G871-7. doi: 10.1152/ajpgi.00145.2007
49. Zamora-Valdés D, Méndez-Sánchez N. Experimental evidence of obstructive sleep apnea syndrome as a second hit accomplice in nonalcoholic steatohepatitis pathogenesis. *Ann Hepatol* 2007;6(4):281-3.
50. Koutsari C, Ali AH, Mundi MS, Jensen MD. Storage of circulating free fatty acid in adipose tissue of postabsorptive humans: quantitative measures and implications for body fat distribution. *Diabetes* 2011;60(8):2032-40. doi: 10.2337/db11-0154
51. Kojima S, Watanabe N, Numata M, Ogawa T, Matsuzaki S. Increase in the prevalence of fatty liver in Japan over the past 12 years: analysis of clinical background. *J Gastroenterol* 2003;38(10):954-61. doi: 10.1007/s00535-003-1178-8
52. Hu X, Huang Y, Bao Z, Wang Y, Shi D, Liu F, et al. Prevalence and factors associated with nonalcoholic fatty liver disease in Shanghai work-units. *BMC Gastroenterol* 2012;12:123. doi: 10.1186/1471-230x-12-123
53. Kobayashi J, Maruyama T, Watanabe H, Kudoh A, Tateishi S, Sasaki T, et al. Gender differences in the effect of type 2 diabetes on serum lipids, pre-heparin plasma lipoprotein lipase mass and other metabolic parameters in Japanese population. *Diabetes Res Clin Pract* 2003;62(1):39-45. doi: 10.1016/s0168-8227(03)00160-8
54. Hashimoto E, Tokushige K. Prevalence, gender, ethnic variations, and prognosis of NASH. *J Gastroenterol* 2011;46 Suppl 1:63-9. doi: 10.1007/s00535-010-0311-8
55. Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 2004;40(6):1387-95. doi: 10.1002/hep.20466
56. Solga SF, Clark JM, Alkhuraishi AR, Torbenson M, Tabesh A, Schweitzer M, et al. Race and comorbid factors predict nonalcoholic fatty liver disease histopathology in severely obese patients. *Surg Obes Relat Dis* 2005;1(1):6-11. doi: 10.1016/j.soard.2004.12.006
57. Ogawa Y, Imajo K, Yoneda M, Nakajima A. [Pathophysiology of NAsh/NAFLD associated with high levels of serum triglycerides]. *Nihon Rinsho* 2013;71(9):1623-9.

58. Toledo FG, Sniderman AD, Kelley DE. Influence of hepatic steatosis (fatty liver) on severity and composition of dyslipidemia in type 2 diabetes. *Diabetes Care* 2006;29(8):1845-50. doi: 10.2337/dc06-0455
59. Adiels M, Taskinen MR, Borén J. Fatty liver, insulin resistance, and dyslipidemia. *Curr Diab Rep* 2008;8(1):60-4. doi: 10.1007/s11892-008-0011-4
60. Nobili V, Alisi A, Panera N, Agostoni C. Low birth weight and catch-up-growth associated with metabolic syndrome: a ten year systematic review. *Pediatr Endocrinol Rev* 2008;6(2):241-7.
61. Sypniewska G. Laboratory assessment of cardiometabolic risk in overweight and obese children. *Clin Biochem* 2015;48(6):370-6. doi: 10.1016/j.clinbiochem.2014.12.024
62. Cotrim HP, Parise ER, Oliveira CP, Leite N, Martinelli A, Galizzi J, et al. Nonalcoholic fatty liver disease in Brazil. Clinical and histological profile. *Ann Hepatol* 2011;10(1):33-7.
63. Hsiao PJ, Kuo KK, Shin SJ, Yang YH, Lin WY, Yang JF, et al. Significant correlations between severe fatty liver and risk factors for metabolic syndrome. *J Gastroenterol Hepatol* 2007;22(12):2118-23. doi: 10.1111/j.1440-1746.2006.04698.x
64. Parker A, Kim Y. The effect of low glycemic index and glycemic load diets on hepatic fat mass, insulin resistance, and blood lipid panels in individuals with nonalcoholic fatty liver disease. *Metab Syndr Relat Disord* 2019;17(8):389-96. doi: 10.1089/met.2019.0038
65. Dasarathy J, Varghese R, Feldman A, Khiyami A, McCullough AJ, Dasarathy S. Patients with nonalcoholic fatty liver disease have a low response rate to vitamin D supplementation. *J Nutr* 2017;147(10):1938-46. doi: 10.3945/jn.117.254292
66. El-Hajj Fuleihan G, Baddoura R, Habib RH, Halaby G, Arabi A, Rahme M, et al. Effect of vitamin D replacement on indexes of insulin resistance in overweight elderly individuals: a randomized controlled trial. *Am J Clin Nutr* 2016;104(2):315-23. doi: 10.3945/ajcn.116.132589
67. Bhatt SP, Misra A, Pandey RM, Upadhyay AD, Gulati S, Singh N. Vitamin D supplementation in overweight/obese Asian Indian women with prediabetes reduces glycemic measures and truncal subcutaneous fat: a 78 weeks randomized placebo-controlled trial (PREVENT-WIN Trial). *Sci Rep* 2020;10(1):220. doi: 10.1038/s41598-019-56904-y
68. Wijarnpreecha K, Lou S, Panjawatanan P, Sanguankeo A, Pungpapong S, Lukens FJ, et al. Nonalcoholic fatty liver disease and urolithiasis. a systematic review and meta-analysis. *J Gastrointestin Liver Dis* 2018;27(4):427-32. doi: 10.15403/jgld.2014.1121.274.nac
69. Einollahi B, Naghii MR, Sepandi M. Association of nonalcoholic fatty liver disease (NAFLD) with urolithiasis. *Endocr Regul* 2013;47(1):27-32. doi: 10.4149/endo_2013_01_27
70. Qin S, Wang S, Wang X, Wang J. Non-alcoholic fatty liver disease and the risk of urolithiasis: a systematic review and meta-analysis. *Medicine (Baltimore)* 2018;97(35):e12092. doi: 10.1097/md.0000000000012092
71. Catanzaro R, Cuffari B, Italia A, Marotta F. Exploring the metabolic syndrome: nonalcoholic fatty pancreas disease. *World J Gastroenterol* 2016;22(34):7660-75. doi: 10.3748/wjg.v22.i34.7660
72. Bi Y, Wang JL, Li ML, Zhou J, Sun XL. The association between pancreas steatosis and metabolic syndrome: a systematic review and meta-analysis. *Diabetes Metab Res Rev* 2019;35(5):e3142. doi: 10.1002/dmrr.3142
73. Shah N, Rocha JP, Bhutiani N, Endashaw O. Nonalcoholic fatty pancreas disease. *Nutr Clin Pract* 2019;34 Suppl 1:S49-S56. doi: 10.1002/ncp.10397
74. Dite P, Blaho M, Bojkova M, Jabandziev P, Kunovsky L. Nonalcoholic fatty pancreas disease: clinical consequences. *Dig Dis* 2020;38(2):143-9. doi: 10.1159/000505366
75. Saeed N, Glass L, Sharma P, Shannon C, Sonnenday CJ, Tincopa MA. Incidence and risks for nonalcoholic fatty liver disease and steatohepatitis post-liver transplant: systematic review and meta-analysis. *Transplantation* 2019;103(11):e345-e54. doi: 10.1097/tp.0000000000002916
76. Manka P, Bechmann L, Best J, Sydor S, Claridge LC, Coombes JD, et al. Low free triiodothyronine is associated with advanced fibrosis in patients at high risk for nonalcoholic steatohepatitis. *Dig Dis Sci* 2019;64(8):2351-8. doi: 10.1007/s10620-019-05687-3
77. Pettinelli P, Arendt BM, Teterina A, McGilvray I, Comelli EM, Fung SK, et al. Altered hepatic genes related to retinol metabolism and plasma retinol in patients with non-alcoholic fatty liver disease. *PLoS One* 2018;13(10):e0205747. doi: 10.1371/journal.pone.0205747
78. Hsu CS, Kao JH. Sarcopenia and chronic liver diseases. *Expert Rev Gastroenterol Hepatol* 2018;12(12):1229-44. doi: 10.1080/17474124.2018.1534586
79. Pacifico L, Osborn JF, Bonci E, Pierimarchi P, Chiesa C. Association between vitamin D levels and nonalcoholic fatty liver disease: potential confounding variables. *Mini Rev Med Chem* 2019;19(4):310-32. doi: 10.2174/1389557518666181025153712
80. Rosenblatt R, Mehta A, Snell D, Hissong E, Kierans AS, Kumar S. Ultrasonographic nonalcoholic fatty pancreas is associated with advanced fibrosis in NAFLD: a retrospective analysis. *Dig Dis Sci* 2019;64(1):262-8. doi: 10.1007/s10620-018-5295-x
81. Qin S, Wang S, Wang X, Wang J. Non-alcoholic

fatty liver disease and the risk of urolithiasis: a systematic review and meta-analysis. *Medicine (Baltimore)* 2018;97(35):e12092. doi: 10.1097/md.0000000000012092

82. Mitchell T, Jeffrey GP, de Boer B, MacQuillan G, Garas G, Ching H, et al. Type and pattern of alcohol consumption is associated with liver fibrosis in patients with non-alcoholic fatty liver disease. *Am J Gastroenterol* 2018;113(10):1484-93. doi: 10.1038/s41395-018-0133-5
83. Duarte SMB, Stefano JT, Miele L, Ponziani FR, Souza-Basqueira M, Okada L, et al. Gut microbiome composition in lean patients with NASH is associated with liver damage independent of caloric intake: aprospective pilot study. *Nutr Metab Cardiovasc Dis* 2018;28(4):369-84. doi: 10.1016/j.numecd.2017.10.014
84. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J Clin Invest* 2005;115(5):1343-51. doi: 10.1172/jci23621
85. Dowman JK, Tomlinson JW, Newsome PN. Pathogenesis of non-alcoholic fatty liver disease. *QJM* 2010;103(2):71-83. doi: 10.1093/qjmed/hcp158
86. Postic C, Girard J. Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice. *J Clin Invest* 2008;118(3):829-38. doi: 10.1172/jci34275
87. Wang Y, Rimm EB, Stampfer MJ, Willett WC, Hu FB. Comparison of abdominal adiposity and overall obesity in predicting risk of type 2 diabetes among men. *Am J Clin Nutr* 2005;81(3):555-63. doi: 10.1093/ajcn/81.3.555
88. Tiniakos DG, Vos MB, Brunt EM. Nonalcoholic fatty liver disease: pathology and pathogenesis. *Annu Rev Pathol* 2010;5:145-71. doi: 10.1146/annurev-pathol-121808-102132
89. de Souza Bruno A, Rodrigues MH, Alvares MC, Nahas-Neto J, Nahas EA. Non-alcoholic fatty liver disease and its associated risk factors in Brazilian postmenopausal women. *Climacteric* 2014;17(4):465-71. doi: 10.3109/13697137.2014.881353
90. Xu Q, Li Y, Shang YF, Wang HL, Yao MX. miRNA-103: molecular link between insulin resistance and nonalcoholic fatty liver disease. *World J Gastroenterol* 2015;21(2):511-6. doi: 10.3748/wjg.v21.i2.511
91. Yuan M, Konstantopoulos N, Lee J, Hansen L, Li ZW, Karin M, et al. Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. *Science* 2001;293(5535):1673-7. doi: 10.1126/science.1061620
92. Edmison J, McCullough AJ. Pathogenesis of non-alcoholic steatohepatitis: human data. *Clin Liver Dis* 2007;11(1):75-104. doi: 10.1016/j.cld.2007.02.011
93. London RM, George J. Pathogenesis of NASH: animal models. *Clin Liver Dis* 2007;11(1):55-74. doi: 10.1016/j.cld.2007.02.010
94. Day CP, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology* 1998;114(4):842-5. doi: 10.1016/s0016-5085(98)70599-2
95. Leclercq IA, Farrell GC, Field J, Bell DR, Gonzalez FJ, Robertson GR. CYP2E1 and CYP4A as microsomal catalysts of lipid peroxides in murine nonalcoholic steatohepatitis. *J Clin Invest* 2000;105(8):1067-75. doi: 10.1172/jci8814
96. Stojsavljević S, Gomerčić Palčić M, Virović Jukić L, Smirčić Duvnjak L, Duvnjak M. Adipokines and proinflammatory cytokines, the key mediators in the pathogenesis of nonalcoholic fatty liver disease. *World J Gastroenterol* 2014;20(48):18070-91. doi: 10.3748/wjg.v20.i48.18070
97. Berg AH, Combs TP, Scherer PE. ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. *Trends Endocrinol Metab* 2002;13(2):84-9. doi: 10.1016/s1043-2760(01)00524-0
98. Kukla M, Mazur W, Bułdak RJ, Zwirska-Korczala K. Potential role of leptin, adiponectin and three novel adipokines--visfatin, chemerin and vaspin--in chronic hepatitis. *Mol Med* 2011;17(11-12):1397-410. doi: 10.2119/molmed.2010.00105
99. Tarantino G, Savastano S, Colao A. Hepatic steatosis, low-grade chronic inflammation and hormone/growth factor/adipokine imbalance. *World J Gastroenterol* 2010;16(38):4773-83. doi: 10.3748/wjg.v16.i38.4773
100. Day CP. Pathogenesis of steatohepatitis. *Best Pract Res Clin Gastroenterol* 2002;16(5):663-78. doi: 10.1053/bega.2002.0333
101. Diehl AM, Li ZP, Lin HZ, Yang SQ. Cytokines and the pathogenesis of non-alcoholic steatohepatitis. *Gut* 2005;54(2):303-6. doi: 10.1136/gut.2003.024935
102. Xu A, Wang Y, Keshaw H, Xu LY, Lam KS, Cooper GJ. The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. *J Clin Invest* 2003;112(1):91-100. doi: 10.1172/jci17797
103. Sanyal AJ, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology* 2001;120(5):1183-92. doi: 10.1053/gast.2001.23256
104. Gusdon AM, Song KX, Qu S. Nonalcoholic fatty liver disease: pathogenesis and therapeutics from a mitochondria-centric perspective. *Oxid Med Cell Longev* 2014;2014:637027. doi: 10.1155/2014/637027
105. Pérez-Carreras M, Del Hoyo P, Martín MA, Rubio JC, Martín A, Castellano G, et al. Defective hepatic mitochondrial respiratory chain in patients with nonalcoholic steatohepatitis. *Hepatology* 2003;38(4):999-1007. doi: 10.1053/jhep.2003.50398

106. Paradies G, Paradies V, Ruggiero FM, Petrosillo G. Oxidative stress, cardiolipin and mitochondrial dysfunction in nonalcoholic fatty liver disease. *World J Gastroenterol* 2014;20(39):14205-18. doi: 10.3748/wjg.v20.i39.14205
107. Hartvigsen K, Binder CJ, Hansen LF, Rafia A, Juliano J, Hörkkö S, et al. A diet-induced hypercholesterolemic murine model to study atherogenesis without obesity and metabolic syndrome. *Arterioscler Thromb Vasc Biol* 2007;27(4):878-85. doi: 10.1161/01.atv.0000258790.35810.02
108. Mansouri A, Gattolliat CH, Asselah T. Mitochondrial dysfunction and signaling in chronic liver diseases. *Gastroenterology* 2018;155(3):629-47. doi: 10.1053/j.gastro.2018.06.083
109. Biedermann L, Rogler G. The intestinal microbiota: its role in health and disease. *Eur J Pediatr* 2015;174(2):151-67. doi: 10.1007/s00431-014-2476-2
110. Wan Y, Tang J, Li J, Li J, Yuan J, Wang F, et al. Contribution of diet to gut microbiota and related host cardiometabolic health: diet-gut interaction in human health. *Gut Microbes* 2020;11(3):603-9. doi: 10.1080/19490976.2019.1697149
111. Bäckhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, et al. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci U S A* 2004;101(44):15718-23. doi: 10.1073/pnas.0407076101
112. Aron-Wisnewsky J, Gaborit B, Dutour A, Clement K. Gut microbiota and non-alcoholic fatty liver disease: new insights. *Clin Microbiol Infect* 2013;19(4):338-48. doi: 10.1111/1469-0691.12140
113. Arslan N. Obesity, fatty liver disease and intestinal microbiota. *World J Gastroenterol* 2014;20(44):16452-63. doi: 10.3748/wjg.v20.i44.16452
114. Falck-Ytter Y, Younossi ZM, Marchesini G, McCullough AJ. Clinical features and natural history of nonalcoholic steatosis syndromes. *Semin Liver Dis* 2001;21(1):17-26. doi: 10.1055/s-2001-12926
115. Doğan Z, Filik L, Ergül B, Sarikaya M, Akbal E. Association between *Helicobacter pylori* and liver-to-spleen ratio: a randomized-controlled single-blind study. *Eur J Gastroenterol Hepatol* 2013;25(1):107-10. doi: 10.1097/MEG.0b013e3283590c10
116. Abo-Amer YE, Sabal A, Ahmed R, Hasan NFE, Refaie R, Mostafa SM, et al. Relationship between *Helicobacter pylori* infection and nonalcoholic fatty liver disease (NAFLD) in a developing country: a cross-sectional study. *Diabetes Metab Syndr Obes* 2020;13:619-25. doi: 10.2147/dmso.s237866
117. Karlas T, Petroff D, Sasso M, Fan JG, Mi YQ, de Lédinghen V, et al. Individual patient data meta-analysis of controlled attenuation parameter (CAP) technology for assessing steatosis. *J Hepatol* 2017;66(5):1022-30. doi: 10.1016/j.jhep.2016.12.022
118. Kinner S, Reeder SB, Yokoo T. Quantitative imaging biomarkers of NAFLD. *Dig Dis Sci* 2016;61(5):1337-47. doi: 10.1007/s10620-016-4037-1
119. Friedman SL, Neuschwander-Tetri BA, Rinella M, Sanyal AJ. Mechanisms of NAFLD development and therapeutic strategies. *Nat Med* 2018;24(7):908-22. doi: 10.1038/s41591-018-0104-9
120. Safadi R, Konikoff FM, Mahamid M, Zelber-Sagi S, Halpern M, Gilat T, et al. The fatty acid-bile acid conjugate Aramchol reduces liver fat content in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2014;12(12):2085-91.e1. doi: 10.1016/j.cgh.2014.04.038