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# Review on the Role of Fructose in Non-Alcoholic Fatty Liver Diseases

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## Abstract

Non-alcoholic fatty liver disease (NAFLD) is a clinical liver condition characterized by accumulation of fat in the liver that can develop into non-alcoholic steatohepatitis (NASH) without any evidence of excessive alcohol consumption. NASH involves liver inflammation, necrosis, fibrosis, cirrhosis, and eventually liver cancer. Excessive intake of fructose is known to promote non-alcoholic fatty liver (NAFLD), because this sugar is both a substrate and an inducer of liver fat regeneration. In addition to the hepatic lipogenic effects, the consumption of fructose can also cause liver inflammation and cellular stress, such as oxidative and endoplasmic stress, which are conducive to the progression of steatosis to non-alcoholic steatohepatitis (NASH). Fructose also has direct and indirect effects on peripheral levels. For example, excessive fructose intake is associated with changes in the gut micro biota, which can lead to a worsening of the disease. Currently, there is no known treatment for NAFLD. Therefore, treatment is based on lifestyle changes, including changes in diet and physical exercise.

**Keywords:** Role, Fructose, Non-Alcoholic, Fatty, Liver, Diseases

## 1. Introduction

Nonalcoholic fatty liver disease (NAFLD) represents a number of diseases, from simple steatosis to nonalcoholic steatohepatitis (NASH), that can progress to fibrosis, cirrhosis and liver cancer (Jegatheesan and De Bandt, 2017) without history of drinking too much alcohol. Other causes of steatosis, such as viral, autoimmune, iron overload, drugs, must be excluded (Munteanu *et al.*, 2016). Accumulation of fat in liver due to excessive alcohol intake is termed Alcoholic fatty liver disease (AFLD).

NAFLD is characterized by hepatic steatosis. Hepatic steatosis is characterized by an accumulation of fat in liver cells without any inflammation and is generally less dangerous (Banini and Sanyal, 2016), Non-alcoholic steatohepatitis (NASH) develops from non-alcoholic fatty liver disease (NAFLD) (Peng *et al.*, 2020). The typical feature of NASH is liver steatosis and fibrotic inflammation caused by metabolic disorders, such as obesity, diabetes, and dyslipidemia, (Peng *et al.*, 2020). NASH patients with significant fibrosis are at increased risk of cirrhosis and liver failure

(Peng *et al.,* 2020). Patients can have NAFLD for many years before proceeding to NASH (Peng *et al.,* 2020). If left untreated, the risk of developing liver cirrhosis and subsequent liver failure and hepatocellular carcinoma will increase, and eventually will lead to death (Alexander *et al.,* 2019).

Currently, it is estimated that approximately 25% of the world's population suffers from NAFLD. Additionally, 20-25% of the patient population with NAFLD will continue to develop in NASH (Peng *et al.,* 2020). It is currently the second most common indication for liver transplantation in the United States (Peng *et al.,* 2020).

NAFLD is often asymptomatic. However, a recent population study of people highlighted that of NASH patients have a higher incidence of fatigue and abdominal discomfort, and of them have been shown to be associated with liver lobule inflammation (Peng *et al.,* 2020). This may be because liver inflammation is associated with elevated plasma inflammatory cytokines (Ajmera *et al.,* 2017), and the metabolically inflamed environment can negatively affect mood (Peng *et al.,* 2020).

Hepatic biopsy (tissue examination) represents the gold standard for the diagnosis and staging of NAFLD/NASH and is required as the primary end-point for later stage clinical trial (Than and Newsome, 2015).

NAFLD is closely related to insulin resistance and other risk factors for metabolic diseases, such as diabetes, central abdominal obesity, and dyslipidemia (Than and Newsome, 2015). Over nutrition and insulin resistance are the main risk factors for the development of NAFLD (Wong *et al.,* 2018). Genetic variation, the state of the gut micro biota, and epigenetic regulation induced by microRNA (miRNA), DNA methylation, histone modification, and ubiquitination can alter susceptibility to NAFLD (Wong *et al.,* 2018). The prevalence of NALD increases with age and male sex, but the relationship between age, sex, and susceptibility to NAFLD has not been determined (Wong *et al.,* 2018). Asians, Hispanics, Indians, and Native Americans seem to be more likely to develop NAFLD than Europeans and Africans (Vancells *et al.,* 2021). The prevalence of the adult population in North America is estimated to be 27-34%, Europe is 25%, and Asia is 15-20% (Vancells *et al.,* 2021).

Human studies indicate that a diet rich in carbohydrates, especially fructose, may be a major cause of NAFLD (Bhattacharjee *et al.,* 2014). A high fructose diet is considered a key reason for the development of NAFLD (Bhattacharjee *et al.,* 2014). Animal studies have shown that increased fructose intake can lead to hepatic steatosis with insulin resistance, elevated plasma triglyceride levels, and hepatic oxidative stress.

## 2. Nonalcoholic Fatty Disease (NAFLD)

Nonalcoholic fatty liver disease (NAFLD) defines a spectrum of liver disease ranging through simple steatosis, nonalcoholic steatohepatitis (NASH), liver fibrosis, and liver cirrhosis (Banini and Sanyal, 2016) with any evidence of heavy alcohol intake. This condition is currently

recognized as the most prevalent liver disease in Western populations with average rate estimated at 20% to 30% (Lisboa *et al.,* 2016) and it is predicted to become the main indication for liver transplantation within the next decade (Kelly *et al.,* 2019). Simple hepatic steatosis is characterized with buildup of fats in the hepatocytes without any inflammation and it is often carrying a relatively favourable clinical course, non-alcoholic steatohepatitis (NASH) is estimated to occurs in 25% of NAFLD patients, involves hepatocellular injury and liver inflammation and a significant risk factor for liver cirrhosis and liver cancer (Peng *et al.,* 2020; Banini and Sanyal, 2016). With the spectrum of NAFLD, NASH give course for worries, as it signifies hepatocellular injury and liver inflammation, leading to hepatic and extrahepatic complications (Banini and Sanyal, 2016). Hepatic steatosis rarely progresses to NASH.

Over nutrition and insulin resistance are the dominant risk factors for development of NAFLD (Wong *et al.,* 2018). Other risks include overweight, metabolic syndrome, a diet high in fructose, being male and older age (Wong *et al.,* 2018). The connection between age, gender and susceptibility to NAFLD remains unsettled (Wong *et al.,* 2018).

Current guidelines do not recommend any medical intervention for patients with simple hepatic steatosis or non-NASH NAFLD, because hepatic steatosis occurs primarily in people who are managed through lifestyle management (Kelly *et al.,* 2019). Vitamin E is the only antioxidant supplement recommended for a subset of patients with biopsy-confirmed NASH, which targets oxidative stress that can cause liver damage (Kelly *et al.,* 2019).

Hepatic biopsy (tissue examination) represents the gold standard for the diagnosis and staging of NAFLD/NASH and is required as the primary end-point for later stage clinical trial (Than and Newsome, 2015).

### 2.1. Pathogenesis and Progression of NAFLD

The pathogenesis of NAFLD is not fully understood. The "multiple hits" hypothesis believes that multiple hits in genetically susceptible subjects work together to induce NAFLD and provide a more precise explanation for the pathogenesis of NAFLD (Kupčová, *et al.,* 2019). Such hits include insulin resistance, types of hormones secreted by fat tissue, nutritional factors, gut microbiota, and genetic and epigenetic factors (Kupčová, *et al.,* 2019). Obesity appears to play an important role in the development of NAFLD, but NAFLD occurs even in lean patients (HaGani *et al.,* 2019; Kupčová, *et al.,* 2019). Obesity can lead to metabolic syndrome and insulin resistance (IR). On the other hand, insulin resistance may be the cause of NAFLD in non-obese patients (Kupčová, *et al.,* 2019).

The first hit reflects the accumulation of triglyceride (TG) and free fatty acids (steatosis) (Than and Newsome, 2015). Once the liver shows fatty infiltration, a second “hit” triggers the progression from steatosis to steatohepatitis (Grant and Lisker-Melman, 2004).

These hits include insulin resistance (IR), elevated plasma-free fatty acids, oxidative stress, liver inflammation, adipose tissue-secreted hormones, nutritional factors, gut microbiota, and genetic

and epigenetic factors. The multiple-hit theory begins with simple steatosis which is reversible and occurs mainly via four mechanisms: increased fatty acid uptake to the liver; reduced-fat transport in the form of very low-density lipoprotein (VLDL) triglycerides; decreased free fatty acid β-oxidation; and increased *de novo* lipogenesis (DNL) in the liver (Vancells *et al.*, 2021).

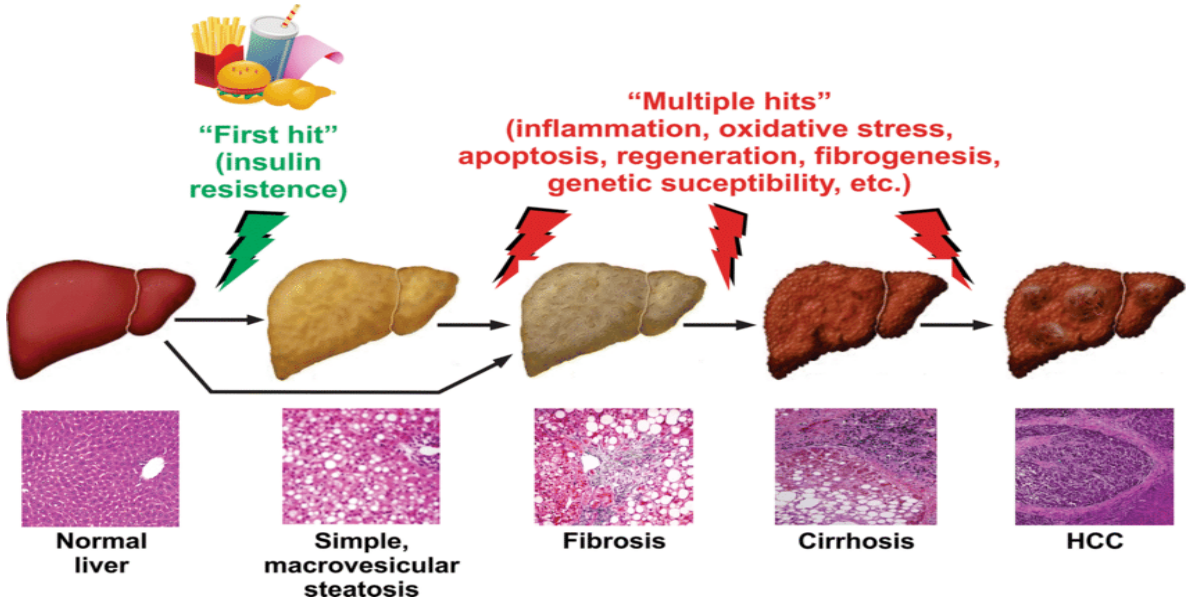


**Figure 2.0: Progression of NAFLD (Bessone *et al.*, 2019).**

There is a reduced insulin sensitivity in the liver, muscle, and adipose tissues of individuals with NAFLD (Than and Newsome, 2015). This plays a significant role in the pathogenesis of NAFLD. Due to IR, adipose tissue becomes resistant to the anti-lipolytic effects of the insulin and results to peripheral lipolysis which leads to an increased delivery of free fatty acids to the liver as well as driving *de novo* lipogenesis (Than and Newsome, 2015). Also, overload of lipids in the B-cells of the pancreas results to dysregulated insulin secretion and changes in the expression of peroxisome proliferator-activated receptor-α(PPAR-α), glucosekinase, the glucose transporter-2 (GLUT-2), pre-pro-insulin and pancreatic duodenal homeoboc-1 (PDX-1), which can lead to IR as a result of FFA-induced B-cell apoptosis (Than and Newsome, 2015).

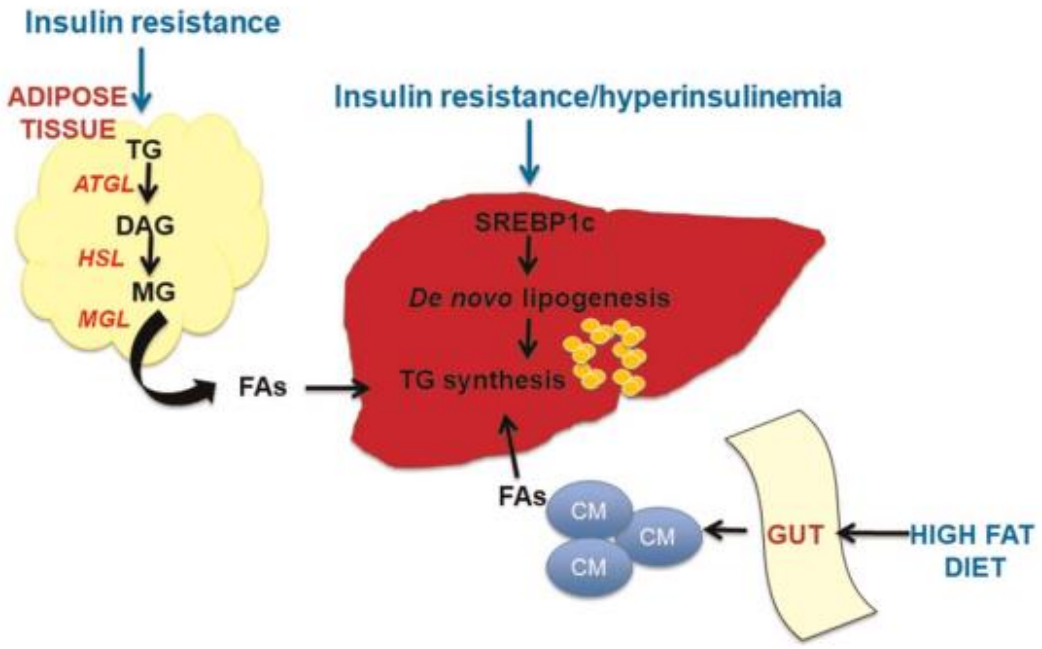


**Figure 2.0: Insulin resistance induces TG breakdown by hydrolase activity of specific enzymes (Pierantonelli and Svegliati-Baroni, 2019).**

It has been shown that around 26% of FFAs in the liver derived from hepatic DNL in individuals with NAFLD while the remaining TG content being derived from lipolysis of adipose tissue store (59%) and from diet (15%) (Than and Newsome, 2015). It has been suggested that hepatic IR can be both a cause and/or a consequence of steatosis in the liver, though the exact underlying mechanism is not completely understood (Than and Newsome, 2015). Chronic hyperinsulinaemia, as found in NAFLD, promotes hepatic DNL through an up-regulation of lipogenic transcription factors (Than and Newsome, 2015).

Adipose tissue is the major source of FFA and responsible for 60% of TG accumulation. Suggestions from previous studies indicate that obese individuals often have enlarged adipocytes due to lipid overload (Than and Newsome, 2015).

Genetic variations play a role in NAFLD (Vancells *et al.,* 2021). It has been revealed that an allele in gene variant rs738409 of PNPLA3 (patatin-like phospholipase domain-containing protein 3) contributes to ancestry-related and interindividual differences in hepatic fat content and predisposition to NAFLD; it also enhances NAFLD severity across the entire histological spectrum, leading to cirrhosis in a higher proportion of subjects (Vancells *et al.,* 2021).

According to Vancells *et al*, (2021), the inflammatory pathways of patients with NAFLD are more activated; this activation of the Kupffer cells occurs via two main classical inflammatory pathways: JNK-AP-1 and IKK-NF-κBDl. The increased levels of free fatty acids cause lipotoxicity and IR and, together with pathogenic drivers such as endotoxins and xenobiotics, activate the release of pro-inflammatory cytokines systemically and locally in the liver (Vancells *et al.,* 2021).

### 2.2. Symptoms of NAFLD

NAFLD is commonly asymptomatic disease and often identified incidentally (Than and Newsome, 2015). The symptoms are usually non-specific when they occur. Some patients have fatigue, malaise, or vague right upper quadrant pain (Grant and Lisker-Melman, 2004). The most common finding on physical examination is obesity. In the later stages of the disease, stigmata of chronic liver disease such as spider nevi, palmar erythema, jaundice, gynecomastia asterixis and muscle wasting are seen (Grant and Lisker-Melman, 2004).

### 2.3. Risk Factors for NAFLD

The increasing epidemics of obesity, dyslipidemia and insulin resistance serve as major risk factors for development of NASH (Peng *et al.*, 2020). Analysis from previous studies involving more than 8.5 million persons from 22 countries show that more than 80% of patients with nonalcoholic steatohepatitis are overweight or obese, 72% have dyslipidemia, and 44% have received a diagnosis of type 2 diabetes mellitus (Diehl *et al.*, 2018). NAFLD appears to be more prevalent in middle-aged to elderly patients as older patients exhibit more characteristics of metabolic syndrome (Peng *et al.*, 2020). Nevertheless, NAFLD can also be diagnosed in children/adolescences who are as young as 13 years old (Peng *et al.*, 2020). In a study of more 250,000 Danish children showed that childhood obesity increased the risk of hepatocellular carcinoma in adulthood (Diehl *et al.*, 2018).

Presence of metabolic syndrome (defined as at least three of the five following medical conditions: abdominal obesity, high blood pressure, high blood sugar, high serum triglycerides, and low serum HDL cholesterol) in an individual is the strongest risk factor for NAFLD and NASH (Friedman *et al.*, 2018). Among the features of metabolic syndrome, diabetes mellitus has the clearest biologic link to the progression NAFLD, up to 75% of patients with type 2 diabetes have NAFLD (Friedman *et al.*, 2018). NASH and advanced fibrosis are more prevalent in diabetic patients with NAFLD than in nondiabetics with NAFLD (Friedman *et al.*, 2018). Individuals with diabetes and NAFLD are at increased risk of developing liver-related complications (Friedman *et al.*, 2018).

#### 2.3.1. Obesity and Type 2 Diabetes (T2D)

The prevalence of NAFLD is increasing in high-risk groups, especially those with obesity and T2D (Marjot *et al*., 2020). In an unselected Italian population sample, 91% of obese patients (BMI ≥ 30 kg/m2) and 67% of overweight patients (BMI 25-30 kg/m2) had evidence of NAFLD in the United States (Marjot *et al*., 2020). Similarly, the overall prevalence of bariatric surgery in patients with NAFLD confirmed by biopsy is 91% (Marjot *et al*., 2020).

In the past 30 years, the number of obese people worldwide has increased, of which are mainly due to new cases in Asia due to urbanization, lifestyle changes, westernized diets and over nutrition (Marjot *et al*., 2020). In 1975, the number of obese people in China was less than 100,000, and in 2014 it increased to 43.2 million, accounting for 16.3% of the global obesity population (WHO 2014; Marjot *et al*., 2020). During the same period, the number of obese people in India increased

from 0.4 to 9.8 million (Pradeepa *et al*., 2015). This is related to the increasing prevalence of NAFLD (20) every year, even in traditional rural areas (21, 22). Among patients with T2DM, two large studies from Italy reported that the prevalence of NAFLD was 60% to 70% (Majumdar *et al*., 2016; Marjot *et al*., 2020), and data from the United Kingdom indicated a prevalence of 42.6% (Marjot *et al*., 2020). A recent meta-analysis of the T2D studies showed that the pooled prevalence of NAFLD was 60%, despite the high degree of heterogeneity among eligible studies, ranging from 29.6% to 87.1% (Dai *et al*., 2017). In a global meta-analysis of the unselected general population, obesity and type 2 diabetes appeared in 51% and 22.5% of NAFLD patients, respectively (Younossi *et al*., 2018). In summary, these data show that cases of obesity and T2D are closely related to NAFLD (Marjot *et al*., 2020).

**2.3.2. Dietary factors**

The prevalence of NAFLD reflects the global prevalence of obesity and T2D, and is related to the consumption of a westernized diet (Kalafati *et al*., 2019; Marjot *et al*., 2020), which is characterized by a large intake of fast food, sweets, refined grains, red meat and meat processed. Whole dairy products and soft drinks (Marjot *et al*., 2020). Compared with healthy controls of the same age and sex, NAFLD patients have a higher mean total daily energy intake (Wehmeyer *et al*., 2017; Marjot *et al*., 2017). In contrast, the Mediterranean diet is characterized by a low intake of saturated fat and cholesterol, a high intake of monounsaturated fatty acids, a balanced ratio of omega6 and omega3 fatty acids, a high content of complex carbohydrates and fiber, has been associated with a lower incidence of NAFLD, NASH, fibrosis, cardiovascular events and cancer (Aller *et al*., 2015; Della *et al*., 2017; Marjot *et al*., 2017). Randomized trials have shown that the Mediterranean diet can reduce plasma alanine aminotransferase (ALT) levels in obese patients with type 2 diabetes, improve insulin sensitivity and hepatic steatosis in patients with NAFLD independent of weight loss as measured by magnetic resonance spectroscopy (MRS) (Marjot *et al*., 2020). The biological mechanisms involved in these improvements may include the anti-inflammatory and lipid-lowering properties of the Mediterranean diet and its impact on the composition of the gut microbiota (Anania *et al*., 2018; Marjot *et al*., 2020). Therefore, the Mediterranean diet is currently the macronutrient composition recommended by NAFLD and is recommended in the joint clinical practice guidelines of the European Association for the Study of the Liver (EASL) and the European Association for the Study of Diabetes (Marjot *et al*., 2020).

Fructose is the main ingredient of the two most commonly used sweeteners: sucrose and high fructose corn syrup (HFCS) (Marjot *et al*., 2020). In the past 100 years, its consumption has increased significantly, mainly in the form of soft drinks, and now accounts for about 15% of the energy consumed as part of a Westernized diet (Marjot *et al*., 2020). Compared with the control group matched with age, gender and BMI, NAFLD patients consume nearly 3 times more fructose (Marjot *et al*., 2020). Compared with milk, dietary glue and water, daily intake of sucrose-containing beverages will significantly increase liver and internal organs accumulation of fat (Maersk *et al*., 2012). Among the 427 adults registered with the NASH CRN, fructose intake was associated with a higher stage of fibrosis (Marjot *et al*., 2020). In addition, low fructose intake can

prevent obese individuals from developing NAFLD in the future (Marjot *et al*., 2020). From a mechanical point of view, fructose seems to play an important role in inducing liver steatosis by stimulating liver *de novo* lipogenesis (DNL) and reducing the oxidation of β-fatty acids (Jensen *et al*., 2018; Marjot *et al*., 2020). Unlike glucose, fructose kinase rapidly phosphorylates fructose, resulting in a decrease in adenosine triphosphate (ATP), this decline in ATP can lead to impaired protein synthesis cascades, oxidative stress, and mitochondrial dysfunction, which are recognized features in the pathogenesis of NASH (Marjot *et al*., 2020). The decrease in intracellular phosphate also promotes the rapid turnover of purine nucleotides, which eventually forms uric acid (Marjot *et al*., 2020). Fructose is the only common carbohydrate that produces uric acid during metabolism (Marjot *et al*., 2020). Fructose is also involved in the pathogenesis of NAFLD by increasing oxidative stress and altering β-oxidation (Choi *et al*., 2014; Marjot *et al*., 2020). Epidemiological studies have shown an association between hyperuricemia and NAFLD, and a meta-analysis has shown that for every 1 mg / dL increase in serum uric acid, the incidence of NAFLD increases by 3% (Marjot *et al*., 2020).

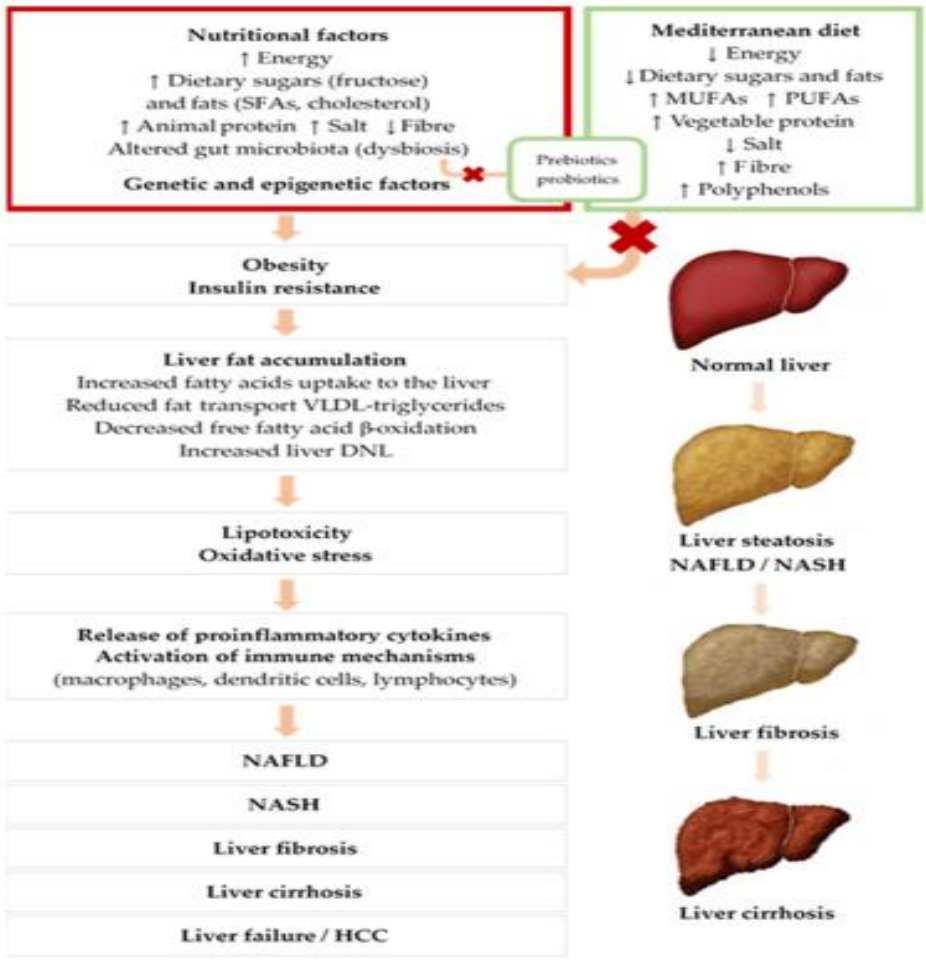


**Figure 2.2: Different phases of NAFLD: progressing from healthy liver to cirrhosis (Peng *et al*., 2020)**

### 2.3.3. Genetic factors

Genetic factors also contribute toward the development of NAFLD. The patatin-like phospholipase domain containing protein 3 (PNPLA3) I148M variant is a strong determinant of hepatic fat content and predisposes to hepatocellular carcinoma in the presence of triggering metabolic risk factors including obesity (Banini and Sanyal, 2016). The risk-associated PNPLA3-I148M variant is resistant to normal proteasomal degradation and accumulates on lipid droplets, which interferes with lipolysis (Friedman *et al.*, 2018). Two independent genome-wide association studies were the first to link the common rs738409 C>G single nucleotide polymorphism, which encodes for the I148M variant of PNPLA3 with hepatic fat content, steatosis, and alanine aminotransferase (ALT) levels (Banini and Sanyal, 2016). The 148M allele, which results in an amino acidic substitution next to the catalytic domain, decreases PNPLA3 enzymatic activity toward glycerolipids and leads to the development of macrovascular steatosis. Individuals with familial hypobetalipoproteinemia, a rare disorder of lipoprotein metabolism, have reduced plasma levels of total cholesterol, low-density lipoprotein cholesterol (LDL-C), and apolipoprotein B with consequently reduced hepatic export of VLDLs, leading to hepatic steatosis (Banini and Sanyal, 2016).

Carriers of this variant affect the risk of liver disease, especially during the developmental age, interacting with dietary factors, such as intake of fructose-rich beverages and lack of physical activity (Armandi and Bugianesi, 2021). Other common gene mutations that regulate liver cell lipids increase the risk of NAFLD (Armandi and Bugianesi, 2021). In Europeans, homozygosity for the mutation is enriched almost nine- fold in patients who develop NAFLD- HCC compared to the general population, while an absence of this variant can exclude the risk of HCC with a high specificity in the general population; polygenic risk scores can help to gain insight into the causal relationship between NAFLD and HCC and to improve HCC risk stratification (Armandi and Bugianesi, 2021).

Mutations in another common genes that regulate liver cell lipids lead to the risk of NAFLD. rs58542926 C> T encodes an E167K variant in member 2 of the trans membrane superfamily 6 (TM6SF2), which contributes to the accumulation of liver fat by reducing the secretion of lipids in very low-density lipoproteins (VLDL), which also leads to Increase susceptibility to liver damage. At the same time, this genetic factor prevents cardiovascular disease by reducing circulating blood lipids.

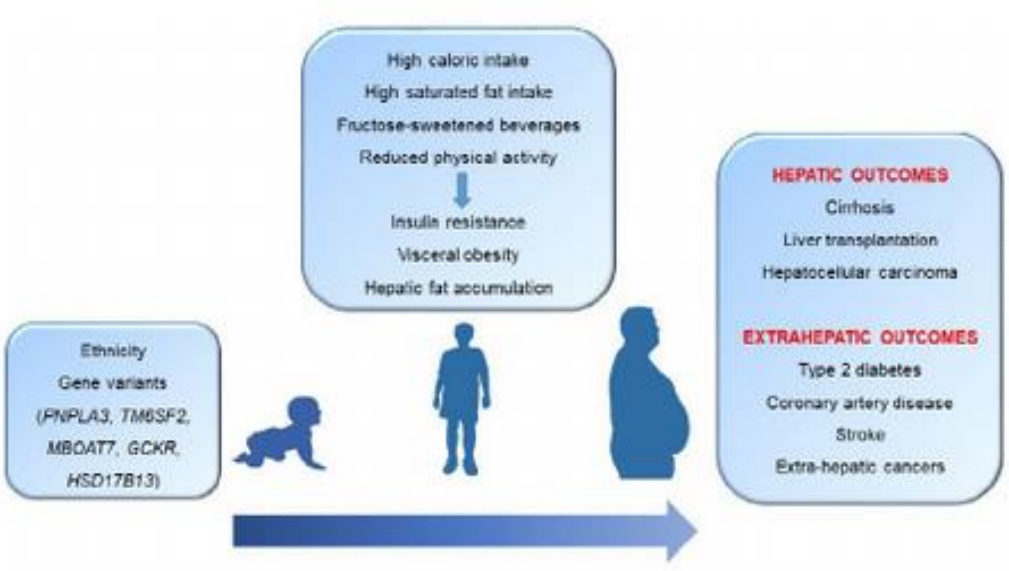


**Figure 2.4: Environmental and genetic factors implied the development of NAFLD and its complications, according to different ages of life (Armandi and Bugianesi, 2021).**

### 2.3.4. Epigenetic factors

Epigenetic events can cause genetic changes in gene expression without directly changing the DNA code (Diehl and Day, 2017). For example, epigenetic mechanisms modify the structure of chromatin (Diehl and Day, 2017). This change alters the accessibility of DNA to factors that control gene transcription, thereby changing the abundance of messenger RNA (mRNA) (Diehl and Day, 2017). RNA fate is also controlled by other genetic factors (for example, microRNAs and RNA-binding proteins), providing alternative epigenetic strategies to affect gene expression (Bian *et al*., 2013). In mice, binge eating during pregnancy induces an epigenetic mechanism that alters the insulin-like growth factor axis during fetal development increasing the susceptibility of offspring to obesity and metabolic syndrome in later life (Diehl and Day, 2017). Similar mechanisms have been proposed to explain the increased incidence of obesity and metabolic syndrome among children whose mothers become pregnant during the famine (Lee, 2013; Diehl and Day, 2017). A study of adult obese families has identified genome-wide epigenetic changes that dysregulate the metabolic pathways that control obesity, insulin sensitivity, and tissue generation or regeneration (Diehl and Day, 2017). It is being investigated whether this epigenetic mechanism will affect the susceptibility to non-alcoholic steatohepatitis, research on rodents has identified an epigenetic mechanism that can regulate the fate determination of liver fibrotic cells and control the susceptibility to cirrhosis across generations (Diehl and Day, 2017). Similar changes in circulating DNA are associated with the severity of liver fibrosis in patients with non-alcoholic steatohepatitis (Diehl and Day, 2017).

**Ethnicity**

The impact of race on the prevalence and severity of NAFLD is complex and remains controversial (Marjot *et al*., 2020). Studies have shown that the prevalence of NAFLD in Hispanic and African-American patients is disproportionately higher than that in white populations (Marjot *et al*., 2020). Importantly, race-related differences in high-risk groups (obesity and type 2 diabetes [T2D]) are smaller compared to population-based cohorts (Rich *et al*., 2018); the prevalence of NAFLD (and NASH) among high-risk Hispanics and Whites are no different (Marjot *et al*., 2020). Studies showed that when Hispanic and white patients were paired with obesity, the severity of NASH and advanced fibrosis did not differ between the two groups (Marjot *et al*., 2020). The data for African Americans are clearer because they have a lower prevalence of NAFLD; however, the frequency of NASH is the same as that of white patients and there is no evidence that there is any difference in the incidence of advanced fibrosis in different races (Bril *et al*., 2018).

Overall, these data suggest that race may have a relatively greater impact on the prevalence of NAFLD than on its severity (Marjot *et al*., 2020). The reasons for the race-related differences in the prevalence and severity of NAFLD are complex (Marjot *et al*., 2020). The reasons are genetic and environmental factors, socioeconomic status, and access to medical care (Marjot *et al*., 2020). Although obesity and type 2 diabetes are recognized risk factors for NAFLD, considering that the metabolic syndrome is more prevalent in blacks than in whites (Menke *et al*., 2015), these factors cannot fully explain the observed racial differences, but the latter has a greater incidence of NAFLD (Marjot *et al*., 2020). Genetic variants may have a greater impact on susceptibility to the prevalence of NAFLD; for example, among different ethnic groups, 49% of Hispanics, 23% of whites, and 17% of blacks have polymorphism in the patatin-like phospholipase domain (PNPLA3) (Marjot *et al*.,2020). A recent systematic review and meta-analysis (including data from 237 studies and> 13 million participants) concluded that in Asia, the prevalence of NAFLD is 29.6%, and the annual incidence is 50 cases/1000 person-years (Li *et al*., 2019). In addition, among Asian patients with NAFLD confirmed by biopsy, NASH was found in approximately 63.5% of cases (Younossi *et al*., 2016; Marjot *et al*., 2020). Among South Asian populations living in Western countries, NAFLD is more common in patients from Bangladesh (Marjot *et al*., 2020). Although the absolute prevalence and incidence of NAFLD and NASH are not significantly different from those of other populations (in fact, their absolute value may be slightly lower for a given body mass index (BMI)) (Fan *et al*., 2017), but the prevalence and severity of NAFLD and NASH NAFLD may be a higher reflection of the observed results of type 2 diabetes (Marjot *et al*., 2020). The reasons for this are not fully understood, but genetic variability, changes in adipose tissue biology are likely, and differences in lifestyle (diet and physical activity) are important (Misra *et al*., 2018).

**Gender and Age**

In the general population, NAFLD is significantly higher in men than in women (Ballestri *et al*., 2017; Marjot *et al*., 2020), although NAFLD with BMI <25 kg/m2 may be more common in women (Marjot *et al*., 2020). In the Dallas Heart Study, the prevalence of NAFLD in white men was approximately twice that of white women (Marjot *et al*., 2020). Several mechanisms may

contribute to this observation, including body fat distribution, liver fatty acid distribution, lifestyle, and sex hormone metabolism (Ballestri *et al*., 2017; Marjot *et al*., 2020). Age-related gender differences also seem to be gender differences (Marjot *et al*., 2020). Males have relatively small differences in the prevalence of NAFLD in different age groups, but females increase significantly after 50 years of age (Marjot *et al*., 2020). Compared with premenopausal women, menopausal status is related to this age-gender interaction, and the incidence of NAFLD in postmenopausal women is twice as high (Florentino *et al*., 2013; Marjot *et al*., 2020). It has been observed that young women who have undergone ovariectomy and young women who have received tamoxifen have an increased risk of NAFLD (Marjot *et al*., 2020) and a reduced risk for women receiving hormone replacement therapy, so this further validates the protective effect of estrogen (Marjot *et al*., 2020). Among NAFLD patients, women may be at higher risk of NASH and Data from the NASH Clinical Research Network (CRN) shows that NASH patients confirmed by biopsy are more likely to be women than men, with a ratio of approximately 2:1 (Marjot *et al*., 2020). Although this may reflect that the burden of disease is higher for women, but this may be due to gender differences between people seeking and receiving medical care (Marjot *et al*., 2020).

Data on the impact of gender on the progression of fibrosis are conflicting (Marjot *et al*., 2020). Although a systematic review of risk factors for fibrosis progression in NASH found to be age-related but not gender-related (Argo *et al*., 2009; Marjot *et al*., 2020), several cross-sectional biopsy studies have shown that compared with men, women have an increased risk of advanced fibrosis regardless of metabolic risk factors (Marjot *et al*., 2020). In addition, the duration of estrogen deficiency in the postmenopausal state puts the risk of fibrosis in NAFLD women (Klair *et al*., 2016; Marjot *et al*., 2020).

Regardless of the cause of liver disease, hepatocellular carcinoma (HCC) is predominantly male, and the male to female ratio is estimated to be 2:1 to 2.5:1 (Marjot *et al*., 2020). However, in related HCC and NASH, the male to female ratio may be lower (1.6:1) compared to hepatitis B and C virus (HCV) and alcohol-related cirrhosis (Marjot *et al*., 2020). Compared with female patients, male patients with NASH also appear to develop HCC in the advanced stages of liver fibrosis (71), and their overall survival rate is worse than female patients (Marjot *et al*., 2020; Wang *et al*., 2016).

### 2.4. Diagnosis of NAFLD

Routine blood test for elevation of the plasma liver enzymes alanine transaminase (ALT) and aspartate aminotransferase (AST) is the first approach to diagnosis of NAFLD (Peng *et al.*, 2020). ALT and AST are used as biomarkers for liver injury, because they are highly expressed in the hepatocytes and are leak into circulation in the event of hepatocyte necrosis (Peng *et al.*, 2020). Presence of other diseases such viral hepatitis, may also induce elevation of ALT and AST. NASH patients may have normal plasma ALT/AST levels (Peng *et al.*, 2020). Several studies had demonstrated that ALT is a poor marker to predict advance fibrosis in individuals with NAFLD (Than and Newsome, 2015).

### 2.4.1. Non-invasive method

The diagnosis of NAFLD is based on a combination of clinical factors and liver imaging (Carr *et al*., 2016). The combination of medical history and serological tests with radiological findings (ultrasound, CT, or MRI) can diagnose NAFLD in most patients (Carr *et al*., 2016). At least 30% of hepatic steatosis is the best option to visualize hepatic steatosis with these commonly used radiological tools, although there are large differences between and within observers (Carr *et al*., 2016).

To confirm the presence of fatty liver, computed tomography (CT) scan or magnetic resonance imaging (MRI) can potentially be used as a non-invasive diagnostic tool to assess the percentage of fat in the liver (Peng *et al.,* 2020). There is no standard radiological method that can detect the presence of steatohepatitis or early fibrosis (Carr *et al*., 2016). Standard radiology protocol was unable to detect advanced stages of NAFLD, leading studies to stage NAFLD severity for other non-invasive strategies (Carr *et al*., 2016). Serologic tests and biomarker panels, Transient Ultrasonic Elastography (TE) and Magnetic Resonance Elastography (MRE) can be used for NAFLD staging (Carr *et al*., 2016). Cytokeratin 18 (CK18) is an intermediate filament of hepatocytes, which is cleaved by caspase during apoptosis, and its serum level is elevated in patients with NASH (Feldstein *et al*., 2009; Carr *et al*., 2016).

### 2.4.2. Hepatic Biopsy

Due to the limitations of noninvasive testing for NAFLD patients, liver biopsy is still the gold standard for NAFLD staging (Carr *et al*., 2016; Kelly *et al.,* 2019). However, the prevalence of NAFLD, the relatively low likelihood of disease progression in most patients, the lack of treatment options, the risk of biopsy, and the uncertain cost-effectiveness of invasive examinations prevent the recommendation of liver biopsy for all patients (Carr *et al*., 2016). Current guidelines limit liver biopsy to patients with uncertain diagnosis or who may have advanced disease based on the non-invasive assessment (Carr *et al*., 2016).

The scope of NAFLD histology includes simple steatosis, steatohepatitis, fibrosis, and cirrhosis. Among them, fibrosis is the histological feature that can most predict mortality from NAFLD (Angulo *et al*., 2015).

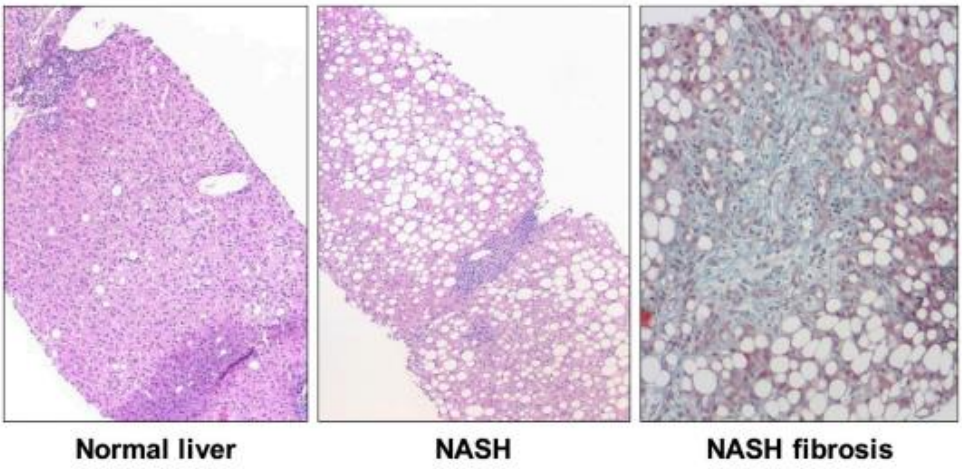


**Figure 2.5: Showing normal liver, NASH and NASH fibrosis**

### 3.0 Fructose and NAFLD

Diets rich in fructose, can quickly lead to almost all the diseases of metabolic syndrome (Roeb and Weiskirchen, 2021). Metabolic syndrome is associated with trunk obesity, arterial hypertension, high serum sugar/impaired glucose tolerance (diabetes), elevated serum triglycerides, and reduced high-density lipoproteins (HDL) (Roeb and Weiskirchen, 2021).

Some natural foods (fruits, vegetables, honey) contain fructose. It is also present in some processed foods. Fructose is a primary component in the most widely used sweeteners (sucrose or high fructose corn syrup [HFCS]) (Vancells *et al.*, 2021). Fructose consumption has increased by 30% in the last 40 years and by 500% over the last century due to the increased consumption of processed foods (Vancells *et al.*, 2021). Today, added sugar intake makes up 15% of total daily calories in the average Western diet (Vancells *et al.*, 2021).

The increased consumption of added sugars, particularly fructose, is a major underlying cause of chronic metabolic diseases, including NAFLD, type 2 diabetes mellitus, obesity, hypertension, and cardiovascular diseases (Vancells *et al.*, 2021). Due to its hepatic metabolism, fructose is suspected to be partly responsible for the development of non-alcoholic fatty liver disease (NAFLD) (Roeb and Weiskirchen, 2021). Many clinical and experimental studies have indicated that high fructose intake is a major risk factor for NAFLD and its consequences (Vancells *et al.*, 2021). Many observational studies have clearly indicated a close link between overconsumption of added sugars and the development of NAFLD in adults and children (Vancells *et al.*, 2021). In a systemic and meta-analysis of seven studies (six cross-sectional studies and one cohort study) involving 4639 subjects demonstrated that sugar-sweetened beverage (SSB) consumers had a 53% increased risk of developing NAFLD compared with non-consumers (Vancells *et al.*, 2021).

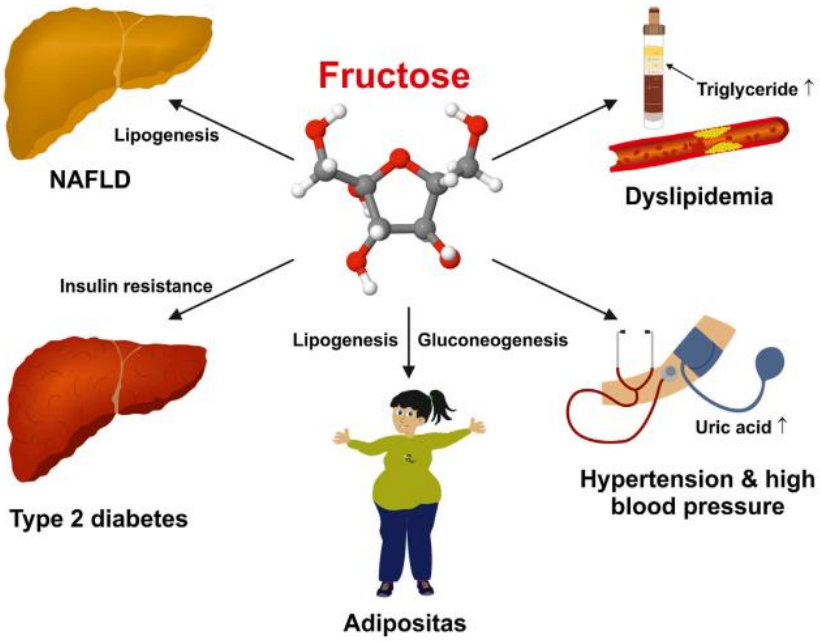


**Figure 3.0: Harmful effects of high fructose intake on human health. (Source: Roeb and Weiskirchen, 2021).**

### 3.1. Role of Fructose in Pathogenesis of NAFLD

#### 3.1.1. Fructose as substrate and inducer of *de novo* lipogenesis

Unlike glycolysis, fructose metabolism in the liver is not a tightly regulated biochemical pathway (Roeb and Weiskirchen, 2021). Glucose is absorbed by intestinal epithelial cells and liver cells through the glucose transporter 2 (GLUT2). In the cytosol, glucose is converted to glucose-6-phosphate (G6P) by glucokinase (GK), (Federico *et al.*, 2021) and G6P is converted into glycogen to store energy or metabolized through glycolysis to produce adenosine triphosphate (ATP) and pyruvate (Federico *et al.*, 2021). Fructose is transported passively by glucose transporter 5 (GLUT5) which is the sole transporter specific for fructose (Nomura and Yamanouchi, 2012). GLUT5, located in the apical of enterocytes, is highly expressed in the small intestine and has a high affinity for fructose, diffusing rapidly into the intestinal capillaries to transport to the liver via the portal vein (Federico *et al.*, 2021). GLUT2, located on the basal surface of intestinal epithelial cells, shows low affinity for fructose. In fact, a study in mice that eliminated the whole body of GLUT2 showed only a slight decrease in fructose absorption (Federico *et al.*, 2021).

Fructose intake disrupts metabolic pathways that might lead to excess accumulation of hepatic fat (Roeb and Weiskirchen, 2021).

In hepatic cells, fructose is converted to fructose-1-phosphate (F1P) by fructokinase (Jegatheesan and De Bandt, 2017; Roeb and Weiskirchen, 2021). Fructokinase has high affinity for fructose, it is not controlled by insulin but induced by fructose (Jegatheesan and De Bandt, 2017). Fructose-1-phosphate is split into D-glyceraldehyde and dihydroxyacetone phosphate (DHAP) by the liver-

specific fructose-1,6-diphosphate aldolase (Roeb and Weiskirchen, 2021). The DHAP can be broken down further via the corresponding reaction of glycolysis or used for gluconeogenesis. Hence, fructose can be broken down into pyruvate more quickly than glucose because the rate-limiting reactions of glycolysis are bypassed (Jegatheesan and De Bandt, 2017; Roeb and Weiskirchen, 2021).

By saturating the glycolytic pathway, high fructose intake might result in an accumulation of glycolysis intermediates which can be converted to glycerol-3-phosphate used in triglyceride (TG) synthesis (Jegatheesan and De Bandt, 2017; Roeb and Weiskirchen, 2021).

Apart from direct fat uptake, TGs derived from *de novo* lipogenesis are reportedly elevated in subjects who were on a high carbohydrate diet (Peng *et al.*, 2020). Chronic fructose consumption leads to activation of the sterol regulatory element-binding protein 1c (SREBP1c) and the carbohydrate-responsive element-binding protein (ChREBP) (Roeb and Weiskirchen, 2021). As a consequence, their key target enzymes regulating lipid synthesis, such as Fatty Acid Synthase (FASN) stearoyl CoA desaturase-1 (SCD-1) and Acetyl-CoA Carboxylase (ACC), also increase as they induce the expression of genes encoding for these enzymes (Jegatheesan and De Bandt, 2017; Nomura and Yamanouchi, 2012).

Also, ChREBP transactivates expression of the apolipoprotein C-III (APOC3) and angiopoietin-like 8 (ANGPTL8), which both lower the activation of lipoprotein lipase (LPL) and limit the clearance of very low-density lipoproteins (VLDL) (Roeb and Weiskirchen, 2021).

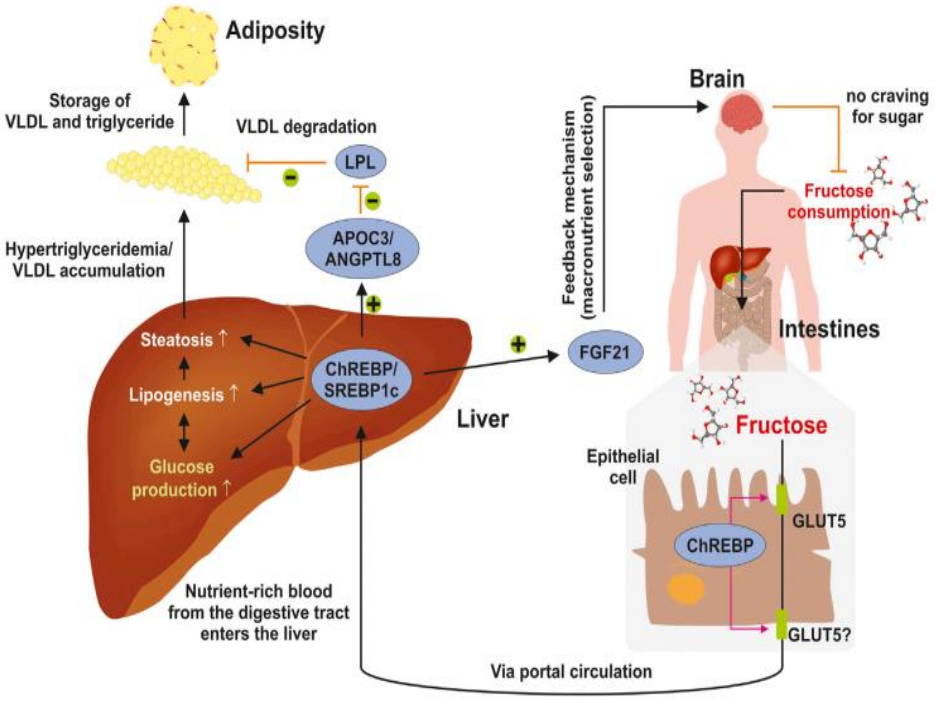


**Figure 3.1: Fructose as inducer and substrate of *de novo* lipogenesis (Source: Roeb and Weiskirchen, 2021).**

### 3.1.2. Fructose induces oxidative stress in hepatocytes

Beside its lipogenic effects in the liver, fructose metabolism induces other specific hepatotoxic effects by inducing an increase in oxidative stress (Skenderian *et al.*, 2020). Hepatocyte damage and cell death induced by oxidative stress have been reported as one of the leading causes of tissue damage in NASH (Peng *et al.*, 2020). Fructose may directly or indirectly promote oxidative stress, in part through mitochondrial dysfunction and endoplasmic reticulum (ER) stress, both contributing to the development of an inflammatory process and the progression of simple steatosis to NASH (Jegatheesan and De Bandt, 2017). Acute fructose load induces a non-enzymatic protein fructosylation, which is seven times faster than glycation. Fuctosylation generates 100 times more reactive oxygen species (ROS) than glycation (Jegatheesan and De Bandt, 2017). Compared to glucose, long-term fructose feeding in mice resulted in increased accumulation of carboxymethylisine (a glycation product) in the liver (Federico *et al.*, 2021; Jegatheesan and De Bandt, 2017). For example, carboxymethylase can interact with the SREBP cleavage activation protein to induce sustained activation of SREBP1c (Federico *et al.*, 2021; Jegatheesan and De Bandt, 2017).

Uric acid stimulates the production of ROS by activating transforming growth factor beta and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 4 (Jegatheesan and De Bandt, 2017). Phosphorylation of fructose consumes more ATP; because the phosphorylation of fructokinase is very fast, and the cleavage reaction of aldolase B is relatively slow, excessive fructose will cause liver phosphate deficiency, leading to AMP accumulation, which leads to uric acid synthesis increase (Jegatheesan and De Bandt, 2017).

Mitochondrial dysfunction can also be caused by lipotoxicity associated with fructose-induced disturbances of liver lipid metabolism (Alwahsh *et al.*, 2014; Jegatheesan and De Bandt, 2017). The mechanism involved may be; Low expression of Peroxisome Proliferator-Activated Receptor α (PPARα) resulting to reduced lipid degradation, which regulates genes involved in beta oxidation, such as carnitine palmitoyl transferase 1 ( CPT1) (Jegatheesan *et al.*,2015), a low expression of peroxisomal proliferator-activated receptor γ coactivator-1-α (PGC1α) (a mitochondrial biological protein) (Jegatheesan and De Bandt, 2017), and reduced expression of microsomal triglyceride transfer protein (MTP) involved in the production of very low-density lipoproteins (VLDL), resulting in reduced lipid clearance(Jegatheesan *et al.*, 2015; Jegatheesan and De Bandt, 2017). However, the exact mechanisms are still debated because, in several studies, an increase in beta-oxidation and clearance of VLDL after consumption of fructose has been reported, suggesting that an accumulation of lipid accumulation in the liver is mainly caused by uncontrolled DNL (Nigro *et al.*, 2017; Jegatheesan and De Bandt, 2017). Therefore, the imbalance between the release of VLDL and DNL can promote the changes in the respiratory chain and the uncoupling of oxidative phosphorylation and the excess of ROS (Jegatheesan and De Bandt, 2017).

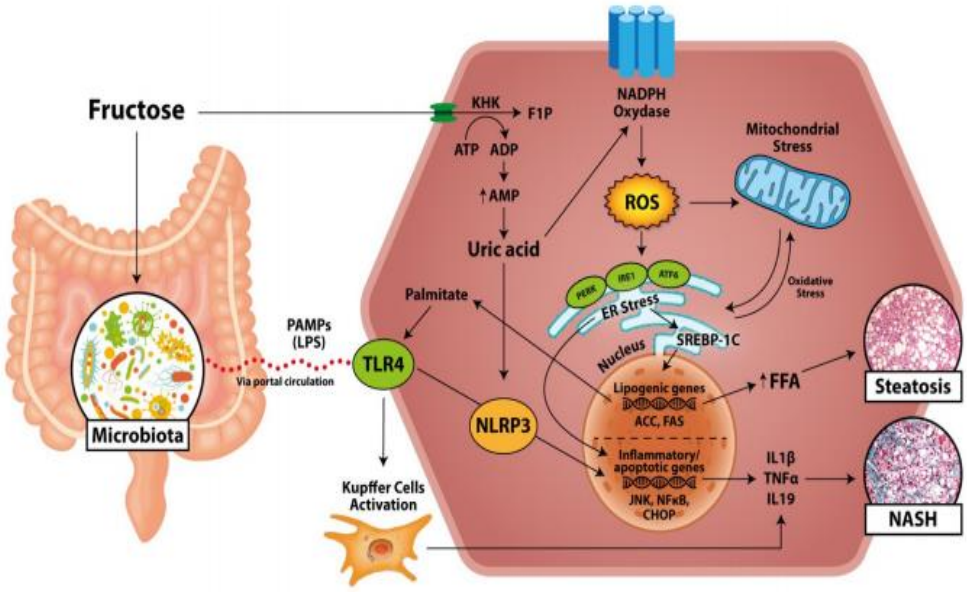


**Figure 3.2: Fructose pathways involved in oxidative stress (Federico *et al.*, 2021)**

### 3.1.3. Fructose and Hepatic Endoplasmic Reticulum (ER) Stress

Hepatic endoplasmic recticulum (ER) stress is closely associated with NASH (Peng *et al.*, 2020). Studies have shown that ER stress is a mechanism that promotes progression from HS to NASH (Jegatheesan and De Bandt, 2017). Chronic consumption of fructose leads to higher stress on the ER through stimulation of lipid metabolism and VLDL-TG production (Jegatheesan and De Bandt, 2017). ER membrane proteins may be fructosylated, or lipids may accumulate into ER membrane resulting to ER stress and the unfolded protein response (UPR) (Jegatheesan and De Bandt, 2017). The unfolded protein response is in an attempt to maintain normal cell function (Peng *et al.*, 2020). With prolonged exposure to fructose, ER stress becomes chronic leading to inflammation, oxidative stress and apoptosis, it also contributes to the progression of fatty liver disease and insulin resistance (Jegatheesan and De Bandt, 2017).

Prolong unresolved ER stress is thought to induce the expression of pro-apoptotic transcription factors C/EBP Homologous protein (Peng *et al.*, 2020).

ER stress further interferes with lipid metabolism in the liver by activating DNL, via the protein kinase activated by dsRNA (PKR)-related Endoplasmic Reticulum Kinase (PERK)/eukaryotic translation Initiation Factor 2α (eIF2α)/Activating Transcription Factor 4 (ATF4) pathway and by limiting the formation and secretion of VLDL, via Inositol Requiring Enzyme 1 (IRE1) pathway (Jegatheesan and De Bandt, 2017).

Finally, endoplasmic reticulum stress promotes the initiation of inflammation and apoptosis pathways through c-Jun N-terminal kinase (JNK), nuclear factor kB (NFkB), and CCAAT /

homologous enhancer binding protein (CHOP), plays an important role in progress (Federico *et al.,* 2021).

### 3.1.4. Fructose and inflammation

Hepatic inflammation is one of the features that differentiates NASH from NAFLD (Peng *et al.,* 2020). The fat production mechanism and oxidative stress induced by fructose consumption led to liver inflammation, which favours progression to NASH (Federico *et al.,* 2021). As evidence of the specific role of fructose in determining liver inflammation, showed that fructokinase knockout mice (KHKA and KHKC) compared with wild-type mice, under high-fat high-sucrose diet did not develop steatohepatitis, the groups were characterized by obesity and mild liver steatosis (Federico *et al.,* 2021).

The contribution of fructose diet to the inflammatory process is well established (Jegatheesan and De Bandt, 2017). Ectopic liver fat accumulation increases hepatocytes vulnerability to cellular stress, therefore initiating an inflammatory process (Jegatheesan and De Bandt, 2017).

Kupffer cells contribute to the progression of NAFLD and amplify the inflammation induced by ROS and toll-like receptor (TLR), especially the activation of TLR4 (Federico *et al.,* 2021). TLR4 can be activated by altered lipid homeostasis and fatty acids (such as palmitate), and can promote its production through fructose (Federico *et al.,* 2021). The activation of TLR4 stimulates nitric oxide synthase and NFkB, and induces the production of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNFα) (Federico *et al.,* 2021; Jegatheesan and De Bandt, 2017). TNFα induces the expression of the monocyte chemoattractant protein 1 (MCP1) which is thought to be elevated in NASH MCP1 patients and its corresponding receptor, the CC chemoattractant receptor type 2 (CCR2) are important for the liver to recruit monocytes Ly6C+, which can amplify inflammation as they mature into macrophages (Peng *et al.,* 2020). In addition to monocytes and Kupffer cells, it has been suggested that neutrophilic myeloperoxidase exacerbates inflammation of the liver by inducing oxidative stress (Peng *et al.,* 2020).

The activation of TLR4 can also activate NOD, LRR and the pyridine domain-containing protein 3 (NLRP3) inflammasome, which is an intracellular polyprotein complex involved in interleukin (IL) 1β and interleukin 18 production (Federico *et al.,* 2021). NLRP3 plays a crucial role in the progression of liver fat overload in NASH, activating immune system modulators responsible for fibrotic stimulation and inflammation (Dallio *et al.,* 2021; Fedrico *et al.,* 2021). The accumulation of uric acid induced by fructose produces ROS, which leads to the release of pro-inflammatory cytokines Federico *et al.,* 2021). In addition, xanthine oxidase is the last enzyme involved in the synthesis of uric acid is induced by the excess substrate provided by fructose can act as an electron donor for oxygen generates ROS and supplies oxidative stress to the liver (Kleiner *et al.,* 2019; Federico *et al.,* 2021). Uric acid can also directly activate the NLRP3 inflammasome and exert the above-mentioned harmful effects (Wan *et al.,* 2016; Federico *et al.,* 2021).

The increase in the number of cytokines (such as TNFα, IL1beta and IL18) produced by excessive consumption of fructose in liver and fat cells can help activate the hypothalamic-pituitary-adrenal (HPA) axis, resulting in the release inhibitors of immunity as glucocorticoids (DiNicolantonio *et al.,* 2018; Federico *et al.,* 2021). Increased cortisol activity may induce insulin resistance, reduce adipogenesis in subcutaneous fat cell tissue, and cause visceral and hepatic fat deposits (Federico *et al.,* 2021).

The overlap of ER stress and inflammation can lead to the production of various mediators, such as cytokines, liver cytokines, carbohydrates and lipid derivatives, collectively referred to as DAMP (damage-related molecular patterns), which send signals throughout the body and help metabolism changes in the entire body (Jegatheesan and De Bandt, 20217). Many studies, including animal models and studies of in NASH patients, indicate that abnormal production of liver factor also plays a key role in the pathogenesis of NASH (Jegatheesan and De Bandt, 2017; Kleiner *et al.,* 2019). Hepatocyte cytokines released by fatty liver, such as fetuin A, fibroblast growth factor 21 (FGF21), leukocyte-derived chemokine 2 (LECT2), and angiopoietin-like protein (ANGPTL) may cause dysfunction of peripheral organs (Jegatheesan and De Bandt, 2017).

Long-term consumption of high fructose diet can cause dysbiosis which can result in a leaky gut, hence enabling endotoxins, such as lipopolysaccharide to travel to the liver, triggering/enhancing liver inflammation during NASH (Peng *et al.,* 2020).

### 3.1.5. Fructose and gut/liver axis

A large number of microorganisms, such as archeae, viruses, phages, yeast, fungi and bacterial made up the intestinal microbiota (Pierantonelli and Svegliati-Baroni, 2019). The bacterial include more than 1000 species, with a number of genes (Pierantonelli and Svegliati-Baroni, 2019). The progression of HS to NASH is also influenced by gut function and the possible translocation of bacterial compounds due to a compromised intestinal barrier (Jegatheesan and De Bandt, 2017).

One study used a stable isotope of 13C fructose or glucose to track the intake and metabolism of fructose and glucose in a mouse model (Federico *et al.,* 2021). The results showed that in the small intestine, the dietary intake of fructose in low doses (0.25-0.5 g/kg) was almost completely eliminated and converted to glucose (Federico *et al.,* 2021). In contrast, high doses (> 1 g/kg) exceeded the intestinal fructose absorption and elimination rate, causing fructose to reach the liver and colon microbiota (Jang *et al.,* 2018; Federico *et al.,* 2021). Importantly, fructose clearance from the small intestine increases with prior exposure to fructose or food consumption Federico *et al.,* 2021).

In a recent study in intestinal-specific KHKC knockout mice induced to eliminate intestinal fructose catabolism, increased hepatic lipogenesis with fatty liver and more severe hyperlipidemia compared with mice mice with KHKC overexpression have been demonstrated (Jang *et al.,* 2020). In addition, the effects of a high dose of fructose bolus (2 g/kg) showed higher fructose spillover

of into the portal circulation and induction of liver lipogenic genes compared with a gap dose of (0.5 g/kg × 4) (Jang *et al.,* 2020; Federico *et al.*, 2021). Thus, it can be assumed that the small intestine acts as a barrier for the liver, protecting it from excessive fructose load (Federico *et al.*, 2021).

Changes in the gut microbiota may be related to different diets, antibiotic use, and alcohol abuse (Pierantonelli and SvegliatiBaroni, 2019). In healthy subjects, the intestinal flora maintains a symbiotic relationship with the host, and intestinal bacteria play a key role in the development and efficiency of the immune system and in the regulation of energy metabolism (Pierantonelli and SvegliatiBaroni, 2019).

However, qualitative and quantitative changes in the gut microbiota (a condition called dysbiosis) may be beneficial to the development of chronic diseases, including NAFLD and its progression to NASH (Pierantonelli and SvegliatiBaroni, 2019). Fructose-induced NAFLD is also associated with changes in the composition of the microbiota, which alter intestinal permeability by reducing the expression of tight junction proteins (Jegatheesan and De Bandt, 2017). Due to this change in intestinal barrier function and dysbiosis, endotoxin translocation increases in patients with NASH and liver cirrhosis (Jegathee De Bandt, 2017).

Changes in the intestinal barrier facilitate the entry of bacteria or bacterial products such as lipopolysaccharide (LPS) into the portal circulation, leading to the progression of NAFLD to NASH, as shown in experiments and studies in humans (Pierantonelli and SvegliatiBaroni, 2019). In addition, in a study of rodents on a high fructose diet, tight junctions were reduced, followed by increased release of bacterial endotoxins (such as lipopolysaccharide (LPS)) into the portal circulation (Federico *et al.,* 2021). In the pediatric cohort of NAFLD patients, acute and chronic exposure to a high fructose diet increased levels of endotoxin, insulin resistance markers, and various inflammatory cytokines compared with obese controls (Federico *et al.,* 2021).

NAFLD can be caused by ecological disorders through a variety of mechanisms (Pirantonelli and SvegliatiBaroni, 2019).

The gut microbiota can directly affect lipid metabolism (Pierantonelli and SvegliatiBaroni, 2019). The most famous effect is the ability of gut bacteria to inhibit the fasting-induced synthesis of adipocyte factor (also known as angiopoietin-related protein 4), which is a specific lipoprotein lipase (LPL) inhibitor (Pierantonelli and SvegliatiBaroni, 2019). Lack of inhibition of LPL leads to a stronger release of FFAs from very low- density lipoprotein particles to the liver, favouring the development of steatosis (Pierantonelli and Svegliati-Baroni, 2019). Exposure of lipopolysaccharides (LPS) and other endotoxins to the liver can cause chronic inflammation, and neutrophils release ROS, proteases, lipocalin 2, and enzymes, leading to worse liver damage (Jegathessan & De Bandt, 2017). LPS and oxidative stress can also activate stellar cells, leading to fibrosis (Jegathessan & De Bandt, 2017).

Changes in the gut microbiota have been shown to affect host metabolism through the synthesis of short chain FA (SCFA) (Pirantonelli and SvegliatiBaroni, 2019). In experimental models of NAFLD and in obese patients, fecal SCFA levels have been shown to increase (Pierantonelli and SvegliatiBaroni, 2019). Short-chain FA is a metabolite of polysaccharide colon bacterial fermentation, which includes acetate, propionate, and butyrate (Pierantonelli and SvegliatiBaroni, 2019). Short-chain FAs can bind specific G-protein–coupled receptors, G-protein coupled receptor 41 (GPR41) and G-protein coupled receptors 43 (GPR43), that are expressed in all organs involved in the pathogenesis of NAFLD, such as AT, liver, and intestine (Pierantonelli and Svegliati-Baroni, 2019). The activation of these receptors by the 3 different SCFAs can induce DNL, synthesis of cholesterol and alterations of glucose homeostasis (Pierantonelli and Svegliati-Baroni, 2019).

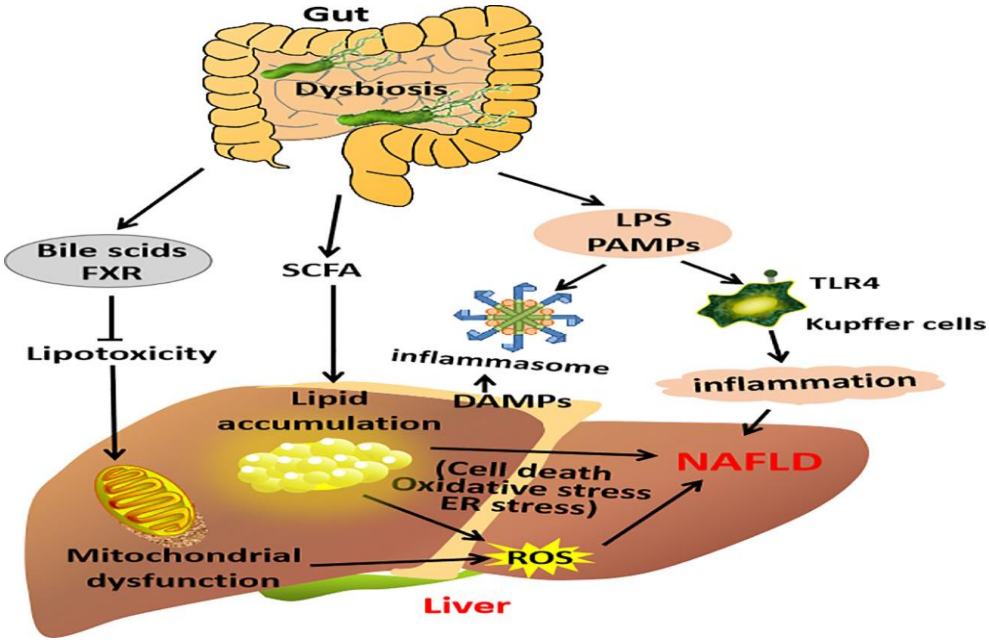


**Figure 3.3: Contribution of Gut/liver axis to NAFLD pathogenesis. (Zhang *et al.*, 2020)**

### 3.1.6. Fructose and Adipose Tissue/Liver Axis

The adipose tissue-liver axis also contributes to the pathogenesis of NAFLD (Zhang *et al.*, 2020). In subjects with NAFLD, adipose tissue has high expression of inflammatory mediators (Zhang *et al.*, 2020). The amount of visceral fat increases with the increase of human fructose diet and experimental models (Jegathessan and De Bandt, 2017), which indicates that the direct metabolism of fructose in visceral fat cells may be exposed to more fructose concentrations than subcutaneous fat cells due to the anastomosis between hepatic portal circulation and the internal organs of the whole body, or the indirect effect caused by the accumulation of lipids from the liver (Jegathessan and De Bandt, 2017).

Fructose-fed rats display an accumulation of MG in epididymal adipose tissue (Jegathessan and De Bandt, 2017). MG alters insulin signaling pathway in visceral adipose tissue in vivo (Jegathessan and De Bandt, 2017).

In vitro, fructose increases adipogenesis and, conversely, the inhibition of fructose transport in mice is associated with reduced epididymal adipose tissue (Jegathessan and De Bandt, 2017). Together these data underline fructose influence on visceral adipose tissue but data in human are missing. Owing to this adipogenic effects, adipokines and cytokines profile would also be changed by fructose diet (Jegathessan and De Bandt, 2017). Adiponectin and leptin are the two common adipokines (Zhang *et al.*, 2020). Adiponectin has anti-fibrotic effect in the liver, mediated by AMPK activity (Zhang *et al.*, 2020), and anti-inflammatory effect, it blocks the activation of NF-κB, increases secretion of anti-inflammatory cytokines, and reduces the release of pro-inflammatory cytokines such as TNF-α and IL-6 (Zhang *et al.*, 2020). Clinical studies have reported that reduced adiponectin and increased leptin levels result in hepatic steatosis and activation of inflammation and fibrogenesis (Zhang *et al.*, 2020).

Increased visceral adiposity results to elevated circulating free fatty acids and proinflammatory mediators (Jegathessan and De Bandt, 2017). This will alter liver function but also other the function of peripheral organs leading to an aggravation of the metabolic disorders (Jegathessan and De Bandt, 2017).

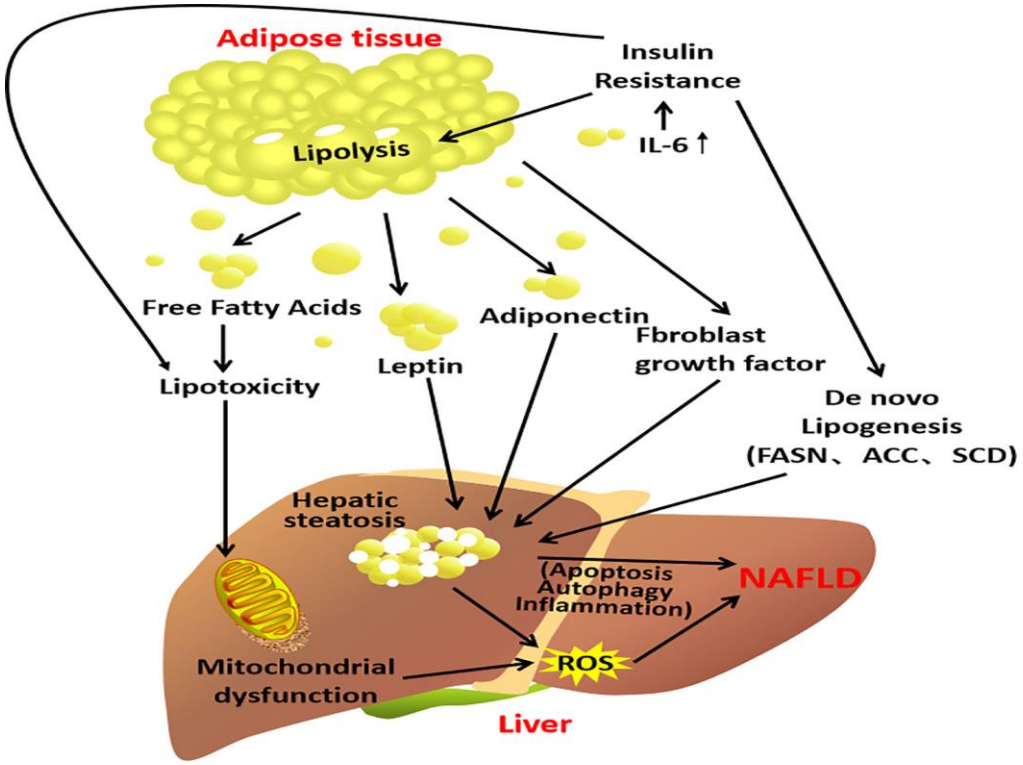

**Figure 3.4: Contributions of adipose tissue/liver axis to NAFLD pathogenesis (Source: Zhang *et al.*, 2020)**

### 3.1.7. Fructose and Muscle/Liver Axis

A high-fructose diet is linked to changes in muscle function in both humans and in rodents (Jegathessan and De Bandt, 2017). Mechanisms of diet-induced sarcopenia may be a decrease in mechanistic target of rapamycine complex (mTORC) 1 activity, and inflammation (Jegatheesan and De Bandt, 2017). This is a key factor involved in disease progression to NASH as the muscle heavily contributes to energy homeostasis (Cabrera *et al.*, 2016).

Disorders of nitrogen homeostasis in situations of stimulated DNL may be an early event following excessive fructose consumption (Softic *et al.*, 2016; Jegatheesan and De Bandt, 2017) as excess fructose may alter liver-muscle axis via its metabolism or via DNL-associated RE stress leading to increased production by the liver of catabolic effectors (Jegatheesan and De Bandt, 2017).

The increase in lipid flux observed with fructose-enriched diet contributes to alter muscle insulin sensitivity (Stephens *et al.*, 2015; Jegatheesan and De Bandt, 2017). Excess fructose may lead to a saturation of its normal metabolism with adverse consequences in terms of increased hepatic release of MG or uric acid. In vitro studies show that MG inhibits insulin signaling in muscle (Jegatheesan and De Bandt, 2017). High fructose diet under hypercaloric feeding conditions has been shown to induce hyperuricemia that contributes to metabolic disorders (Jegatheesan and De Bandt, 2017). Uric acid inhibits muscle insulin signaling and induces insulin resistance in mice as well as in severely obese subjects (Jegatheesan and De Bandt, 2017; Fabbrini *et al.*, 2014). Hepatic ER is associated with enhanced production of pro-inflammatory cytokines and hepatokines suspected to be involved in alterations in energy homeostasis and insulin-resistance (Jegatheesan and De Bandt, 2017). ER stress markedly stimulates liver production of Fetuin A and of insulin-like growth factor binding protein 1 (IGFBP1) (Jegatheesan and De Bandt, 2017).

Fetuin A is an endogenous inhibitor of the insulin receptor tyrosine kinase in muscle while IGFBP1 is a modulator of insulin-like growth factor 1 (IGF-1) action associated with hyperinsulinemia and glucose intolerance (Jegatheesan and De Bandt, 2017). ER stress regulates fibroblast growth factor 21 (FGF21) expression in the liver (Jiang *et al.*, 2014). FGF21 is a mediator mainly produced by the liver that contributes to the regulation of peripheral energy metabolism and insulin sensitivity (Liu *et al.*, 2015). It is now recognized as a key player in the adaptive response to starvation and feeding (Hong *et al.*, 2014). Consumption of fructose leads to decreased liver production of anabolic factors such as insulin-like growth factor (IGF)1 (Cabrera *et al.*, 2016). Another factor contributing to these alterations of protein metabolism is a reorientation of AA fluxes as suggested by NAFLD-associated changes in plasma amino acids (AAs) profile (Jegatheesan *et al.*, 2015). In hypertriglyceridemic patients, fructose increased plasma arterial AA concentrations but also their splanchnic extraction (Jegatheesan and De Bandt, 2017). These interorgan AA fluxes probably

correspond to a reorientation of AAs towards the liver in order to enable the synthesis of inflammatory proteins and the elevated gluconeogenesis (Jegatheesan and De Bandt, 2017).

In the case of fructose overfeeding, energy metabolism will tend to increase gluconeogenesis and DNL and reduce lipid catabolism (Jegatheesan and De Bandt, 2017). On the other hand, experimental studies and in humans have shown that the availability of AA has a regulatory effect on liver DNL, because a greater availability of AA prevents the accumulation of lipids in the liver by (i) reducing DNL by reducing expression of the genes ChREBP, SREBP1c and FAS (ii) when increasing Gene expression of PPARα increases β oxidation; (iii) VLDL production increases with increasing MTP gene expression (Jegatheesan *et al.*, 2015).

### 3.1.8. Lean NAFLD

Based on the body mass index (BMI) of a particular area, the term "lean NAFLD" refers to liver steatosis in lean or normal weight patients (Roeb and Weiskirchen, 2021). Similar to the pathogenesis of NAFLD in obese people, fructose consumption may also play an important role in the development of NAFLD in lean people (Kumar & Mohan, 2017). For example, in the absence of traditional risk factors, soft drink consumption appears to be associated with NAFLD (Roeb and Weiskirchen, 2021).

Regardless of the diagnosis of metabolic syndrome, the consumption of soft drinks in NAFLD patients increased according to a 2017 study (Roeb and Weiskirchen, 2021). The study showed that compared with healthy controls, NAFLD patients consume 5 times more carbohydrates in soft drinks (40% 8%, $p < 0.001$), approximately 7% of patients only drink 1 cup of soda a day, more than 50% of patients drink 2 to 3 cups of soda a day, and 38% of patients drink more than 4 cups of soda a day in the six months (Roeb and Weiskirchen, 2021). The most popular drink is Coca-Cola (regular: 32%; diet: 21%) and the second most popular juice (47%) (Roeb and Weiskirchen, 2021). On the other hand, patients with fructose-1 phosphate aldolase B deficiency characterized by hereditary intolerance to fructose also showed an increase in the accumulation of intrahepatic triglycerides, indicating that the increase in the concentration of fructose-1 phosphate and the decrease of β-oxidation appear to be both involved in the pathogenesis of NAFLD (Roeb and Weiskirchen, 2021). In patients with this type of inborn error, higher intrahepatic triglyceride concentrations compared to the control group are associated with glucose intolerance (Roeb and Weiskirchen, 2021). Accumulation of fructose breakdown intermediates can lead to triglyceride accumulation in the liver through impaired β-oxidation (Simons *et al*., 2019).

## 4. Management of NAFLD

### 4.1. Lifestyle modification

Lifestyle adjustment, is still the most effective treatment for NAFLD/NASH, including a combination of diet adjustment and physical activity (Cardoso *et al.*, 2021), especially when they enable to lose weight (Jegatheesan and De Bandt, 2017). It is well known that low levels of

moderate-intensity physical activity and large amounts of sedentary time are associated with insulin resistance (IR), metabolic syndrome, type 2 diabetes mellitus (T2DM) and NAFLD (Cardoso *et al.,* 2021). The relationship between diet and the development of NAFLD is complex, and of course it is related to diet patterns and the amount of food (Cardoso *et al.,* 2021).

Increased physical activity will reduce the intrahepatic triglyceride content of NAFLD patients and hepatocyte damage markers, is not associated with weight loss (Cardoso *et al.,* 2021). In NASH patients, after 200 minutes of moderate intensity physical activity per week for 48 weeks, disease severity markers were reduced and correlated with a balanced diet (Jegatheesan and De Bandt, 2017). A systematic review of 17 studies on the effects of physical exercise and related weight loss on intrahepatic triglycerides (IHTG) in NAFLD patients and a meta-analysis showed that exercise reduced IHTG levels regardless of significant changes in weight (Cardoso *et al.,* 2021). However, when weight is lost, the benefits gained increase significantly (Cardoso *et al.,* 2021). In addition to exercise, it is also important to reduce sedentary time, because sedentary time increases for all reasons the mortality rate is independent of physical activity (Munteanu *et al.,* 2016). Sedentary time also predicts higher IR levels (Munteanu *et al.,* 2016). There are currently no studies on sedentary time in patients with NAFLD, but minimizing sedentary time should be a general recommendation for these patients (Munteanu *et al.,* 2016). There are very few randomized controlled trials (RCTs) of diet and lifestyle interventions in NASH patients (Munteanu *et al.,* 2016). In a well-designed small RCT, 32 patients randomly received diet and healthy lifestyle interventions for 48 weeks, in which dietitians, dietitians, psychologists, and physical coaches participated (Munteanu *et al.,* 2016). The authors concluded that a 7% weight loss resulted in histological improvements in NASH, including steatosis, balloon lesions, lobular inflammation, and NAS score (a composite score of steatosis and NASH histological activity) (Munteanu *et al.,* 2016). However, is such a broad lifestyle intervention, involves such a complex intervention team, is unlikely to be implemented in daily practice (Munteanu *et al.,* 2016). Given the histological benefits of weight loss, weight loss also resulted in improvement of ALT (Munteanu *et al.,* 2016).

Calorie restriction seems to be the most important factor in dietary intervention, as it is the main driver of weight loss, liver and subcutaneous fat loss, and visceral obesity (Munteanu *et al.,* 2016). Macronutrient composition does not seem to have an effect on weight loss, provided that it is achieved (Ratziu *et al.,* 2015). Recent data indicate that the Mediterranean diet can provide some benefits in liver fat and liver IR, even without weight loss (Munteanu *et al.,* 2016). This emphasizes the importance of monounsaturated and polyunsaturated fatty acids in a healthy diet (Munteanu *et al.,* 2016). In addition to limiting caloric intake, these patients should also avoid diets rich in saturated fatty acids, sucrose, and alcohol (Jegatheesan and De Bandt, 2017). For example, a Mediterranean diet rich in monounsaturated fatty acids may be effective (Jegatheesan and De Bandt, 2017). In NAFLD patients without diabetes, it has been shown to reduce liver steatosis and improve insulin sensitivity Jegatheesan and De Bandt, 2017). A diet rich in omega3 polyunsaturated fatty acids can also reduce fatty degeneration (Jegatheesan and De Bandt, 2017).

A more compelling weight loss strategy is bariatric surgery. This is an effective procedure, primarily to reduce calorie intake to improve insulin resistance and glucose metabolism, thereby reducing weight and liver steatosis (Jegatheesan and De Bandt, 2017). However, bariatric surgery with NASH is not recommended as a first-line treatment, and NASH is not a contraindication in obese patients requiring bariatric surgery (Munteanu *et al.*, 2016).

Possible alternatives are probiotics and prebiotics, due to their impact on the intestinal microbiota and / or intestinal barrier function, there is a growing interest in the management of these patients (Jegatheesan and De Bandt, 2017). For instance, *Lactobacillus rhamnosus* GG prevents the development of fructose-induced NAFLD by preserving the gut microbiota, thus restoring the intestinal barrier by increasing the expression of close-binding proteins Claudine1 and Occludine (Jegatheesan and De Bandt, 2017). Finally, people are more and more interested in natural products and plant extracts, which may be effective in some aspects of fructose-induced NAFLD, though its clinical effectiveness has not yet been evaluated (Cheng *et al.*, 2017; Jegatheesan and De Bandt, 2017).

### 4.2. Pharmacological Treatment

There are no drugs approved by regulatory agencies for NASH. Therefore, any specific treatment cannot be strongly recommended, and any drug treatment will be off-label (Cardoso *et al.*, 2021). Several drugs that have been tried for the treatment of NAFLD have not yet been recommended (Munteanu *et al.*, 2016). These include pentoxifylline, ursodeoxycholic acid (UDCA), omega3 fatty acids, and metformin (Munteanu *et al.*, 2016). All of these therapies have inconsistent results, so further research is needed to confirm their efficacy (Munteanu *et al.*, 2016).

#### 4.2.1. Pentoxifylline

Pentoxifylline (PTX) is a tumor necrosis factor agonist that reduces the production of anaerobic free radicals (Handy *et al.*, 2015). Animal models also show that has anti-fibrosis effects and can significantly reduce steatohepatitis (Handy *et al.*, 2015).

#### 4.2.2. Vitamin E and Pioglitazone

A 2011 study showed that compared with placebo, vitamin E and pioglitazone treatment had beneficial effects on non-diabetic patients over 2 years (Cardoso *et al.*, 2021). Vitamin E (800 IU/d) improved steatosis, inflammation, and inflammation in 36% of patients (21% in placebo group) and induced regression of NASH (Cardoso *et al.*, 2021). Compared with placebo, pioglitazone improved all histological characteristics (except fibrosis) and induced NASH regression more frequently (Cardoso *et al.*, 2021). The histological benefit is combined with the improvement of ALT and the partial correction of insulin resistance. Despite its powerful antioxidant effects, vitamin E should be used with caution because it is associated with all causes of death and due to its possible side effects, such as prostate cancer (men over 50 years old) and

bleeding (Cardoso *et al.,* 2021). In view of these risks, the current American Association for the Study of Liver Diseases Guide recommends that vitamin E be considered in non-diabetic adults with NASH, but not in diabetics or children (Handy *et al.,* 2015). Pioglitazone may be associated with weight gain, congestive heart failure (rarely), and bone loss. Like vitamin E, the benefits of pioglitazone must be balanced with the reported risks. In addition, the optimal duration of treatment is unknown. Only liraglutide (a glucagon-like peptide 1 agonist used to reduce weight) was found in a phase II study of to improve liver histology in patients with NASH (Armstrong *et al.,* 2016; Cardoso *et al.,* 2021).

Although preliminary data from small studies indicate that supplementation with Omega-3 polyunsaturated fatty acids can reduce liver fat content, a large trial tested two doses of pentaethyl eicosapentaenoic acid didn't show any histological effect (Hardy *et al.,* 2015).

### 4.2.3. Metformin

Metformin is a biguanide that can improve IR and hyperinsulinemia by reducing liver glucose production, increasing peripheral muscle glucose uptake, and reversing the IR induced by tumor necrosis factor (Lisboa *et al*., 2016). However, a recent meta-analysis concluded that the use of metformin does not promote the continued benefit of patients with hepatic steatosis (Lisboa *et al*., 2016). Therefore, its use is reserved for the treatment of fatty liver and related type 2 diabetes patients, because it can improve metabolic parameters and help moderate weight loss (Lisboa *et al*., 2016)

### 4.2.4. Omega-3 Fatty Acids

Omega 3 fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are effective activators of nuclear receptor proteins such as PPARα and PPARγ, and can regulate several factors involved in stimulating the oxidation of fatty acids genes, they regulate pro-inflammatory genes, such as TNFα and IL6, and improve insulin sensitivity (Lisboa *et al*., 2016). Regarding the impact on NAFLD, a recent systematic review and meta-analysis found heterogeneity between studies and concluded that although omega3 supplementation can reduce fat in the liver (no effect on aminotransferase levels), the dose optimal has yet to be determined (Lisboa *et al*., 2016).

### 4.2.5. DNL Enzyme Inhibitors

Enzymes of the *de novo* lipogenic pathway are another popular target for drugs in development (Peng *et al.,* 2020). Aramchol is a synthetic molecule produced by combining bile acid and arachidic acid (Peng *et al.,* 2020). Aramchol works by inhibiting the SCD1 enzyme, which is a key rate-limiting enzyme responsible for converting FA to TG (Softic *et al.,* 2016; Peng *et al.,* 2020). Aramchol has shown antioxidant and anti-fibrosis effects in animal studies, while reducing hepatic steatosis (Peng *et al.,* 2020). (NCT01094158) Phase II clinical trial showed that, compared

to the placebo group, NASH patients taking 300 mg aramchol daily had a 12.6 to 22.1% reduction in liver fat and, compared to the placebo group, liver fat increased by 6.4-36.3% (Peng *et al.*, 2020). Aramchol is, currently in phase III trial (NCT04104321), with an estimated completion date of June 2022 (Peng *et al.*, 2020).

### 4.2.6. Use of Anti-Inflammatory and Anti-Apoptotic Drugs in NAFLD

Inflammation of the liver is one of the hallmarks of NASH and one of the popular targets of drugs in development. Several drugs that target inflammation, such as emricasan, a pan-caspase inhibitor, have been shown to fail to meet the primary endpoint of the clinical trial (Harrison *et al.*, 2020). Similarly, selonsertib, an inhibitor of apoptotic signal-regulated kinase 1 (ASK1) used to prevent apoptosis of hepatocytes, has shown promising results in reversing fibrosis and reducing liver inflammation in several models. Preclinical (Alexander *et al.*, 2019; Challa *et al.*, 2019). However, selonsertib did not meet its primary clinical endpoint in any of its phase III trials (STELLAR3: NCT03053050, STELLAR4: NCT03053063). It is worth noting that, compared to humans, animal models have a limited lifespan. It is difficult to determine precisely whether treatment in animal models is reducing fibrosis or simply delaying its progression. Cenicriviroc, a dual CCR2 / CCR5 inhibitor, is currently in phase III trials with an estimated completion date of approximately October 2021 (NCT03028740). CCR2 is one of the main recruitment mechanisms for extrahepatic inflammatory cells (Peng *et al.*, 2020). In animal studies, CCR2 inhibition has shown anti-inflammatory effects in the liver (Krenkel *et al.*, 2018; Peng *et al.*, 2020). More importantly, in their phase II clinical trial, 20% (23/145) of patients who received 150 mg of cinicivirol per day had a reduction in fibrosis, compared to 10% of those who received placebo (14/144) reduced fibrosis (Lefere *et al.*, 2020; Peng *et al.*, 2020). Overall, compared to the control group, patients who received cenicriviroc had a reduction of levels of inflammation (Lefere *et al.*, 2020; Peng *et al.*, 2020).

#### 4.2.6.1. PPAR Antagonist

Elafibranor is a dual agonist of alpha/delta receptor (PPAR alpha/delta) activated by peroxisome proliferation, which was shown to improve the histological efficacy of NASH in its phase II trial on 274, patients One of the drugs (Peng *et al.*, 2020; Ratziu *et al.*, 2016). The preclinical models used to validate elafibranor include db/db mice, CCL4-induced liver fibrosis, and WD-conjugated hApoE2KI mice (Staels *et al.*, 2013). PPARα activation improves NASH by increasing FFA oxidation Stienstra *et al.*, 2007) and reduce inflammation through negative crosstalk with NFκB (Delerive *et al.*, 1999). PPAR δ is responsible for improving liver and systemic insulin sensitivity (Lee *et al.*, 2006). Elafibranor reduced fibrosis in a CCL4-induced liver fibrosis model (Staels *et al.*, 2013; Tsuchida *et al.*, 2018). In addition, the increased expression of TG, VLDL, and inflammation genes exhibited by the WDfed hApoE2KI model was also standardized by elafibranor (ShiriSverdlov *et al.*, 2006; Staels *et al.*, 2013). However, neither CCL4 nor WD + hApoE2KI models showed obesity or hyperglycemia (ShiriSverdlov *et al.*, 2006; Tsuchida *et al.*,

2018). The efficacy of Elafibranor in improving glucose homeostasis and insulin sensitivity has been individually demonstrated in obese db/db mice (Hanf *et al.*, 2014). Despite this, elafibranor did not meet its primary clinical endpoint (RESOLVEIT: NCT02704403) in its recently completed 72-week phase III trial. Results from the RESOLVEIT trial and interim analysis showed that there were no significant differences between the placebo group and the treatment group (120 mg / day) (GENFIT S.A, 2020). However, the full dataset will not be released until the second half of 2020 at the International Hepatology Conference (GENFIT S.A, 2020). Although there are many reasons that may cause 4,444 drug candidates not to be successfully transferred from preclinical to clinical research, the use of animal models that only partially mimic the NASH phenotype (as highlighted in the model portion) can be a important factor. However, the complete data set of the phase III clinical study of elafibranor will be published at the International Hepatology Congress (GENFIT S.A, 2020) in the second half of 2020. Further analysis of existing clinical data is needed to determine the long-term therapeutic effect of elafibranor treatment in a large trial population.

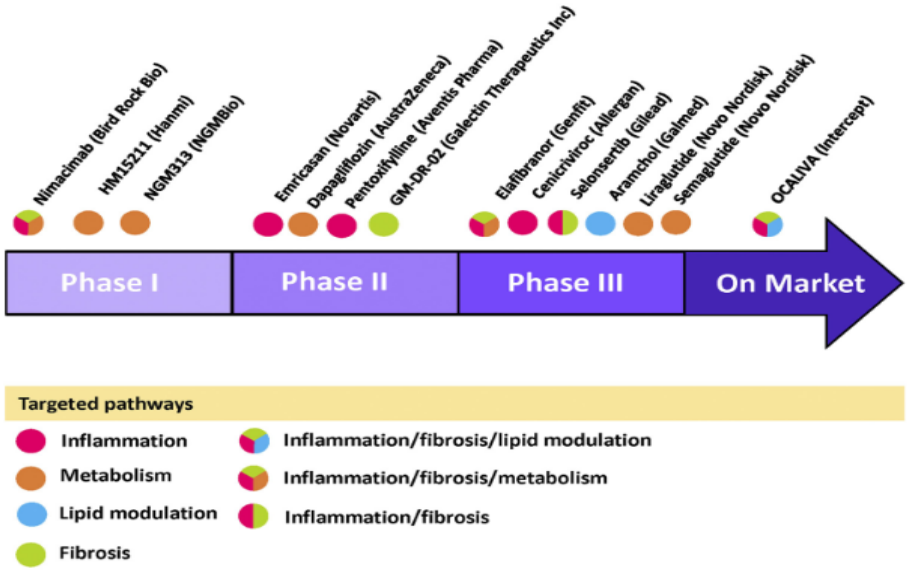


**Figure 4.0: Current NASH/NAFLD pipeline drugs with targeted pathways. (Peng *et al., 2020*)**

### 4.2.6. Glucagon-Like Peptide 1 Receptor Antagonists

Synthetic long-acting glucagon-like peptide 1 (GLP1) receptor agonists, such as liraglutide and simaglutide, were initially approved for the treatment of type 2 diabetes (Pearson *et al.*, 2019).

Recently, both liraglutide and semaglutide have received attention for their efficacy in reducing insulin resistance, hyperglycemia, and liver lipotoxicity in patients with NASH (Armstrong *et al.*, 2016; Peng *et al.*, 2020). GLP1, a hormone secreted by the small intestine after meals, has been shown to restore insulin sensitivity and reduce hyperglycemia in humans (Peng *et al.*, 2020). In

preclinical and clinical studies, treatment of NASH with GLP1 receptor agonists has been reported to improve hepatic steatosis (Peng *et al.*, 2020). Novo Nordisk has completed its 48-week phase II clinical trial (NCT02970942) to evaluate the efficacy of taking 1.8 mg liraglutide daily and is preparing for its phase III clinical trial. In addition, semaglutide is a structurally related analogue of GLP 1 receptor agonists, which can significantly reduce body weight and liver enzymes in obese and type 2 diabetes patients (Newsome *et al.,* 2019). Information from a 72-week phase II multicenter trial of semaglutide (NCT02970942) showed that 33 of 56 NASH patients who received 0.4 mg semaglutide had remission, while 10 of 58 patients who received placebo (Newsome *et al.,* 2020). Semaglutide was well tolerated, and reported adverse events were gastrointestinal events (Newsome *et al.,* 2020).

### 4.3. Use of Plant-Based Natural Products in NAFLD Management

In recent years, people have become more and more interested in using natural extracts or plant-derived products to treat NASH (Peng *et al.*, 2020). Many of these products are widely used as traditional Chinese medicine, and their potential beneficial effects on NASH in preclinical models are currently being investigated (Sun *et al.*, 2017; Peng *et al.*, 2020). Plants including *acanthopanax senticosus* (Siberian ginseng) (Peng *et al.,* 2020) and glycyrrhizic acid (Sun *et al.,* 2017) showed reduced *de novo* liver lipogenesis and improved insulin in the NASH mouse model Sensitivity. In addition, some naturally-derived analogs are also being tested to determine the therapeutic potential of in diet-induced NASH mice, and have been shown to reduce liver adipogenesis as well as endoplasmic reticulum stress and oxidative stress (Peng *et al.,* 2020; Rao *et al.,* 2020).

In addition, Zhang *et al*. (2020), have thoroughly reviewed the use of herbs to induce autophagy as a treatment for NASH/NAFLD. (2018). However, large-scale clinical trials with participants of multiple ethnic origins are needed to confirm the therapeutic potential of natural plant-based products against NASH.

## 5. Conclusion

Fructose metabolism is related to the development and progress of NAFLD and the use of high fructose sweeteners (such as HFCS) is increasing in the food industry because is inexpensive and easy to add to food. The metabolism of fructose in the liver is unrestricted, bypassing glycolysis regulatory enzymes and causing to effectively induce adipogenesis. Furthermore, fructose, independently of negative feedback regulation of insulin signaling, indirectly induces increased hepatic *de novo* adipogenesis and gluconeogenesis through SREBP1c and ChREBP, leading to increased resistance to liver insulin.

Fructose consumption leads to liver inflammation, so NASH progresses through ROS production, uric acid accumulation, and induction of stress ER (Federico *et al.,* 2021).

Furthermore, chronic high fructose intake will change the gut microbiota, which will cause an ecological microbial imbalance and changes in gut permeability, which will lead to the translocation of bacteria and PAMP and will induce and promote inflammation of the liver. So far, animal studies have vastly improved our understanding of fructose metabolism in the body and its accidental effects on NAFLD, but in humans, some problems remain unsolved. Therefore, clinical trials for fructose-induced NAFLD will be a key strategy for to fully reveal and understand the pathophysiology of fructose-induced changes and the underlying molecular mechanism of clinical diagnosis and treatment.

**Author:** "I, as the Corresponding Author, declare and undertake that in the study titled as Review on the Role of Fructose in Non-Alcoholic Fatty Liver Diseases, scientific, ethical and citation rules were followed; Turkish Online Journal of Qualitative Inquiry Journal Editorial Board has no responsibility for all ethical violations to be encountered, that all responsibility belongs to the author/s and that this study has not been sent to any other academic publication platform for evaluation."

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