

## REVIEW ARTICLE

### Non-Alcoholic Fatty Liver Disease (NAFLD): Molecular Mechanism, Pathological Progression and Treatment

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#### Abstract

Non-alcoholic fatty liver disease (NAFLD), currently is considered the most common liver disease in the world, affects up to a quarter of the people. NAFLD, characterized by hepatic steatosis, is associated with numerous adverse outcomes and high mortality. Furthermore, fatty acid uptake and de novo lipogenesis production outstrips fatty acid oxidation and export, leading to hepatic steatosis. Hepatic fatty acid uptake beside de novo lipogenesis are enhanced in NAFLD, whereas compensatory fatty acid oxidation is inadequate to normalize lipid levels and, by inducing oxidative stress, may contribute to cellular damage and disease progression, especially when the function of mitochondrial is impaired and increased peroxisomal and cytochromal oxidation. Although lipid output initially increases, it levels off and may decrease as the disease progresses, thereby promoting fat gain. NAFLD is closely associated with many current lifestyle-related diseases, as hepatic steatosis can lead to systemic metabolic imbalances affecting multiple organs. This overview focuses on four major pathways that promote hepatic lipid homeostasis while we discuss the molecular causes of NAFLD

#### Keywords:

NAFLD, Mechanism, Oxidative stress, Pathology, Nano-particles.

#### Introduction

Non-alcoholic fatty liver disease (NAFLD) and alcoholic liver disease (ALD) are the two most common types of chronic liver disease, share this characteristic [1]. The most frequent cause of morbidity, ALD often coexists with other alcohol use disorders and is frequently associated with concomitant psychiatric conditions. [2, 3]. Unlike NAFLD, which is defined as liver fat accumulation (in excess of 5%) not related to alcohol or drugs [4]. Simple steatosis to non-alcoholic steatohepatitis (NASH) are just a few of the many liver illnesses that are included in the metabolic

condition known as NAFLD [5]. Insulin resistance plus a more serious metabolic imbalance are linked to NASH [6]. The high incidence of NAFLD in developed nations is associated with the prevalence of risk factors, including medication use, gastric bypass surgery, parenteral nutrition, and inherited metabolic diseases such as obesity, type 2 diabetes, dyslipidemias, and insulin resistance [7, 8].

Fat builds up in the liver parenchyma as a result of poor fatty acid metabolism, which includes: (1) insufficient apolipoprotein and triglyceride synthesis and secretion [9]; (2) delivery of more

free fatty acids (FFAs) to the liver than can be digested; and (3) increased mitochondrial lipid oxidation. According to research on liver biopsy in obese individuals, 30–40% of patients have more severe steatosis [10]. Just 30% of NASH patients will develop cirrhosis at the time of their primary liver biopsy, while 74% of them will have fibrosis [10, 11]. Due to these facts, the risk factors that indicate the emergence of NASH were found. A working hypothesis known as the "two-hit" theory suggests that basic steatosis can advance into cirrhosis, fibrosis, or NASH. The hepatic fat accumulation brought on by insulin resistance (IR) is the "initial hit." Although this phase is insufficient to cause NASH, it is sufficient to put the liver at risk for chronic inflammation. Patients with metabolic syndrome frequently have it.

The oxidative stress due to reactive oxygen species (ROS) [12], gut-derived lipopolysaccharide, and soluble mediators synthesized both from the immune system cells and from the adipose tissue cells have been indicated as risk factors responsible for the "second hit" [13]. Although the "two-hit theory" paradigm quickly gained acceptance in the scientific community, it is clear that several elements are interacting. The molecular and physiological alterations required for the transition from steatosis to steatohepatitis have recently been the subject of new research utilizing NAFLD animal models. Studies show that complex interactions between genetic determinants, dysmetabolism, and dietary factors in NAFLD development led to hepatic damage and eventual liver disease [14].

Lipotoxic effects of lipid intermediates and free fatty acids (FFAs) interfere with the normal functions of the organelles in

the liver cell, including the production of ROS, the activation of pro-inflammatory defense mechanisms, and ultimately apoptosis. When inflammatory lipids and cytokines are released, insulin signalling is compromised. As a result, very low-density lipoprotein (VLDL) production and liver secretion are impaired [14]. The ineffective regulation of many nuclear receptors' activation is the molecular cause of the developing metabolic insufficiency in NAFLD [15]. The regulation of insulin signalling is necessary for the progression of steatohepatitis from steatosis to inflammation, lipid peroxidation, and liver destruction [16, 17].

Organelle activities during the metabolic load cycle are compromised by the liver's fight against oxidative stress. The first organelle to breakdown is the mitochondria, according to decreased fatty acid oxidation in mitochondria and increased fatty acid oxidation in peroxisome [18]. Moreover, oxidative stress makes the hepatocytes more vulnerable to toxic stimuli and stress [19].

The activation of cellular defense mechanisms produces additional stress stimuli by altering oxidation of lipid and the redox state intracellularly and may steer hepatocytes away from survival and towards death [20]. Activated Kupffer cells drive the creation of large amounts of collagen by hepatic stellate cells (HSCs), which results in liver fibrosis and cirrhosis. The goal of this review was to present a summary of the molecular basis of NAFLD. As a result, we will talk about the primary reason causing NAFLD as well as the part that pathologic modifications in lipid metabolism play during the entire course of the illness.

### ***Prevalence of NAFLD***

Because to lifestyle factors and risk factors like metabolic syndrome (MS), type 2 diabetes, hypertriglyceridemia, and obesity, NAFLD has a significant incidence among the general population [21]. Almost 25% of people worldwide have NAFLD [22].

### ***Risk factors of NAFLD***

Type 2 diabetes, dyslipidemia, insulin resistance (IR) and central obesity are the key risk factors for NAFLD, just as they are for the other elements of the metabolic syndrome. NAFLD is regarded as the metabolic syndrome's hepatic component [23]. Hepatic inflammation histologically is one of the most significant risk factors. Other variables that have been linked to disease progression or extensive fibrosis include: Older age [24,25], diabetes mellitus [26], raised serum aminotransferases [23, 27], body mass index (BMI)  $\geq 28$  kg/m<sup>2</sup>, ballooning degeneration and Mallory hyaline on biopsy [28]. Higher index of visceral adiposity, that accounts for high-density lipoprotein levels, triglycerides, and waist circumference [29]. Genetic: -A family investigation of 157 people with familial combined hyperlipidemia found that both dyslipidemic patients and family members with normal lipid levels had higher rates of fatty liver and alanine transaminase (ALT) [30]. A decreased risk of advancement has been linked to coffee drinking [31].

### ***Molecular mechanism of hepatic lipid accumulation in NAFLD***

#### ***1. Hepatic lipid uptake***

Passive diffusion is only a small part of the process by which circulating fatty acids are taken up by the liver via fatty acid transporters [32]. The transfer is predominantly mediated by caveolins, cluster of differentiation36 (CD36) and

fatty acid transport protein (FATP) [33]. In mice, FATP2 knockdown decreases uptake of fatty acid and hastens the development of hepatic steatosis brought on by a high-fat diet [34]. In a similar manner, FATP5 knockdown in mice reduces fatty acid absorption by hepatocyte, decreases triglyceride content of the liver, and inhibits steatosis [35].

Long-chain fatty acid transport is facilitated by CD36, which is regulated by peroxisome proliferator activated receptor (PPAR), liver X receptor, and pregnane X receptor [36]. Both diet-induced and hereditary steatosis are lessened by the liver-specific deletion of CD36 [37]. By having unusually elevated CD36 levels in NAFLD patients, this shows that CD36 plays a causal role in steatosis [38]. A family of three membrane proteins called the caveolins are involved in the production of lipid droplets and lipid trafficking [33]. In the centrilobular zone 3 of the mice livers with NAFLD, where the steatosis was severe, caveolin 1 levels were elevated [39].

As hydrophobic fatty acids are difficult to diffuse in the cytoplasm, they must be transported between different organelles via fatty acid binding proteins (FABP), of which FABP1, the main liver isoform [32]. FABP1 improves fatty acids and their acyl-CoA derivatives transit, storage, and utilization by binding to cytotoxic free fatty acids and assisting in their oxidation or incorporation into triglycerides. This might protect against lipotoxicity [40]. When compared to non-NAFLD controls, NAFLD patients had greater levels of the hepatic FABP1, FABP4, and FABP5 mRNA [41]. In Figure 1, the four main pathways are depicted for the regulation of lipid intake, de novo lipogenesis (DNL), fatty acid oxidation (FAO), and the export of lipids in very low-density lipoproteins (VLDL).

Hepatic fat accumulation is caused by an imbalance between lipid synthesis and lipid oxidation [42].

## 2. *De novo lipogenesis*

Acetyl-CoA is converted by the liver into new fatty acids with the help of *de novo* lipogenesis (DNL). Malonyl-CoA is created by acetyl coA carboxylase (ACC), which is then converted into palmitate by fatty acid synthase (FASN). New fatty acids undergo a multitude of processes, including esterification, elongation, desaturation, and storage as triglycerides or VLDL particles. Elevated DNL can therefore lead to hepatic steatosis and/or hypertriglyceridemia since saturated fatty acids like palmitate cause inflammation and apoptosis. On the other hand, steatohepatitis can also develop [43]. Moreover, DNL was independently correlated with intrahepatic lipid concentrations [44], and suppression of DNL during fasting may be a key feature in NAFLD patients [44].

The importance of DNL in NAFLD is shown by studies showing that approximately 26% of hepatic triglycerides in obese NAFLD patients were generated by DNL and that these individuals were unable to modify DNL during the change from a fasted to a fed state [45]. The two main molecules that regulate the transcription of DNL are carbohydrate regulatory element-binding protein (ChREBP), which is activated by carbohydrates, and sterol regulatory element-binding protein 1c (SREBP1c), which is activated by insulin and the liver X receptor [46, 47]. Due to its lipogenic action, SREBP1c expression is elevated in NAFLD patients, and transgenic mice overexpressing SREBP1c had greater hepatic triglyceride levels than SREBP1c knockout animals [48]. However, in SREBP1c knockout mice, expression of

lipogenic enzymes decreased [49]. ChREBP promotes carbohydrate-induced DNL but not fat-induced DNL due to the fact that high-fat diets do not increase ChREBP and may even diminish ChREBP activity [50]. ChREBP has been demonstrated to be knocked out in mice, which causes delayed glucose clearance, insulin resistance, and extreme intolerance to simple sugars like sucrose and fructose. As a result of the animals' failure to shift fructose into glycolytic pathways, most of the animals die [51]. Furthermore, a 65% decrease in hepatic fatty acid production in knockout mice as compared to wild-type controls has been observed. This implies that ChREBP is essential for a typical lipogenic response following carbohydrate delivery and supports the critical function of ChREBP in both glucose and lipid metabolisms [51]. ChREBP may be hepatoprotective by lowering cytotoxic-free cholesterol levels and the resulting liver damage [52].

An effective defense mechanism against more liver damage and the emergence of NASH may be represented by elevated ChREBP levels in NAFLD. This theory is backed by the fact that lipogenesis and the development of NASH are distinct processes, indicating that high DNL may promote steatosis while being advantageous for the advancement of the illness [53]. When a liver-specific ACC1 gene was knocked out, the accumulation of fat in mice's livers and DNL in their hepatocytes was decreased [54]. Although mutant mice were not protected from hepatic steatosis brought on by a high-fat diet, this may have been because of an increase in ACC2, an inhibitor of mitochondrial-oxidation, which inhibited fatty acid oxidation [54]. Consequently, to advance hepatic steatosis in mice, both ACC1 and ACC2 needed to be inhibited [55].

Saturated fatty acids are expected to be partitioned to mono-unsaturated fatty acids, which is thought to help halt the progression of NAFLD [56]. While not everyone with fatty livers progresses to developing NASH, some of them require protective measures against lipotoxicity, such as lipid desaturation and reduction of lipid-induced inflammation [57]. Diacylglycerides are detrimental in the early stages of NAFLD development because NAFLD/NASH progression does not promote further alterations in lipid metabolism linked to diacylglycerides until steatosis has started [58]. In conclusion, lipogenesis encourages lipid accumulation in NAFLD and raises the possibility that DNL might be a good therapeutic target.

### 3. Oxidation of fatty acids

Particularly when levels of circulating glucose are low, the synthesis of adenosine triphosphate (ATP) is powered by fatty acid oxidation (FAO), which is predominantly controlled by PPAR and occurs mitochondrially [33, 59]. Peroxisomes, cytochromes, and mitochondria in mammalian cells all have a role in FAO [58, 60]. Fatty acids cannot enter mitochondria without the enzyme carnitine palmitoyl transferase 1 (CPT1), which is located in the outer membrane of the mitochondria [61].

Very long chain fatty acids, on the other hand, are broken down through peroxisomal-oxidation since the mitochondria are unable to oxidize them [62]. NAFLD is also influenced by the cytochrome-oxidation [60]. Yet, the quantity of oxidative stress, ROS, and toxic dicarboxylic acids generated by these activities increases the likelihood of inflammation and the onset of disease [60]. PPAR's role in regulating hepatic lipid metabolism is supported by the

hepatic steatosis that PPAR knockout animals experience [63]. Hepatic PPAR levels are similar in healthy volunteers and human steatotic patients [41]. In contrast to patients with steatosis and healthy individuals, NASH patients had downregulated PPAR, and PPAR expression decreased with rising NAFLD activity score and fibrosis stage [64, 65] and decreased PPAR expression [66]. Due to the fact that decreased PPAR in NASH also enhanced the binding of DNA to c-Jun N-terminal kinase 1 (JNK1) and nuclear factor kappa-light-chain enhancer of activated B cells (NF-B), increasing hepatic inflammation, it may be related to several aspects of NASH progression, modulating both inflammation and lipid homeostasis [67]. Depending on how bad the condition is, different fatty acids may oxidize differently. Moreover, FAO capacity may differ amongst people, making some subjects more prone to NAFLD. Patients with more severe steatosis expressed more peroxisomal, mitochondrial, and oxidative genes than others [68]. Enhanced FAO may be a corrective action taken by NAFLD patients in an effort to reduce lipid excess and lipotoxicity. Hence, in addition to FAO, NASH patients also had elevated hepatic oxidative stress and alterations in mitochondrial ultrastructure [69]. In the mitochondria of NAFLD animal models as well as the liver biopsies of NAFLD patients, a decrease in superoxide dismutase (SOD), glutathione, glutathione peroxidase was found [67].

Oxidation of lipid and mitochondrial DNA oxidative damage further impair mitochondrial activities, creating a self-reinforcing feedback loop that exacerbates mitochondrial malfunction and oxidative stress [62]. When compared to controls, obese NASH patients had lower levels of mitochondrial respiratory chain activity

[70]. This decline in the function of mitochondria may have prompted the deployment of substitute FAO routes [71]. Increased cytochromal FAO may thus be a significant steatosis and NASH event due to the excessive amount of ROS that the cytochrome P (CYP) enzymes produce, which exacerbates hepatic oxidative stress and subsequently worsens liver damage. Peroxisome is the third and last organelle in the chain of three necessary for hepatic lipid homeostasis and fatty acid metabolism [72].

Targeting this system, peroxisomal FAO is confirmed to have a role in NAFLD and NASH through hepatic lipid buildup, fibrosis, oxidative stress, and inflammation, either through a defect in acyl coA oxidase (ACOX) or hepatocyte-specific deletion of peroxisomes [72]. To sum up, FAO produces extremely high levels of ROS in damaged mitochondria and may also help FAO utilize peroxisomes and cytochromes. By causing oxidative stress and inflammation, this promotes the spread of disease.

#### 4. Lipid export

Triglyceride export is the only method for lowering hepatic lipid level other than FAO [73]. Fatty acids cannot be exported from the liver on their own because they are hydrophobic; instead, they must be coupled with cholesterol, apolipoproteins, and phospholipids to create water-soluble very low density lipoprotein (VLDL) particles [74]. Microsomal triglyceride transfer protein's (MTTP) enzymatic activity causes the endoplasmic reticulum, where apolipoprotein B100 (apoB100) is lipidated, to produce VLDL particles. After being delivered to the Golgi apparatus, the growing VLDL particle continues to be lipidated there until the entire VLDL particle is created [75]. The

triglyceride concentration of VLDL particles can vary significantly, despite the fact that one apoB100 molecule is associated with each and required for VLDL export [74, 76]. Hence, MTTP and apoB100 are necessary for maintaining hepatic lipid homeostasis and hepatic VLDL secretion. Therefore, hepatic steatosis brought on by impaired triglyceride export is hence common in those with apo B or MTTP gene mutations (hypo-beta-lipoproteinemia or a betaproteinemia, respectively) [77].

Although extended exposure to fatty acids led to posttranslational degradation of apoB100 and endoplasmic reticulum stress (ER stress), which in turn reduced apoB100 secretion, researchers were able to relate ER stress to the evolution of NAFLD through apoB100 suppression [78]. PPAR controls the transcription of the MTTP gene, and an alteration in apoB100 secretion enhanced MTTP [79]. PPAR therefore regulates lipoprotein metabolism in addition to FAO to exert its catabolic effect. Contrarily, insulin adversely regulates both apoB100 and MTTP, causing apoB100 to degrade and MTTP production to be suppressed, both of which reduce hepatic lipid export [76].

Increased insulin levels decreased the production of hepatic VLDL in the postprandial period, favoring the transport of dietary lipids to the periphery via chylomicrons [76]. Yet, in NAFLD patients, the selective hepatic insulin resistance allows for an increase in DNL without affecting the synthesis of VLDL [80]. NAFLD patients secreted more VLDL [75, 81, 82], and triglyceride levels in the liver were strongly correlated with VLDL-TG secretion rates [83]. While the export of VLDL-TG increased as intrahepatic lipid level increased, the secretion peaked when hepatic fat content exceeded 10% and was beyond the

capacity of compensatory mechanisms to avoid growing hepatic lipid accumulation [75].

While VLDL-apoB100 secretion remained unaltered, patients with hepatic steatosis secreted more VLDL-TG than healthy individuals, which suggests that NAFLD patients create larger, more triglyceride-rich VLDL particles rather than more VLDL [75]. Due to the fact that particularly large VLDL particles cannot be released if their diameter exceeds that of sinusoidal endothelium pores, the restriction may result in fat retention and NAFLD [84]. When compared to controls, apoB100 mRNA levels and MTTP were found to be higher in NAFLD patients [85], but apoB100 synthesis rates were shown to be lower in NASH patients [86]. Inadequate levels of apoB100 as a NAFLD causing factor could be indicated by a failure to increase the amount of released VLDL particles.

By comparing to healthy controls, NAFLD patients who had more advanced steatosis (> 30%) exhibited lower levels of MTTP, raising the possibility that the considerable intracellular lipid buildup may also directly limit lipid export [86].

### ***Pathology of non-alcoholic fatty liver disease***

#### ***1. Non-alcoholic fatty liver (NAFLD)***

Steatosis alone or steatosis combined with mild lesions that are not severe enough to be classified as non-alcoholic steatohepatitis (NASH) characterize a substantial portion of those with non-alcoholic fatty liver. Based on studies, people with steatosis alone are more likely to experience non-hepatic cancer-related illnesses but not to die from liver-related causes [87]. Steatosis may be regarded as pathological when lipid droplets are present in at least 5% of

hepatocytes. There are two types of steatosis: macro vesicular and medio-vesicular. The lipid vacuole fills the entire hepatocyte in macro vesicular steatosis, forcing the nucleus to one side. These cells resemble adipocytes at their most severe [88]. When small vacuoles are present in the cystol, medio-vesicular steatosis develops. Typically, these vacuoles may be easily separated from one another and are few enough to count. The micro vesicular steatosis, in which a large number of tiny vacuoles replaced the hepatocyte and gave a foamy appearance to the cell, should be separated from the medium vesicular steatosis [89].

#### ***2. Non-alcoholic steatohepatitis (NASH)***

Those having a histological pattern of advanced fibrosis and steatohepatitis are more likely to experience end-stage liver disease or liver-related death [24, 90]. Epidemiological studies indicate the presence of two distinct entities with a potential shift from one to the other and in both directions. Pure steatosis may evolve to steatohepatitis. In order to diagnose steatohepatitis, liver tissue must be examined. If a liver biopsy is required to confirm the existence of NASH, it must be done [91].

It is now well accepted that inflammation of lobules and clarification/ballooning of liver cells are necessary beside steatosis [92]. Additional histological traits might occur, although they might not be useful in making the diagnosis of NASH. Presence of more than 5% of mainly macro-vesicular hepatocellular steatosis in liver tissue sections is the minimal histologic indicator of NAFLD [93].

#### ***3. Lobular inflammation***

Little clusters of inflammatory cells, primarily macrophages and lymphocytes that are occasionally coupled with apoptotic bodies or hepatocyte dropout make up lobular inflammation. Neutrophil aggregates are uncommon and only start to dominate when there are lots of Mallory-Denk bodies present [94].

#### 4. Ballooning hepatocellular injury

Another important steatohepatitis diagnostic sign is hepatocellular ballooning. The balloon-shaped cytoplasm of inflated hepatocytes is caused by the removal of sharp angles in the liver cell, which is transparent, non-vacuolar, and flocculent. It's possible that hepatocytes are larger than typical hepatocytes [94]. Ballooned hepatocytes are typically found in zone 3 in adult NASH, where perisinusoidal collagen fibers are frequently associated with them [95]. In the presence of a steatosed liver, lobular inflammation and hepatocyte ballooning are both required and enough features for NASH diagnosis.

#### 5. Fibrosis

Many studies have shown that, regardless of the presence or severity of other histological indicators, the stage of fibrosis has an impact on both overall mortality and mortality attributable to the liver alone making it a significant characteristic [90]. Yet, while NAFL might have any level of fibrosis without having any NASH symptoms, NASH is often always linked to some degree of fibrosis.

#### 6. Cirrhosis

The pathological precursor to hepatic cirrhosis, which is the last stage of several chronic liver diseases is fibrosis [96]. Although there are numerous contributing factors to liver cirrhosis, there are specific clinical characteristics that are present in

all instances, including liver parenchyma replacement by regenerating nodules and fibrotic tissues as well as liver function loss [97, 98]. The known information about the natural history of NAFLD is displayed and summarized in Figure 2[99].

#### *Effects of oxidative stress on non-alcoholic fatty liver disease*

Nonalcoholic fatty liver disease is accompanied by intrahepatic alterations such as medium credits, inflammation-induced injury, angiogenesis, death of parenchymal cell, fibrosis, and the accumulation of fat. NAFLD also affects tissues other than the liver, although these changes also reduce the liver's ability to renew and increase portal hypertension [21]. Hence, cirrhotic cardiomyopathy (CCM), hepatorenal syndrome, peripheral neuropathy (PN), sarcopenia, portal hypertension, hepatic encephalopathy (HE), and cirrhotic cardiomyopathy are the main intra- and extrahepatic consequences linked to the advanced stage of NAFLD. Additional dysfunctions include coagulopathy, liver malignancy, spontaneous bacterial peritonitis, ascites, gastroesophageal varices, coagulopathy, fragility, malnutrition, and immune system abnormalities [100, 101].

As oxidative species grow and antioxidant systems decline, NAFLD's intra- and extrahepatic problems are mostly caused by oxidative stress (OS) [102]. One significant reason that can affect NAFLD's antioxidant system and increase ROS is increased levels of free fatty acids and lipid overload in the liver. The key mechanisms underlying OS include mitochondrial dysfunction, endothelial damage, and cellular organelle failure, particularly stress on the endoplasmic reticulum (ER). Because of these structural and functional



abnormalities of liver tissue brought on by ROS, the deleterious effects on extrahepatic tissues and organs are affected [103, 104].

### ***Role of oxidative stress in the liver during NAFLD***

In NAFLD, liver experiences steatosis, damage, hepatocyte ballooning, inflammation, fibrosis, and cell death by apoptosis [105]. The functions of the liver are also changed. As suggested by multiple-hit theory [106, 107], a number of variables are involved in the pathophysiology of NAFLD. According to this idea, environmental factors can lead to weight gain, increased free fatty acid (FFA) mobilization, insulin resistance, and fat deposition that may encourage lipolysis and the development of ongoing low-grade inflammation. By increasing the FFA flow to the liver and hepatic de novo lipogenesis, these alterations cause hepatic steatosis. By increasing the flow of FFA into the liver and initiating hepatic lipogenesis from scratch, these alterations lead to hepatic steatosis (Figure 3). Through causing lipotoxicity and OS, FFA and triglycerides damage the liver by causing hepatic inflammation, mitochondrial dysfunction, hepatocyte death, and fibrosis [108, 109].

The pathophysiology of NAFLD is also influenced by environmental variables. In this way, the development of NAFLD is made more likely by endocrine disruptors like bisphenol A (BPA), which interfere with normal hormonal transmission. Plastics made from polycarbonate and epoxy resin contain the food-contaminant BPA. As a result of IR, enhanced de novo lipogenesis, and an imbalanced lipid homeostasis that favors the pathogenesis of NAFLD, exposure to BPA causes epigenetic modifications in

the liver that are able to determine triglyceride accumulation [110, 111]. Moreover, a secondary effect of NAFLD-induced liver failure that adversely affects extrahepatic tissues is an increase in blood ammonia. As a result of the liver's inability to convert ammonia to glutamine and urea, ammonia builds up in the blood, leading to hyperammonemia (HA) [111].

### ***Signs and laboratory findings of NAFLD***

In most cases, NAFLD is symptomless. when it does, they could consist of fatigue, aches and pains in the upper right abdomen [112]. While in NASH and severe scarring (cirrhosis) could show these symptoms, stomach bloating (ascites), bigger blood vessels under the skin's surface and exaggerated spleen [113]. Regarding clinical finding of NAFLD, serum TG and TC levels were higher in the HFD group than in the normal diet group, at the same time LDL level was higher in the HFD group than in the normal diet group. While, HDL level was lower in the HFD group than in the normal diet group [114]. Serum ALT and AST levels were higher in the HFD group than in the mice that were fed the control diet [115].

### ***Screening methods for NAFLD***

Ultra sound is appropriate as a NAFLD screening technique. Nevertheless, steatosis below 10% of hepatocytes is not recognized, and up to 20% is unreliably detected [116]. Good sensitivity (85%–96%) and specificity (up to 98%) are attained in moderate and severe hepatic steatosis [117]. Computed tomography (CT), which can only be used in facilities with substantial medical equipment, reliably ascertains the liver's fat level by assessing organ density [118]. The performance of CT was very underwhelming in a meta-analysis that compared several radiological techniques,

with a sensitivity of 46-72% [119]. If the density ratio between the liver and spleen on native CT has a threshold value more than 1.1[120], then at least severe hepatic fatty degeneration can be identified. Even in comparison to magnetic resonance imaging, dual-energy CT has been able to demonstrate good findings for estimating the amount of fat in the liver in smaller cohorts [121].

The huge medical gadget known as magnetic resonance imaging (MRI) is a radiologic imaging technique that does not expose users to radiation. The liver's fat content and stage of fibrosis can both be identified using specific MRI modalities with high accuracy [118]. Compared to US-based elastography methods, MR elastography evaluates liver stiffness with a markedly higher degree of accuracy [122,123]. Compared to TE, MR elastography also showed stronger correlations with clinical fibrosis measures and scores [124]. In a recent, thorough investigation, multiparametric MRI with fat content (by PDFF or spectroscopy) and fibrosis (by MR elastography) determination was superior to the corresponding Fibro-Scan-based non-MR procedures (CAP for steatosis and TE for fibrosis) [125].

### *1. Natural treatment*

The natural treatment of NAFLD includes: the therapeutic actions of L. contain liver support, antibacterial, lowering of lipid and lowering effects on cholesterol, nitric oxide synthase gene expression is encouraged, and endothelial cells are encouraged and improved in the repair of atherosclerosis[126]. According to the findings of a clinical trial investigation, Cynara appears to be an effective herbal supplement for treating NAFLD problems while also appearing to alleviate the laboratory symptoms of fatty

liver [127]. Furthermore, Vitamin D controls insulin secretion, the immune system, and the metabolism of minerals. It might also have anti-inflammatory effects [128]. According to studies, up to 75% of those with metabolic syndrome (MetS) have vitamin D insufficiency, and there is a link between it and the occurrence of NAFLD.

Individuals with hepatic steatosis and chronically elevated liver function tests (LFTs) identified by ultrasonography (US) were suggested to have a relationship between histologically severe NASH and low vitamin D levels [128]. Moreover, several metabolic and intracellular processes, including the transfer of fatty acids entering the mitochondria, the lipid levels in serum are lower, cell membrane stability, are significantly influenced by L-carnitine [129]. Furthermore, it has the ability to change the inflammatory response and regulate the energy balance in tissues that mostly obtain their energy from oxidation of fatty acid. L-carnitine supplements appear to be effective in treating liver cirrhosis [130].

L-carnitine supplementation can help treat NASH when combined with a change in lifestyle. L-carnitine supplementation (500 mg twice daily) for 1 year as a NAFLD treatment has not been associated with any significant changes in liver function tests (LFTs) or US grade, according to data from randomized controlled trials (RCT) published more recently [131]. There are numerous meals that include betaine, a human vitamin. The liver uses betaine to convert homocysteine into methionine, which raises methionine levels while lowering homocysteine levels [132]. Only one randomized controlled trial (RCT) examining the benefits of betaine supplementation to patients with NAFLD found no difference between the betaine

group and the placebo group in liver function tests (LFTs) or histology [132].

## 2. Chemical treatment

A lipid-lowering drug, atorvastatin is administered to NASH patients at a dose of 10 mg/day for 12 months. As well as reducing the NAFLD activity score and raising the liver to spleen density ratio, atorvastatin dramatically decreased levels of liver transaminase, GGT, LDL-C, TGs, type IV collagen, and tumor necrosis factor (TNF)-alpha [133]. Moreover, N-acetylcysteine (NAC) is a precursor of glutathione (GSH) which acts as a major endogenous antioxidant [134]. NAC protective effects of are evident in chronic diseases where decreased oxidative stress or GSH such as NASH occur [135].

## 3. Nano-treatment

When it comes to the transfer of a medicine to the target site, nanoparticles are small objects that operate as a single unit [136]. Pharmaceutical, therapeutic, and medical diagnostic nanoparticles offer a wide range of possible applications. By using more modern drug delivery techniques, research on nanoparticles is very helpful in treating diseases and performing surgeries [137]. The ultra-small size, higher surface area to mass ratio, and improved reactivity of nanoparticles are their distinctive physical and chemical characteristics. The limitations imposed by conventional medicinal and diagnostic agents are overcome by nanoparticles thanks to these characteristics [138]. Nanoparticles are drug carriers that have an enhanced capacity that gives the drug contained therein more stability, improving drug bioavailability and reducing dosage frequency [139].

The highly reactive species known as nanoparticles have unusual characteristics

like a small, controlled size and a high surface to mass ratio [140]. They enhance the pharmacological and pharmacokinetic features of the therapeutic molecule, making it more appropriate for targeted drug delivery [141]. By preserving the medicine in systemic circulation and confining drug approval to specific areas, nanoparticles enable more effective drug use. Moreover, they reduce undesired side effects and deliver the medication at a controlled rate to the site of action [142]. The most popular drug administration method, with a greater rate of patient compliance, is oral drug delivery. Nonetheless, it is still associated with a variety of barriers, including the stomach's acidic pH and digestive enzymes [143]. By oral administration, nanoparticles may enable the effective delivery of drugs whose action is constrained by low bioavailability [144].

### 3. a. Nano-silymarin

A combination of flavonoids known as silymarin is obtained from the milk thistle plant, *Silybum marianum*. Since the sixteenth century, it has been employed in the treatment of liver ailments. Its extract has a flavonoid content of 65% to 80%, a fatty acid and polyphenolic compound content of 20% to 35% (fatty acids with metabolic regulating actions) [145].

Silymarin can support the antioxidant defenses in a variety of ways. First, by scavenging free radicals. Second, either by inhibiting the enzymes that produce free radicals or by maintaining the integrity of the mitochondrial electron-transport chain under stressful situations. Thirdly, by aiding in the maintenance of the cell's ideal redox status by triggering a variety of enzymatic and nonenzymatic antioxidants, primarily via way of the transcription factors Nrf2 and NF-B. Finally, by activating a range of vitamin

genes that produce defense mechanisms such as heat shock proteins (HSP), sirtuins, thioredoxin (Trx), etc. and provide further protection in tense circumstances [145].

The pharmacological profile of silymarin has been extensively established, and its hepatoprotective effects have been researched in vitro and in animals. Studies have shown that certain substances have antioxidant and free radical scavenging activities, as well as improved antioxidant defense by limiting glutathione depletion and anti-fibrotic action [146]. Silymarin has a variety of effects, including reducing leukotriene synthesis, downregulating Kupffer cells, and using prostaglandins [147]. It also inhibits neutrophil migration. Silymarin's weak water solubility restricts its oral absorption ratio (23–47%) [148] while its bioavailability is (0.73%) [149], which makes it difficult to use in clinical settings. It remains difficult to increase silymarin's oral bioavailability while maintaining therapeutic efficacy. Despite the fact that several dosage forms and nanocarriers have been created to increase silymarin bioavailability, there is not enough information on the possibility that these nanocarriers may improve the lipid-lowering efficiency of silymarin as a hepatoprotectant [150].

### 3.b. Nano-chitosan

Chitosan oligosaccharides, which are water-soluble byproducts of the enzymatic digestion of chitosan, have demonstrated a number of biological characteristics, including anti-inflammatory and antioxidant capabilities [151]. Low molecular weight chitosan oligosaccharide supplementation also markedly reduced overall plasma cholesterol level and intestinal absorption of dietary fat [152]. In comparison to

chitosan-particles, cells of fatty liver more readily absorbed chitosan nanoparticles by a factor of 1.92. Also, chitosan lipid polymer-nanoparticles dramatically lowered the levels of triglyceride (TG) in fatty liver cells during an in vitro lipid deposition assay, indicating the possibility of their potential cholesterol-lowering effects. In vivo, chitosan lipid polymer-nanoparticles effectively reduced blood TG levels, improved aspartate transaminase (AST) and ALT, and reduced lipid synthesis in mouse livers. After treatment with chitosan lipid polymer-nanoparticles, macro-vesicular steatosis in diseased tissue decreased, showing their preventative action against liver steatosis in NAFLD [153]. Mice were fed a diet high in fat, chitosan with a low molecular weight lowers liver triacylglycerol and cholesterol levels. In addition, studies on hyperlipidemic animal models have demonstrated that chitosan effectively lowers blood lipid levels and prevents weight gain in obese animals [154]. Chitosan was predicted to enhance silymarin's hepatoprotective, lipid-lowering, and oral absorption properties [155]. Chitosan nano-particles have undergone extensive research in the lab for the disinfection of water and the immobilization of enzymes [156, 157].

Chitosan supplementation also decreased high-fat diet-induced lipedema due to its hypolipidemic function, improved silymarin oral bioavailability, and improved lipid-lowering efficiency for treating NAFLD [158]. On the other hand, this marine polysaccharide's ability to quench free radicals has also been well researched [159].

### 3.c. Cerium oxide nanoparticles (CeO<sub>2</sub>NPs)

In experimental liver illness, in recent years, cerium oxide nanoparticles

(CeO<sub>2</sub>NPs) have become a brand-new, powerful antioxidant with medicinal applications. There have been reports of CeO<sub>2</sub>NPs acting as ROS and nitric oxide (NOS) scavengers [160] and mimicking the actions of a number of enzymes, including peroxidase, catalase, and SOD [161]. As a result, the positive benefits of A number of medical specialties have recorded the use of CeO<sub>2</sub>NPs, including hepatology and neurology [162]. CeO<sub>2</sub>NPs, in contrast to other antioxidants, are inactive and harmless in healthy cells, becoming active only at pathogenic ROS levels [163]. According to research, CeO<sub>2</sub>NPs can reduce steatosis [162], reduce oxidative stress [154], and exhibit anti-inflammatory effects in various experimental animal models of NAFLD [165].

### Conclusion

One of the most prevalent liver disorders is NAFLD, estimated to affect many people worldwide. In this review, we summarized its molecular mechanism and described the key player involved in NAFLD onset. Moreover, pathological steps of NAFLD progression and its nano-treatments were illustrated. Regarding clinical finding of NAFLD, serum TG, TC, LDL, ALT and AST levels were higher in the HFD group than in the normal diet group. While, HDL level was lower in the HFD group than in the normal diet group. Nanoparticles are drug carriers that have an enhanced capacity giving the drug contained therein more stability, improving drug bioavailability and reducing dosage frequency including nano-silymarin, nano-chitosan and silymarin loaded chitosan nanoparticles.

### Conflict of interest

Authors have no conflict of interest.

### References:

- [1] Asrani, S.K.; Devarbhavi, H.; Eaton, J. and Kamath, P.S. (2019): Burden of liver diseases in the world. *J Hepatol*, 70(1): 151-171.
- [2] Rehm, J.; Samokhvalov, A.V. and Shield, K.D. (2013): Global burden of alcoholic liver diseases. *J Hepatol*, 59(1): 160-168.
- [3] World Health, O. WHO. Global status report on alcohol and health 2014. Geneva: World Health Organization; 2014.
- [4] Chalasani, N.; Younossi, Z.; Lavine, J.E.; Charlton, M.; Cusi, K.; Rinella, M.; Harrison, S.A.; Brunt, E.M. and Sanyal, A.J. (2018): The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. *Hepatology*, 67(1): 328-357.
- [5] Angulo, P. (2002): Nonalcoholic fatty liver disease. *New Engl J Med*, 346(16): 1221-1231.
- [6] Brunt, E.M. (2004): Nonalcoholic steatohepatitis. *Semin Liver Dis*, 24(1): 3-20.
- [7] Cave, M.; Deaciuc, I.; Mendez, C.; Song, Z.; Joshi-Barve, S.; Barve, S. and McClain, C. (2007): Nonalcoholic fatty liver disease: predisposing factors and the role of nutrition. *J Nutr Biochem*, 18(3): 184-195.
- [8] Imai, Y.; Varela, G.M.; Jackson, M.B.; Graham, M.J.; Croke, R.M. and Ahima, R.S. (2007): Reduction of hepatosteatosis and lipid levels by an adipose differentiation-related protein antisense oligonucleotide. *Gastroenterology*, 132(5): 1947-1954.
- [9] Koruk, M.; Savas, M.C.; Yilmaz, Ö.; Taysi, S.; Karakok, M.; Gündogdu, C. and Yilmaz, A. (2003): Serum lipids, lipoproteins and apolipoproteins levels in patients with nonalcoholic

- steatohepatitis. *J Clin Gastroenterol*, 37(2): 177-182.
- [10] McCullough, A.J. (2006): Pathophysiology of nonalcoholic steatohepatitis. *J Clin Gastroenterol*, 40: S17-S29.
- [11] Clark, J.M. (2006): The epidemiology of nonalcoholic fatty liver disease in adults. *J Clin Gastroenterol*, 40: S5-S10.
- [12] Day, C.P. (2006): From fat to inflammation. *Gastroenterology*, 130(1): 207-210.
- [13] Lalor, P.F.; Faint, J.; Aarbodem, Y.; Hubscher, S.G. and Adams, D.H. (2007): The role of cytokines and chemokines in the development of steatohepatitis. *Semin Liver Dis*, 27(2): 173-193.
- [14] Bastard, J.-P.; Maachi, M.; Lagathu, C.; Kim, M.J.; Caron, M.; Vidal, H.; Capeau, J. and Feve, B. (2006): Recent advances in the relationship between obesity, inflammation, and insulin resistance. *Eur Cytokine Netw*, 17(1): 4-12.
- [15] George, J. and Liddle, C. (2008): Nonalcoholic fatty liver disease: pathogenesis and potential for nuclear receptors as therapeutic targets. *Mol Pharm*, 5(1): 49-59.
- [16] Lu, S. and Archer, M.C. (2007): Celecoxib decreases fatty acid synthase expression via down-regulation of c-Jun N-terminal kinase-1. *Exp Biol Med*, 232(5): 643-653.
- [17] Schattenberg, J.M.; Singh, R.; Wang, Y.; Lefkowitz, J.H.; Rigoli, R.M.; Scherer, P.E. and Czaja, M.J. (2006): JNK1 but not JNK2 promotes the development of steatohepatitis in mice. *Hepatology*, 43(1): 163-172.
- [18] Begriche, K.; Igoudjil, A.; Pessayre, D. and Fromenty, B. (2006): Mitochondrial dysfunction in NASH: causes, consequences and possible means to prevent it. *Mitochondrion*, 6(1): 1-28.
- [19] Diehl, A.M. (2005): Lessons from animal models of NASH. *Hepatol Res*, 33(2): 138-144.
- [20] Garcia-Ruiz, C. and Fernandez-Checa, J.C. (2006): Mitochondrial glutathione: Hepatocellular survival–death switch. *J Gastroenterol Hepatol*, 21: S3-S6.
- [21] Perumpail, B.J.; Khan, M.A.; Yoo, E.R.; Cholankeril, G.; Kim, D. and Ahmed, A. (2017): Clinical epidemiology and disease burden of nonalcoholic fatty liver disease. *World J Gastroenterol*, 23(47): 8263.
- [22] Younossi, Z.M.; Koenig, A.B.; Abdelatif, D.; Fazel, Y.; Henry, L. and Wymer, M. (2016): Global epidemiology of nonalcoholic fatty liver disease—meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology*, 64(1): 73-84.
- [23] Younossi, Z.M.; Stepanova, M.; Afendy, M.; Fang, Y.; Younossi, Y.; Mir, H. and Srishord, M. (2011): Changes in the prevalence of the most common causes of chronic liver diseases in the United States from 1988 to 2008. *Clin Gastroenterol Hepatol*, 9(6): 524-530.
- [24] Singh, S.; Allen, A.M.; Wang, Z.; Prokop, L.J.; Murad, M.H. and Loomba, R. (2015): Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. *Clin Gastroenterol Hepatol*, 13(4): 643-654.
- [25] Noureddin, M.; Yates, K.P.; Vaughn, I.A.; Neuschwander-Tetri, B.A.; Sanyal, A.J.; McCullough, A.; Merriman, R.; Hameed, B.; Doo, E. and Kleiner, D.E. (2013): Clinical and histological determinants of nonalcoholic steatohepatitis and advanced fibrosis in elderly patients. *Hepatology*, 58(5): 1644-1654.
- [26] Hamaguchi, E.; Takamura, T.; Sakurai, M.; Mizukoshi, E.; Zen, Y.; Takeshita,

- Y.; Kurita, S.; Arai, K.; Yamashita, T. and Sasaki, M. (2010): Histological course of nonalcoholic fatty liver disease in Japanese patients: tight glycemic control, rather than weight reduction, ameliorates liver fibrosis. *Diabetes Care*, 33(2): 284-286.
- [27] Wong, V.W.-S.; Wong, G.L.-H.; Choi, P.C.-L.; Chan, A.W.-H.; Li, M.K.-P.; Chan, H.-Y.; Chim, A.M.-L.; Yu, J.; Sung, J.J.-Y. and Chan, H.L.-Y. (2010): Disease progression of non-alcoholic fatty liver disease: a prospective study with paired liver biopsies at 3 years. *Gut*, 59(7): 969-974.
- [28] Argo, C.K.; Northup, P.G.; Al-Osaimi, A.M.S. and Caldwell, S.H. (2009): Systematic review of risk factors for fibrosis progression in non-alcoholic steatohepatitis. *J Hepatol*, 51(2): 371-379.
- [29] Bellentani, S.; Saccoccio, G.; Masutti, F.; Crocè, L.S.; Brandi, G.; Sasso, F.; Cristanini, G. and Tiribelli, C. (2000): Prevalence of and risk factors for hepatic steatosis in Northern Italy. *Ann Intern Med*, 132(2): 112-117.
- [30] Loomba, R.; Bettencourt, R. and Barrett-Connor, E. (2009): Synergistic association between alcohol intake and body mass index with serum alanine and aspartate aminotransferase levels in older adults: the Rancho Bernardo Study. *Aliment Pharmacol Ther*, 30(11-12): 1137-1149.
- [31] Saab, S.; Mallam, D.; Cox, G.A. and Tong, M.J. (2014): Impact of coffee on liver diseases: a systematic review. *Liver Int*, 34(4): 495-504.
- [32] Mashek, D.G. (2013): Hepatic fatty acid trafficking: multiple forks in the road. *Adv Nutr*, 4(6): 697-710.
- [33] Koo, S.-H. (2013): Nonalcoholic fatty liver disease: molecular mechanisms for the hepatic steatosis. *Clin Mol Hepatol*, CMH, 19(3): 210.
- [34] Falcon, A.; Doege, H.; Fluit, A.; Tsang, B.; Watson, N.; Kay, M.A. and Stahl, A. (2010): FATP2 is a hepatic fatty acid transporter and peroxisomal very long-chain acyl-CoA synthetase. *Am J Physiol Endocrinol Metab*, 299(3): E384-E393.
- [35] Doege, H.; Grimm, D.; Falcon, A.; Tsang, B.; Storm, T.A.; Xu, H.; Ortegon, A.M.; Kazantzis, M.; Kay, M.A. and Stahl, A. (2008): Silencing of hepatic fatty acid transporter protein 5 in vivo reverses diet-induced non-alcoholic fatty liver disease and improves hyperglycemia. *J Biol Chem*, 283(32): 22186-22192.
- [36] Silverstein, R.L. and Febbraio, M. (2009): CD36, a scavenger receptor involved in immunity, metabolism, angiogenesis, and behavior. *Sci Signal*, 2(72): re3-re3.
- [37] Wilson, C.G.; Tran, J.L.; Erion, D.M.; Vera, N.B.; Febbraio, M. and Weiss, E.J. (2016): Hepatocyte-specific disruption of CD36 attenuates fatty liver and improves insulin sensitivity in HFD-fed mice. *Endocrinology*, 157(2): 570-585.
- [38] Greco, D.; Kotronen, A.; Westerbacka, J.; Puig, O.; Arkkila, P.; Kiviluoto, T.; Laitinen, S.; Kolak, M.; Fisher, R.M. and Hamsten, A. (2008): Gene expression in human NAFLD. *Am J Physiol Gastrointest Liver Physiol*, 294(5): G1281-G1287.
- [39] Qiu, Y.; Liu, S.; Chen, H.-T.; Yu, C.-H.; Teng, X.-D.; Yao, H.-T. and Xu, G.-Q. (2013): Upregulation of caveolin-1 and SR-B1 in mice with non-alcoholic fatty liver disease. *Hepatobiliary Pancreat Dis Int*, 12(6): 630-636.
- [40] Wang, G.; Bonkovsky, H.L.; de Lemos, A. and Burczynski, F.J. (2015): Recent insights into the biological functions of liver fatty acid binding protein 1. *J Lipid Res*, 56(12): 2238-2247.

- [41] Westerbacka, J.; Kolak, M.; Kiviluoto, T.; Arkkila, P.; Sirén, J.; Hamsten, A.; Fisher, R.M. and Yki-Järvinen, H. (2007): Genes involved in fatty acid partitioning and binding, lipolysis, monocyte/macrophage recruitment, and inflammation are overexpressed in the human fatty liver of insulin-resistant subjects. *Diabetes*, 56(11): 2759-2765.
- [42] Ipsen, D.H.; Lykkesfeldt, J. and Tveden-Nyborg, P. (2018): Molecular mechanisms of hepatic lipid accumulation in non-alcoholic fatty liver disease. *Cell Mol Life Sci*, 75: 3313-3327.
- [43] Listenberger, L.L.; Han, X.; Lewis, S.E.; Cases, S.; Farese Jr, R.V.; Ory, D.S. and Schaffer, J.E. (2003): Triglyceride accumulation protects against fatty acid-induced lipotoxicity. *PNAS*, 100(6): 3077-3082.
- [44] Lambert, J.E.; Ramos-Roman, M.A.; Browning, J.D. and Parks, E.J. (2014): Increased de novo lipogenesis is a distinct characteristic of individuals with nonalcoholic fatty liver disease. *Gastroenterology*, 146(3): 726-735.
- [45] Donnelly, K.L.; Smith, C.I.; Schwarzenberg, S.J.; Jessurun, J.; Boldt, M.D. and Parks, E.J. (2005): Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J Clin Invest*, 115(5): 1343-1351.
- [46] Sanders, F.W.B. and Griffin, J.L. (2016): De novo lipogenesis in the liver in health and disease: more than just a shunting yard for glucose. *Biol Rev BIOL REV*, 91(2): 452-468.
- [47] Eberlé, D.; Hegarty, B.; Bossard, P.; Ferré, P. and Foufelle, F. (2004): SREBP transcription factors: master regulators of lipid homeostasis. *Biochimie*, 86(11): 839-848.
- [48] Kohjima, M.; Enjoji, M.; Higuchi, N.; Kato, M.; Kotoh, K.; Yoshimoto, T.; Fujino, T.; Yada, M.; Yada, R. and Harada, N. (2007): Re-evaluation of fatty acid metabolism-related gene expression in nonalcoholic fatty liver disease. *Int J Mol Med*, 20(3): 351-358.
- [49] Liang, G.; Yang, J.; Horton, J.D.; Hammer, R.E.; Goldstein, J.L. and Brown, M.S. (2002): Diminished hepatic response to fasting/refeeding and liver X receptor agonists in mice with selective deficiency of sterol regulatory element-binding protein-1c. *J Biol Chem*, 277(11): 9520-9528.
- [50] Uyeda, K. and Repa, J.J. (2006): Carbohydrate response element binding protein, ChREBP, a transcription factor coupling hepatic glucose utilization and lipid synthesis. *Cell Metab*, 4(2): 107-110.
- [51] Iizuka, K.; Bruick, R.K.; Liang, G.; Horton, J.D. and Uyeda, K. (2004): Deficiency of carbohydrate response element-binding protein (ChREBP) reduces lipogenesis as well as glycolysis. *PNAS*, 101(19): 7281-7286.
- [52] Zhang, D.; Tong, X.; VanDommelen, K.; Gupta, N.; Stamper, K.; Brady, G.F.; Meng, Z.; Lin, J.; Rui, L. and Omary, M.B. (2017): Lipogenic transcription factor ChREBP mediates fructose-induced metabolic adaptations to prevent hepatotoxicity. *J Clin Invest*, 127(7): 2855-2867.
- [53] Ducheix, S.; Vegliante, M.C.; Villani, G.; Napoli, N.; Sabbà, C. and Moschetta, A. (2016): Is hepatic lipogenesis fundamental for NAFLD/NASH? A focus on the nuclear receptor coactivator PGC-1 $\beta$ . *Cell Mol Life Sci*, 73(20): 3809-3822.
- [54] Mao, J.; DeMayo, F.J.; Li, H.; Abu-Elheiga, L.; Gu, Z.; Shaikenov, T.E.; Kordari, P.; Chirala, S.S.; Heird, W.C. and Wakil, S.J. (2006): Liver-specific deletion of acetyl-CoA carboxylase 1 reduces hepatic triglyceride



- accumulation without affecting glucose homeostasis. PNAS, 103(22): 8552-8557.
- [55] Benhamed, F.; Denechaud, P.-D.; Lemoine, M.; Robichon, C.; Moldes, M.; Bertrand-Michel, J.; Ratziu, V.; Serfaty, L.; Housset, C. and Capeau, J. (2012): The lipogenic transcription factor ChREBP dissociates hepatic steatosis from insulin resistance in mice and humans. *J Clin Invest*, 122(6): 2176-2194.
- [56] Kitade, H.; Chen, G.; Ni, Y. and Ota, T. (2017): Nonalcoholic fatty liver disease and insulin resistance: new insights and potential new treatments. *Nutrients*, 9(4): 387.
- [57] Stefan, N.; Staiger, H. and Häring, H.U. (2011): Dissociation between fatty liver and insulin resistance: the role of adipose triacylglycerol lipase. *Diabetologia*, 54: 7-9.
- [58] Walenbergh, S.M.A. and Shiri-Sverdlov, R. (2015): Cholesterol is a significant risk factor for non-alcoholic steatohepatitis. *Expert Rev Gastroenterol Hepatol*, 9(11): 1343-1346.
- [59] Reddy, J.K. and Hashimoto, T. (2001): Peroxisomal  $\beta$ -oxidation and peroxisome proliferator-activated receptor  $\alpha$ : an adaptive metabolic system. *Annu Rev Nutr*, 21(1): 193-230.
- [60] Rao, M.S. and Reddy, J.K. (2001): Peroxisomal beta-oxidation and steatohepatitis. *Semin Liver Dis*, 21(1): 43-55.
- [61] Nassir, F. and Ibdah, J.A. (2014): Role of mitochondria in nonalcoholic fatty liver disease. *Int J Mol Sci*, 15(5): 8713-8742.
- [62] Reddy, J.K. and Sambasiva Rao, M. (2006): Lipid metabolism and liver inflammation. II. Fatty liver disease and fatty acid oxidation. *Am J Physiol Gastrointest Liver Physiol*, 290(5): G852-G858.
- [63] Lee, S.S.; Pineau, T.; Drago, J.; Lee, E.J.; Owens, J.W.; Kroetz, D.L.; Fernandez-Salguero, P.M.; Westphal, H. and Gonzalez, F.J. (1995): Targeted disruption of the alpha isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. *Mol Cell Biol*, 15(6): 3012-3022.
- [64] Fujita, K.; Nozaki, Y.; Wada, K.; Yoneda, M.; Fujimoto, Y.; Fujitake, M.; Endo, H.; Takahashi, H.; Inamori, M. and Kobayashi, N. (2009): Dysfunctional very-low-density lipoprotein synthesis and release is a key factor in nonalcoholic steatohepatitis pathogenesis. *Hepatology*, 50(3): 772-780.
- [65] Francque, S.; Verrijken, A.; Caron, S.; Prawitt, J.; Paumelle, R.; Derudas, B.; Lefebvre, P.; Taskinen, M.-R.; Van Hul, W. and Mertens, I. (2015): PPAR $\alpha$  gene expression correlates with severity and histological treatment response in patients with non-alcoholic steatohepatitis. *J Hepatol*, 63(1): 164-173.
- [66] Videla, L.A. and Pettinelli, P. (2012): Misregulation of PPAR functioning and its pathogenic consequences associated with nonalcoholic fatty liver disease in human obesity. *PPAR res*, 2012.
- [67] von Loeffelholz, C.; Doecke, S.; Lock, J.F.; Lieske, S.; Horn, P.; Kriebel, J.; Wahl, S.; Singmann, P.; de las Heras Gala, T. and Grallert, H. (2017): Increased lipogenesis in spite of upregulated hepatic 5'AMP-activated protein kinase in human non-alcoholic fatty liver. *Hepatol Res*, 47(9): 890-901.
- [68] Sanyal, A.J.; Campbell-Sargent, C.; Mirshahi, F.; Rizzo, W.B.; Contos, M.J.; Sterling, R.K.; Luketic, V.A.; Shiffman, M.L. and Clore, J.N. (2001): Nonalcoholic steatohepatitis: association

- of insulin resistance and mitochondrial abnormalities. *Gastroenterology*, 120(5): 1183-1192.
- [69] Begrich, K.; Massart, J.; Robin, M.A.; Bonnet, F. and Fromenty, B. (2013): Mitochondrial adaptations and dysfunctions in nonalcoholic fatty liver disease. *Hepatology*, 58(4): 1497-1507.
- [70] Koliaki, C.; Szendroedi, J.; Kaul, K.; Jelenik, T.; Nowotny, P.; Jankowiak, F.; Herder, C.; Carstensen, M.; Krausch, M. and Knoefel, W.T. (2015): Adaptation of hepatic mitochondrial function in humans with non-alcoholic fatty liver is lost in steatohepatitis. *Cell Metab*, 21(5): 739-746.
- [71] Rector, R.S.; Morris, E.M.; Ridenhour, S.; Meers, G.M.; Hsu, F.F.; Turk, J. and Ibdah, J.A. (2013): Selective hepatic insulin resistance in a murine model heterozygous for a mitochondrial trifunctional protein defect. *Hepatology*, 57(6): 2213-2223.
- [72] Dirx, R.; Vanhorebeek, I.; Martens, K.; Schad, A.; Grabenbauer, M.; Fahimi, D.; Declercq, P.; Van Veldhoven, P.P. and Baes, M. (2005): Absence of peroxisomes in mouse hepatocytes causes mitochondrial and ER abnormalities. *Hepatology*, 41(4): 868-878.
- [73] Perry, R.J.; Samuel, V.T.; Petersen, K.F. and Shulman, G.I. (2014): The role of hepatic lipids in hepatic insulin resistance and type 2 diabetes. *Nature*, 510(7503): 84-91.
- [74] Fabbrini, E.; Mohammed, B.S.; Magkos, F.; Korenblat, K.M.; Patterson, B.W. and Klein, S. (2008): Alterations in adipose tissue and hepatic lipid kinetics in obese men and women with nonalcoholic fatty liver disease. *Gastroenterology*, 134(2): 424-431.
- [75] Kawano, Y. and Cohen, D.E. (2013): Mechanisms of hepatic triglyceride accumulation in non-alcoholic fatty liver disease. *J Gastroenterol*, 48: 434-441.
- [76] Fabbrini, E. and Magkos, F. (2015): Hepatic steatosis as a marker of metabolic dysfunction. *Nutrients*, 7(6): 4995-5019.
- [77] Tanoli, T.; Yue, P.; Yablonskiy, D. and Schonfeld, G. (2004): Fatty liver in familial hypobetalipoproteinemia: roles of the APOB defects, intra-abdominal adipose tissue, and insulin sensitivity. *J Lipid Res*, 45(5): 941-947.
- [78] Zhang, X.-Q.; Xu, C.-F.; Yu, C.-H.; Chen, W.-X. and Li, Y.-M. (2014): Role of endoplasmic reticulum stress in the pathogenesis of nonalcoholic fatty liver disease. *World J Gastroenterol*, 20(7): 1768.
- [79] Améen, C.; Edvardsson, U.; Ljungberg, A.; Asp, L.; Åkerblad, P.; Tuneld, A.; Olofsson, S.-O.; Lindén, D. and Oscarsson, J. (2005): Activation of peroxisome proliferator-activated receptor  $\alpha$  increases the expression and activity of microsomal triglyceride transfer protein in the liver. *J Biol Chem*, 280(2): 1224-1229.
- [80] Tessari, P.; Coracina, A.; Cosma, A. and Tiengo, A. (2009): Hepatic lipid metabolism and non-alcoholic fatty liver disease. *Nutr Metab Cardiovasc Dis*, 19(4): 291-302.
- [81] Adiels, M.; Taskinen, M.R.; Packard, C.; Caslake, M.J.; Soro-Paavonen, A.; Westerbacka, J.; Vehkavaara, S.; Häkkinen, A.; Olofsson, S.O. and Yki-Järvinen, H. (2006): Overproduction of large VLDL particles is driven by increased liver fat content in man. *Diabetologia*, 49: 755-765.
- [82] Fabbrini, E.; Magkos, F.; Mohammed, B.S.; Pietka, T.; Abumrad, N.A.; Patterson, B.W.; Okunade, A. and Klein, S. (2009): Intrahepatic fat, not visceral fat, is linked with metabolic

- complications of obesity. PNAS, 106(36): 15430-15435.
- [83] Magkos, F.; Fabbrini, E.; Mohammed, B.S.; Patterson, B.W. and Klein, S. (2010): Increased whole-body adiposity without a concomitant increase in liver fat is not associated with augmented metabolic dysfunction. *Obesity*, 18(8): 1510-1515.
- [84] Horton, J.D.; Shimano, H.; Hamilton, R.L.; Brown, M.S. and Goldstein, J.L. (1999): Disruption of LDL receptor gene in transgenic SREBP-1a mice unmasks hyperlipidemia resulting from production of lipid-rich VLDL. *J Clin Invest*, 103(7): 1067-1076.
- [85] Higuchi, N.; Kato, M.; Tanaka, M.; Miyazaki, M.; Takao, S.; Kohjima, M.; Kotoh, K.; Enjoji, M.; Nakamuta, M. and Takayanagi, R. (2011): Effects of insulin resistance and hepatic lipid accumulation on hepatic mRNA expression levels of apoB, MTP and L-FABP in non-alcoholic fatty liver disease. *Exp Ther Med*, 2(6): 1077-1081.
- [86] Charlton, M.; Sreekumar, R.; Rasmussen, D.; Lindor, K. and Nair, K.S. (2002): Apolipoprotein synthesis in nonalcoholic steatohepatitis. *Hepatology*, 35(4): 898-904.
- [87] Matteoni, C.A.; Younossi, Z.M.; Gramlich, T.; Boparai, N.; Liu, Y.C. and McCullough, A.J. (1999): Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology*, 116(6): 1413-1419.
- [88] Tiniakos, D.G.; Vos, M.B. and Brunt, E.M. (2010): Nonalcoholic fatty liver disease: pathology and pathogenesis. *Annu Rev Pathol*, 5: 145-171.
- [89] Schwimmer, J.B.; Behling, C.; Newbury, R.; Deutsch, R.; Nievergelt, C.; Schork, N.J. and Lavine, J.E. (2005): Histopathology of pediatric nonalcoholic fatty liver disease. *Hepatology*, 42(3): 641-649.
- [90] Angulo, P.; Kleiner, D.E.; Dam-Larsen, S.; Adams, L.A.; Bjornsson, E.S.; Charatcharoenwittaya, P.; Mills, P.R.; Keach, J.C.; Lafferty, H.D. and Stahler, A. (2015): Liver fibrosis, but no other histologic features, is associated with long-term outcomes of patients with nonalcoholic fatty liver disease. *Gastroenterology*, 149(2): 389-397.
- [91] Ludwig, J.; Viggiano, T.R.; McGill, D.B. and Oh, B.J. (1980). Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. *Mayo Clin Proc*, 55(7): 434-438.
- [92] Sanyal, A.J.; Brunt, E.M.; Kleiner, D.E.; Kowdley, K.V.; Chalasani, N.; Lavine, J.E.; Ratziu, V. and McCullough, A. Endpoints and clinical trial design for nonalcoholic steatohepatitis. Wiley Online Library; 2011.
- [93] Brunt, E.M.; Kleiner, D.E.; Carpenter, D.H.; Rinella, M.; Harrison, S.A.; Loomba, R.; Younossi, Z.; Neuschwander-Tetri, B.A.; Sanyal, A.J. and American Association for the Study of Liver Diseases, N.T.F. (2021): NAFLD: reporting histologic findings in clinical practice. *Hepatology*, 73(5): 2028-2038.
- [94] Bedossa, P.; Poitou, C.; Veyrie, N.; Bouillot, J.L.; Basdevant, A.; Paradis, V.; Tordjman, J. and Clement, K. (2012): Histopathological algorithm and scoring system for evaluation of liver lesions in morbidly obese patients. *Hepatology*, 56(5): 1751-1759.
- [95] Lackner, C.; Gogg-Kamerer, M.; Zatloukal, K.; Stumptner, C.; Brunt, E.M. and Denk, H. (2008): Ballooned hepatocytes in steatohepatitis: the value of keratin immunohistochemistry for diagnosis. *J Hepatol*, 48(5): 821-828.
- [96] Asrani, S.K.; Larson, J.J.; Yawn, B.; Therneau, T.M. and Kim, W.R. (2013): Underestimation of liver-related

- mortality in the United States. *Gastroenterology*, 145(2): 375-382.
- [97] Wanless, I.R.; Nakashima, E. and Sherman, M. (2000): Regression of human cirrhosis: morphologic features and the genesis of incomplete septal cirrhosis. *Arch Pathol Lab Med*, 124(11): 1599-1607.
- [98] Elsharkawy, A.M.; Oakley, F. and Mann, D.A. (2005): The role and regulation of hepatic stellate cell apoptosis in reversal of liver fibrosis. *Apoptosis*, 10: 927-939.
- [99] Goh, J.G.B.-B. and Arthur J. McCullough. (2016): Natural History of Nonalcoholic Fatty Liver Disease *Dig Dis Sci*, 61: 1226–1233.
- [100] Bhanji, R.A.; Narayanan, P.; Allen, A.M.; Malhi, H. and Watt, K.D. (2017): Sarcopenia in hiding: the risk and consequence of underestimating muscle dysfunction in nonalcoholic steatohepatitis. *Hepatology*, 66(6): 2055-2065.
- [101] Gan, D.; Wang, L.; Jia, M.; Ru, Y.; Ma, Y.; Zheng, W.; Zhao, X.; Yang, F.; Wang, T. and Mu, Y. (2020): Low muscle mass and low muscle strength associate with nonalcoholic fatty liver disease. *Clin Nutr*, 39(4): 1124-1130.
- [102] Chen, Z.; Yu, Y.; Cai, J. and Li, H. (2019): Emerging molecular targets for treatment of nonalcoholic fatty liver disease. *Trends Endocrinol Metab*, 30(12): 903-914.
- [103] Zhang, L.; Wang, X.; Cueto, R.; Effi, C.; Zhang, Y.; Tan, H.; Qin, X.; Ji, Y.; Yang, X. and Wang, H. (2019): Biochemical basis and metabolic interplay of redox regulation. *Redox Biol*, 26: 101284.
- [104] Sies, H. (2015): Oxidative stress: a concept in redox biology and medicine. *Redox Biol*, 4: 180-183.
- [105] Brunt, E.M.; Wong, V.W.S.; Nobili, V.; Day, C.P.; Sookoian, S.; Maher, J.J.; Bugianesi, E.; Sirlin, C.B.; Neuschwander-Tetri, B.A. and Rinella, M.E. (2015): Nonalcoholic fatty liver disease. *Nat Rev Dis Primers*, 1(1): 1-22.
- [106] Friedman, S.L.; Neuschwander-Tetri, B.A.; Rinella, M. and Sanyal, A.J. (2018): Mechanisms of NAFLD development and therapeutic strategies. *Nat Med*, 24(7): 908-922.
- [107] Gonzalez, A.; Huerta-Salgado, C.; Orozco-Aguilar, J.; Aguirre, F.; Tacchi, F.; Simon, F. and Cabello-Verrugio, C. (2020): Role of oxidative stress in hepatic and extrahepatic dysfunctions during nonalcoholic fatty liver disease (NAFLD). *Oxid Med Cell Longev*, 2020.
- [108] Takaki, A.; Kawai, D. and Yamamoto, K. (2013): Multiple hits, including oxidative stress, as pathogenesis and treatment target in non-alcoholic steatohepatitis (NASH). *Int J Mol Sci*, 14(10): 20704-20728.
- [109] Spahis, S.; Delvin, E.; Borys, J.-M. and Levy, E. (2017): Oxidative stress as a critical factor in nonalcoholic fatty liver disease pathogenesis. *Antioxid Redox Signal*, 26(10): 519-541.
- [110] Dallio, M.; Diano, N.; Masarone, M.; Gravina, A.G.; Patanè, V.; Romeo, M.; Di Sarno, R.; Errico, S.; Nicolucci, C. and Abenavoli, L. (2019): Chemical effect of bisphenol A on non-alcoholic fatty liver disease. *Int J Env Res Public Health*, 16(17): 3134.
- [111] Strakovsky, R.S.; Wang, H.; Engeseth, N.J.; Flaws, J.A.; Helferich, W.G.; Pan, Y.-X. and Lezmi, S. (2015): Developmental bisphenol A (BPA) exposure leads to sex-specific modification of hepatic gene expression and epigenome at birth that may exacerbate high-fat diet-induced hepatic steatosis. *Toxicol Appl Pharmacol*, 284(2): 101-112.
- [112] Assarrar, I.; Messaoudi, N.; Ongoth, F.E.M.; Abdellaoui, W.; Malki, A.;

- Rouf, S.; Abda, N.; Ismaili, Z. and Latrech, H. (2022): Prevalence and Specific Manifestations of Non-alcoholic Fatty Liver Disease (NAFLD) and Diabetes Mellitus Type 2 Association in a Moroccan Population: A Cross-sectional Study. *Rev Diabet Stud*, 18(3): 140-145.
- [113] Franco, L.; Jones-Pauley, M.; Tamimi, O.; Neshatian, L.; Nguyen, D.; Graviss, E.; Quigley, E.M.M. and Victor Iii, D. (2022): Irritable Bowel Syndrome Symptoms in Nonalcoholic Fatty Liver Disease Patients Are an Indicator of Depression and Anxiety. *J Clin Gastroenterol*: 10-1097.
- [114] Islam, A.S.M.M.; Muttalib, M.A.; Islam, M.N.; Haque, M.R. and Hoque, M.R. (2022): Study of Serum Lipid Profile and Aminotransferases (ALT and AST) in Non-obese, Non-diabetic Nonalcoholic Fatty Liver Disease. *J Rangpu Med Coll*, 7(2): 35-39.
- [115] El Amrousy, D.; Abdelhai, D. and Shawky, D. (2022): Vitamin D and nonalcoholic fatty liver disease in children: a randomized controlled clinical trial. *Eur J Pediatr*, 181(2): 579-586.
- [116] Ryan, C.K.; Johnson, L.A.; Germin, B.I. and Marcos, A. (2002): One hundred consecutive hepatic biopsies in the workup of living donors for right lobe liver transplantation. *Liver Transpl*, 8(12): 1114-1122.
- [117] Dasarathy, S.; Dasarathy, J.; Khiyami, A.; Joseph, R.; Lopez, R. and McCullough, A.J. (2009): Validity of real time ultrasound in the diagnosis of hepatic steatosis: a prospective study. *J Hepatol*, 51(6): 1061-1067.
- [118] Roeb, E.; Steffen, H.-M.; Bantel, H.; Baumann, U.; Canbay, A.; Demir, M.; Drebber, U.; Geier, A.; Hampe, J. and Hellerbrand, C. (2015): S2k-Leitlinie nicht alkoholische Fettlebererkrankungen. *Z Gastroenterol*, 53(07): 668-723.
- [119] Bohte, A.E.; Van Werven, J.R.; Bipat, S. and Stoker, J. (2011): The diagnostic accuracy of US, CT, MRI and 1 H-MRS for the evaluation of hepatic steatosis compared with liver biopsy: a meta-analysis. *Eur Radiol*, 21: 87-97.
- [120] Iwasaki, M.; Takada, Y.; Hayashi, M.; Minamiguchi, S.; Haga, H.; Maetani, Y.; Fujii, K.; Kiuchi, T. and Tanaka, K. (2004): Noninvasive evaluation of graft steatosis in living donor liver transplantation. *Transplantation*, 78(10): 1501-1505.
- [121] Hyodo, T.; Yada, N.; Hori, M.; Maenishi, O.; Lamb, P.; Sasaki, K.; Onoda, M.; Kudo, M.; Mochizuki, T. and Murakami, T. (2017): Multimaterial decomposition algorithm for the quantification of liver fat content by using fast-kilovolt-peak switching dual-energy CT: clinical evaluation. *Radiology*, 283(1): 108-118.
- [122] Furlan, A.; Tublin, M.E.; Yu, L.; Chopra, K.B.; Lippello, A. and Behari, J. (2020): Comparison of 2D shear wave elastography, transient elastography, and MR elastography for the diagnosis of fibrosis in patients with nonalcoholic fatty liver disease. *Am J Roentgenol*, 214(1): W20-W26.
- [123] Hsu, C.; Caussy, C.; Imajo, K.; Chen, J.; Singh, S.; Kaulback, K.; Le, M.-D.; Hooker, J.; Tu, X. and Bettencourt, R. (2019): Magnetic resonance vs transient elastography analysis of patients with nonalcoholic fatty liver disease: a systematic review and pooled analysis of individual participants. *Clin Gastroenterol Hepatol*, 17(4): 630-637.
- [124] Choi, S.J.; Kim, S.M.; Kim, Y.S.; Kwon, O.S.; Shin, S.K.; Kim, K.K.; Lee, K.; Park, I.B.; Choi, C.S. and Chung, D.H. (2021): Magnetic resonance-based assessments better capture

- pathophysiologic profiles and progression in nonalcoholic fatty liver disease. *Diabetes Metab J*, 45(5): 739-752.
- [125] Lee, Y.-S.; Yoo, Y.J.; Jung, Y.K.; Kim, J.H.; Seo, Y.S.; Yim, H.J.; Kim, I.H.; Lee, S.Y.; Kim, B.-H. and Kim, J.W. (2020): Multiparametric MR is a valuable modality for evaluating disease severity of nonalcoholic fatty liver disease. *Clin Transl Gastroenterol*, 11(4).
- [126] Lupattelli, G.; Marchesi, S.; Lombardini, R.; Roscini, A.R.; Trinca, F.; Gemelli, F.; Vaudo, G. and Mannarino, E. (2004): Artichoke juice improves endothelial function in hyperlipemia. *Life Sci*, 76(7): 775-782.
- [127] Kalvandi, R.; Rajabi, M.; Kahramfar, Z. and Chaleh Cheleh, T. (2020): Investigation of the effect of artichoke (*Cynara Scolymus L.*) on characteristics of the fatty liver. *CompMediJ*, 10(2): 134-147.
- [128] Targher, G.; Bertolini, L.; Scala, L.; Cigolini, M.; Zenari, L.; Falezza, G. and Arcaro, G. (2007): Associations between serum 25-hydroxyvitamin D3 concentrations and liver histology in patients with non-alcoholic fatty liver disease. *Nutr Metab Cardiovasc Dis*, 17(7): 517-524.
- [129] Flanagan, J.L.; Simmons, P.A.; Vehige, J.; Willcox, M.D.P. and Garrett, Q. (2010): Role of carnitine in disease. *Nutr Metab (Lond)*, 7: 1-14.
- [130] Malaguarnera, M.; Gargante, M.P.; Russo, C.; Antic, T.; Vacante, M.; Malaguarnera, M.; Avitabile, T.; Volti, G.L. and Galvano, F. (2010): L-carnitine supplementation to diet: a new tool in treatment of nonalcoholic steatohepatitis—a randomized and controlled clinical trial. *Am J Gastroenterol*, 105(6): 1338-1345.
- [131] Somi, M.H.; Fatahi, E.; Panahi, J. and Havasian, M.R. (2014): Data from a randomized and controlled trial of LCarnitine prescription for the treatment for Non-Alcoholic Fatty Liver Disease. *Bioinformation*, 10(9): 575.
- [132] Abdelmalek, M.F.; Sanderson, S.O.; Angulo, P.; Soldevila-Pico, C.; Liu, C.; Peter, J.; Keach, J.; Cave, M.; Chen, T. and McClain, C.J. (2009): Betaine for nonalcoholic fatty liver disease: results of a randomized placebo-controlled trial. *Hepatology*, 50(6): 1818-1826.
- [133] Hyogo, H.; Yamagishi, S.-i.; Maeda, S.; Kimura, Y.; Ishitobi, T. and Chayama, K. (2012): Atorvastatin improves disease activity of nonalcoholic steatohepatitis partly through its tumour necrosis factor- $\alpha$ -lowering property. *Dig Liver Dis*, 44(6): 492-496.
- [134] Buckley, N.A.; Buckley, N.; Whyte, I.M.; O'Connell, D.L. and Dawson, A.H. (1999): Oral or intravenous N-acetylcysteine: which is the treatment of choice for acetaminophen (paracetamol) poisoning? *J Toxicol: Clin Toxicol*, 37(6): 759-767.
- [135] Ronis, M.J.J.; Butura, A.; Sampey, B.P.; Shankar, K.; Prior, R.L.; Korourian, S.; Albano, E.; Ingelman-Sundberg, M.; Petersen, D.R. and Badger, T.M. (2005): Effects of N-acetylcysteine on ethanol-induced hepatotoxicity in rats fed via total enteral nutrition. *Free Radical Biol Med*, 39(5): 619-630.
- [136] Hasan, S. (2015): A review on nanoparticles: their synthesis and types. *Res J Recent Sci*, 2277: 2502.
- [137] Klaus, T.; Joerger, R.; Olsson, E. and Granqvist, C.-G. (1999): Silver-based crystalline nanoparticles, microbially fabricated. *PNAS*, 96(24): 13611-13614.
- [138] Zhang, L.; Gu, F.X.; Chan, J.M.; Wang, A.Z.; Langer, R.S. and Farokhzad, O.C. (2008): Nanoparticles in medicine: therapeutic applications and developments. *Clin Pharmacol Ther*, 83(5): 761-769.

- [139] Zhang, L.; Pornpattananankul, D.; Hu, C.M. and Huang, C.M. (2010): Development of nanoparticles for antimicrobial drug delivery. *Curr Med Chem*, 17(6): 585-594.
- [140] Singh, S.; Pandey, V.K.; Tewari, R.P. and Agarwal, V. (2011): Nanoparticle based drug delivery system: advantages and applications. *Indian J Sci Technol*, 4(3): 177-180.
- [141] Sajja, H.K.; East, M.P.; Mao, H.; Wang, Y.A.; Nie, S. and Yang, L. (2009): Development of multifunctional nanoparticles for targeted drug delivery and noninvasive imaging of therapeutic effect. *Curr Drug Disc Technol*, 6(1): 43-51.
- [142] Vineela, C.H. and Krishna, S.K. (2014): Preparation of ibuprofen-loaded Eudragit S100 nanoparticles by solvent evaporation technique. *IJPSR*, 5(7): 375-384.
- [143] Pridgen, E.M.; Alexis, F. and Farokhzad, O.C. (2014): Polymeric nanoparticle technologies for oral drug delivery. *Clin Gastroenterol Hepatol*, 12(10): 1605-1610.
- [144] Pridgen, E.M.; Alexis, F.; Kuo, T.T.; Levy-Nissenbaum, E.; Karnik, R.; Blumberg, R.S.; Langer, R. and Farokhzad, O.C. (2013): Transepithelial transport of Fc-targeted nanoparticles by the neonatal fc receptor for oral delivery. *Sci Transl Med*, 5(213): 213ra167-213ra167.
- [145] Surai, P.F. (2015): Silymarin as a natural antioxidant: an overview of the current evidence and perspectives. *Antioxidants*, 4(1): 204-247.
- [146] Hamza, R.Z. and Al-Harbi, M.S. (2015): Amelioration of paracetamol hepatotoxicity and oxidative stress on mice liver with silymarin and *Nigella sativa* extract supplements. *Asian Pac J Trop Biomed*, 5(7): 521-531.
- [147] Evren, E. and Yurtcu, E. (2015): In vitro effects on biofilm viability and antibacterial and antiadherent activities of silymarin. *Folia Microbiol*, 60: 351-356.
- [148] Blumenthal, M.; Goldberg, A. and Brinckmann, J. Herbal medicine. Expanded commission E monographs: Integrative Medicine Communications; 2000.
- [149] Wu, J.-W.; Lin, L.-C.; Hung, S.-C.; Chi, C.-W. and Tsai, T.-H. (2007): Analysis of silibinin in rat plasma and bile for hepatobiliary excretion and oral bioavailability application. *J Pharm Biomed Anal*, 45(4): 635-641.
- [150] Yang, X.-Z.; Dou, S.; Wang, Y.-C.; Long, H.-Y.; Xiong, M.-H.; Mao, C.-Q.; Yao, Y.-D. and Wang, J. (2012): Single-step assembly of cationic lipid-polymer hybrid nanoparticles for systemic delivery of siRNA. *ACS Nano*, 6(6): 4955-4965.
- [151] Jo, S.-H.; Ha, K.-S.; Moon, K.-S.; Kim, J.-G.; Oh, C.-G.; Kim, Y.-C.; Apostolidis, E. and Kwon, Y.-I. (2013): Molecular weight dependent glucose lowering effect of low molecular weight chitosan oligosaccharide (GO2KA1) on postprandial blood glucose level in SD rats model. *Int J Mol Sci*, 14(7): 14214-14224.
- [152] Jo, S.-H.; Ha, K.-S.; Lee, J.-W.; Kim, Y.-C.; Apostolidis, E. and Kwon, Y.-I. (2014): The reduction effect of low molecular weight chitosan oligosaccharide (GO2KA1) on postprandial blood glucose levels in healthy individuals. *Food Sci Biotechnol*, 23: 971-973.
- [153] Kang, N.-H.; Lee, W.K.; Yi, B.-R.; Park, M.-A.; Lee, H.-R.; Park, S.-K.; Hwang, K.-A.; Park, H.K. and Choi, K.-C. (2012): Modulation of lipid metabolism by mixtures of protamine and chitooligosaccharide through pancreatic

- lipase inhibitory activity in a rat model. *Lab Anim Res*, 28(1): 31-38.
- [154] Liao, A.-H.; Ma, W.-C. and Wu, M.-F. (2013): Evaluation of ultrasound combined with chitosan for the control of weight and local fat in mice. *Ultrasound Med Biol*, 39(10): 1794-1803.
- [155] Li, J.Z.; Huang, Y.; Karaman, R.; Ivanova, P.T.; Brown, H.A.; Roddy, T.; Castro-Perez, J.; Cohen, J.C. and Hobbs, H.H. (2012): Chronic overexpression of PNPLA3 I148M in mouse liver causes hepatic steatosis. *J Clin Invest*, 122(11): 4130-4144.
- [156] Abdelhakim, M.; Elsayed, A. and Alkhulaqi, T. (2014): Activity and stability of immobilized *Candida rugosa* lipase on chitosan coated Fe<sub>3</sub>O<sub>4</sub> nanoparticles in aqueous and organic media. *JAC*, 10(3): 2478-2483.
- [157] Abdelhakim, M.; Ramadan, M. and Basaad, F. (2016): Removing of heavy metals from water by chitosan nanoparticles. *JAC*, 11: 3765-3771.
- [158] Abd-Elhakeem, M.A.; Farag, N. and Maurice, M. (2016): Effects of dietary chitosan nanoparticles on serum lipid concentration in hyperlipidemic rats induced by a high-fat diet. *EJAPS*, 54(4): 17-21.
- [159] Yan, Y.; Wanshun, L.; Baoqin, H.; Bing, L. and Chenwei, F. (2006): Protective effects of chitosan oligosaccharide and its derivatives against carbon tetrachloride-induced liver damage in mice. *Hepatol Res*, 35(3): 178-184.
- [160] Dowding, J.M.; Dosani, T.; Kumar, A.; Seal, S. and Self, W.T. (2012): Cerium oxide nanoparticles scavenge nitric oxide radical ( $\cdot$ NO). *ChemComm*, 48(40): 4896-4898.
- [161] Heckert, E.G.; Seal, S. and Self, W.T. (2008): Fenton-like reaction catalyzed by the rare earth inner transition metal cerium. *Environ Sci Technol*, 42(13): 5014-5019.
- [162] Oró, D.; Yudina, T.; Fernández-Varo, G.; Casals, E.; Reichenbach, V.; Casals, G.; de la Presa, B.G.; Sandalinas, S.; Carvajal, S. and Puentes, V. (2016): Cerium oxide nanoparticles reduce steatosis, portal hypertension and display anti-inflammatory properties in rats with liver fibrosis. *J Hepatol*, 64(3): 691-698.
- [163] Oostingh, G.J.; Casals, E.; Italiani, P.; Colognato, R.; Stritzinger, R.; Ponti, J.; Pfaller, T.; Kohl, Y.; Ooms, D. and Favilli, F. (2011): Problems and challenges in the development and validation of human cell-based assays to determine nanoparticle-induced immunomodulatory effects. *Part Fibre Toxicol*, 8(1): 1-21.
- [164] Ibrahim, H.G.; Attia, N.; Fatma El Zahraa, A.H. and El Heneidy, M.A.R. (2018): Cerium oxide nanoparticles: In pursuit of liver protection against doxorubicin-induced injury in rats. *Biomed Pharmacother*, 103: 773-781.
- [165] Adebayo, O.A.; Akinloye, O. and Adaramoye, O.A. (2020): Cerium oxide nanoparticles attenuate oxidative stress and inflammation in the liver of diethylnitrosamine-treated mice. *Biol Trace Elem Res*, 193: 214-225.



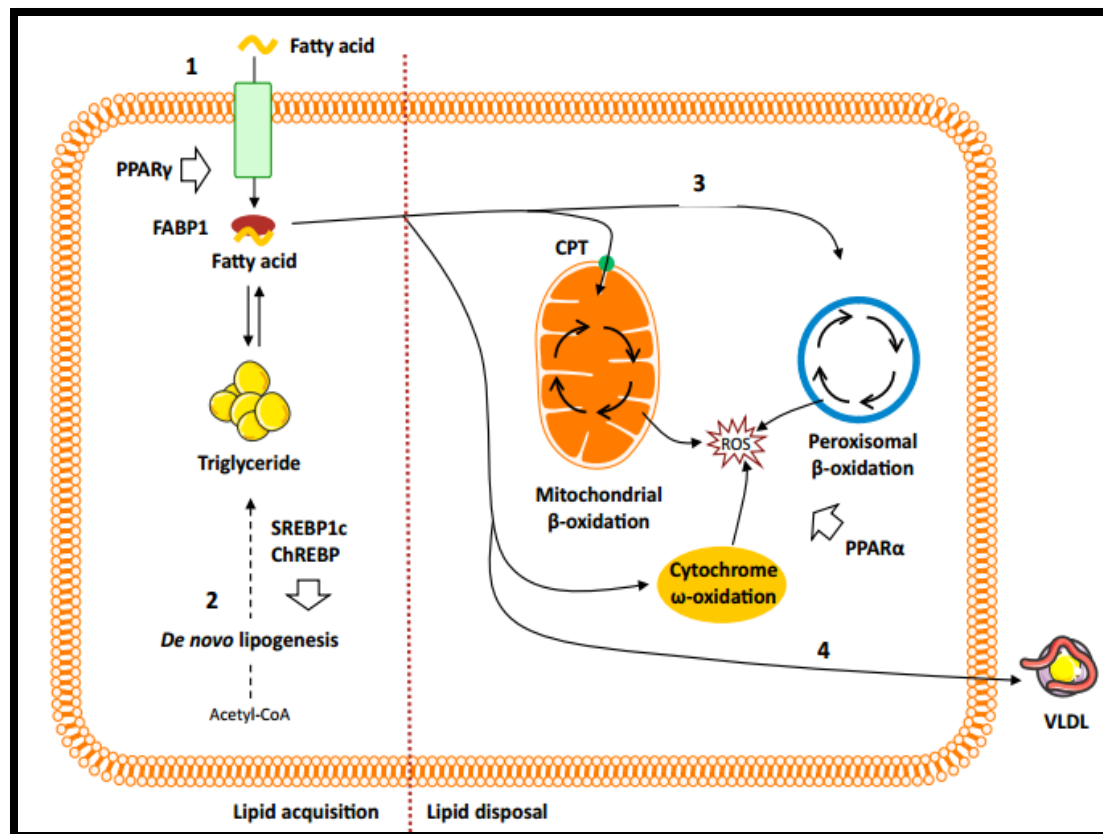
## الملخص العربي

### بعض الدراسات البيولوجية الجزيئية على متلازمة الكبد الدهنية

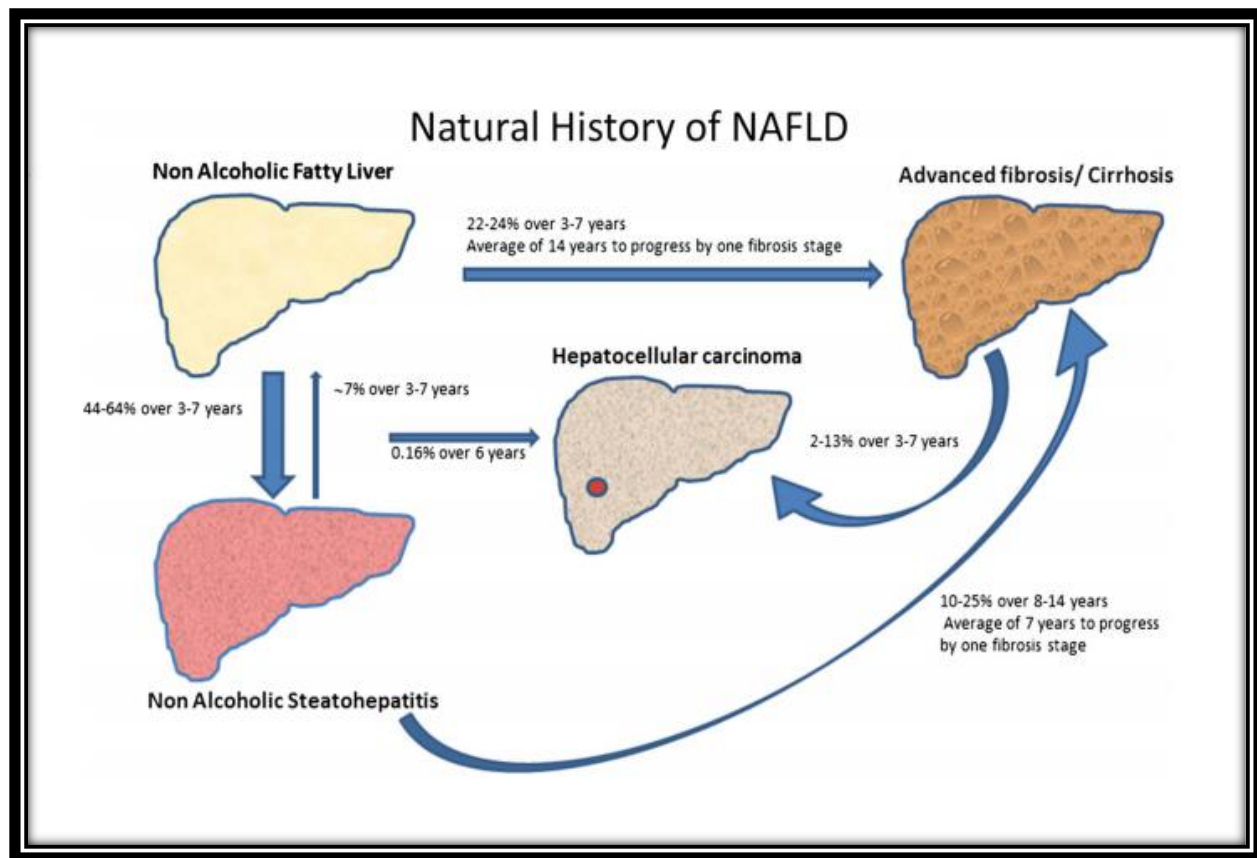
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قسم الكيمياء الحيوية والبيولوجيا الجزيئية، كلية الطب البيطري، جامعة الزقازيق. زقازيق 44519, مصر.

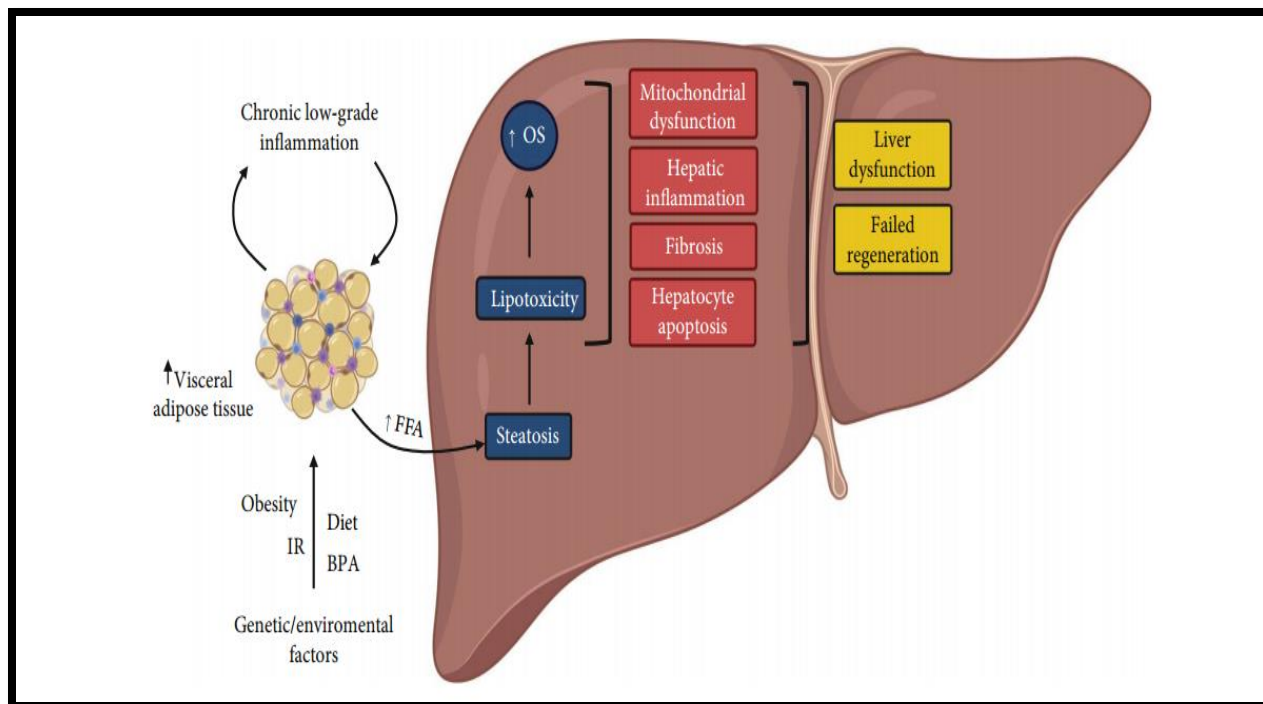
يعاني ما يصل إلى ربع السكان حاليًا من مرض الكبد الدهني غير الكحولي (NAFLD)، والذي يعد حاليًا أكثر أمراض الكبد انتشارًا في جميع أنحاء العالم. يرتبط الكبد الدهني غير الكحولي، الذي يتميز بالتنكس الدهني الكبدي، بالعديد من النتائج السلبية وارتفاع معدل الوفيات. علاوة على ذلك، فإن امتصاص الأحماض الدهنية وإنتاج دهون de novo تفوق أكسدة وتصدير الأحماض الدهنية، مما يؤدي إلى تنكس دهني كبدي. يتم تعزيز الامتصاص الكبدي للأحماض الدهنية وتكوين الدهون في الكبد الدهني غير الكحولي، في حين أن أكسدة الأحماض الدهنية التعويضية غير كافية لإعادة مستويات الدهون إلى طبيعتها وقد تعزز الضرر الخلوي وتطور المرض عن طريق إحداث الإجهاد التأكسدي، خاصة عندما تكون وظيفة الميتوكوندريا ضعيفة وأكسدة بيروكسيسومال وسيتوكرومال مرتفع. على الرغم من ارتفاع تصدير الدهون (lipid export) في البداية، إلا أنه يستقر وقد ينخفض مع تقدم المرض، مما يشجع على تراكم الدهون. يرتبط الكبد الدهني غير الكحولي ارتباطًا وثيقًا بالعديد من الاضطرابات الحالية المتعلقة بنمط الحياة لأن التنكس الدهني الكبدي يمكن أن يؤدي إلى خلل في التمثيل الغذائي النظامي يؤثر سلبيًا على العديد من الأعضاء. في هذه النظرة العامة، يتم إبراز المسارات الأربعة الرئيسية التي تساهم في استتباب الدهون في الكبد بينما نتحدث عن الأسباب الجزيئية لـ الكبد الدهني غير الكحولي.



**Figure 1:** An overview of hepatic lipid metabolism. (1) Fatty acid transportation. (2) De novo lipogenesis creates new fatty acids from acetyl-CoA. (3) By using lipids as an energy source, fatty acid oxidation, which is regulated by peroxisome proliferated activated receptor, lowers the amount of fat stored in the liver. (4) Lipids can be exported from the liver in the form of water-soluble very low-density lipoprotein particles.[42].



**Figure 2:** Available data regarding the natural history of nonalcoholic fatty liver disease illustrating steps of nonalcoholic fatty liver disease including, nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, cirrhosis, fibrosis and hepatocellular carcinoma (HCC)[106].



**Figure 3:** Intrahepatic complications in non-alcoholic fatty liver disease. Hepatic steatosis is one of the primary changes in nonalcoholic fatty liver disease. Hence, when steatosis progresses, free fatty acid levels rise, raising intrahepatic triglyceride levels. Lipotoxicity and oxidative stress are caused by this large increase in lipids within the liver. Oxidative stress and lipotoxicity increase profibrotic factors that cause liver fibrosis by causing mitochondrial malfunction, hepatocyte death, and hepatic inflammation [107].