Proteins involved in lipid metabolism as possible biomarkers or predisposing factors for non-alcoholic fatty liver disease

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is caused by the accumulation of lipids inside hepatocytes ; it can be simple or it can be inflammatory. Its prevalence has been increasing in recent years and is predicted to become the most common indication for liver transplantation by 2030. NAFLD is a multifactorial disease, although patients with metabolic disorders are at higher risk of developing it, and in more severe forms. Liver fat originates from the increase of free fatty acids (FFA), from de novo lipogenesis and from diet. This review compile evidence of the involvement of unregulated enzymes in the synthesis of fatty acids (FA), the alteration of FA transport proteins, and the presence of diverse polymorphisms, which may be associated with an increased risk of NAFLD. (Acta gastroenterol. belg., **2020**, 83, **622-630**).

Key words : Fatty acids, triglycerides, NAFLD, NASH.

Introduction

The liver performs several functions : it filters and stores blood ; metabolizes carbohydrates, proteins, fats, and hormones ; produces bile, stores vitamins and iron, synthetizes coagulation factors, and detoxifies exogenous substances. In the United States, according to the Center for Disease Control and Prevention (CDC), the age-adjusted death rate of chronic liver disease, including cirrhosis, was 10.8 per 100,000 in 2015 ; in males, it was the tenth leading cause of death (1). About 29 million people in the European Union have chronic liver disease, according to data from studies on the burden of liver disease in Europe (2). In Mexico, liver diseases are the fifth leading cause of mortality, with 39,287 deaths per year, of which only 13,942 (35.4%) are related to alcohol consumption (3) .

The role of the liver in fat metabolism

The liver plays a key role in the systemic metabolism of lipids, mainly by performing the following functions : oxidizing fatty acids (FA) to provide energy for other bodily functions ; synthesizing large amounts of cholesterol, phospholipids, and almost all lipoproteins ; synthesizing fat from proteins and carbohydrates (4).

Lipids can accumulate in hepatocytes via three pathways : excessive deposition of lipids (diet), impaired lipid metabolism, and decreased cellular lipid excretion (5). In non-alcoholic fatty liver disease (NAFLD), most hepatic fat comes from free fatty acids (FFA) (59%), but 26% comes from *de novo* lipogenesis (DNL) (Figure 1), and 15% comes the from diet (6).

Liver steatosis : physiopathology

Insulin resistance is the primary disorder of NAFLD ; it increases lipolysis due to the increased lipase, glycogenolysis and gluconeogenesis activity. The resulting hyperglycemia and hyperinsulinemia stimulate the expression of lipogenic transcription factors such as the sterol regulatory element-binding protein 1c (SREBP 1c), which in turn promotes DNL, inducing the synthesis of FFA and exceeding the liver's capacity to oxidize and export them ; this leads to the development of oxidative stress and, as a consequence, to the formation of free radicals, which causes lipid peroxidation, inflammation (6) and steatosis, which is defined as liver fat exceeding 5% of the total liver weight or 5% of hepatocytes containing lipid droplets (7).

In the presence of irreversible cell damage, hepatocytes undergo necrosis. When this happens, the transforming growth factor beta (TGF-β) promotes the differentiation of hepatic stellate cells (HSCc) into myofibroblasts, which deposit collagen in the space of Disse, being the HSCs key players in this process. Several features associated with the metabolic syndrome (MetS) can induce HSCs activation and liver fibrosis (8).

The presence of steatosis, creates a barrier to diffusion between sinusoidal circulation and the surface of hepatocytes, interfering with the transhepatic blood flow and increasing the hydrostatic pressure in the hepatic portal vein, which causes more blood to flow through collateral blood vessels. Histologically, hepatocellular steatosis can be of two types : macrovesicular (a single large vacuole in the center of the cell, with peripheral displacement of the nucleus), or microvesicular (several small vacuoles that appear as foamy cytoplasm, without displacement of the nucleus) (9). NAFLD is classified as simple hepatic steatosis (NAFL) or steatohepatitis (NASH) (10).

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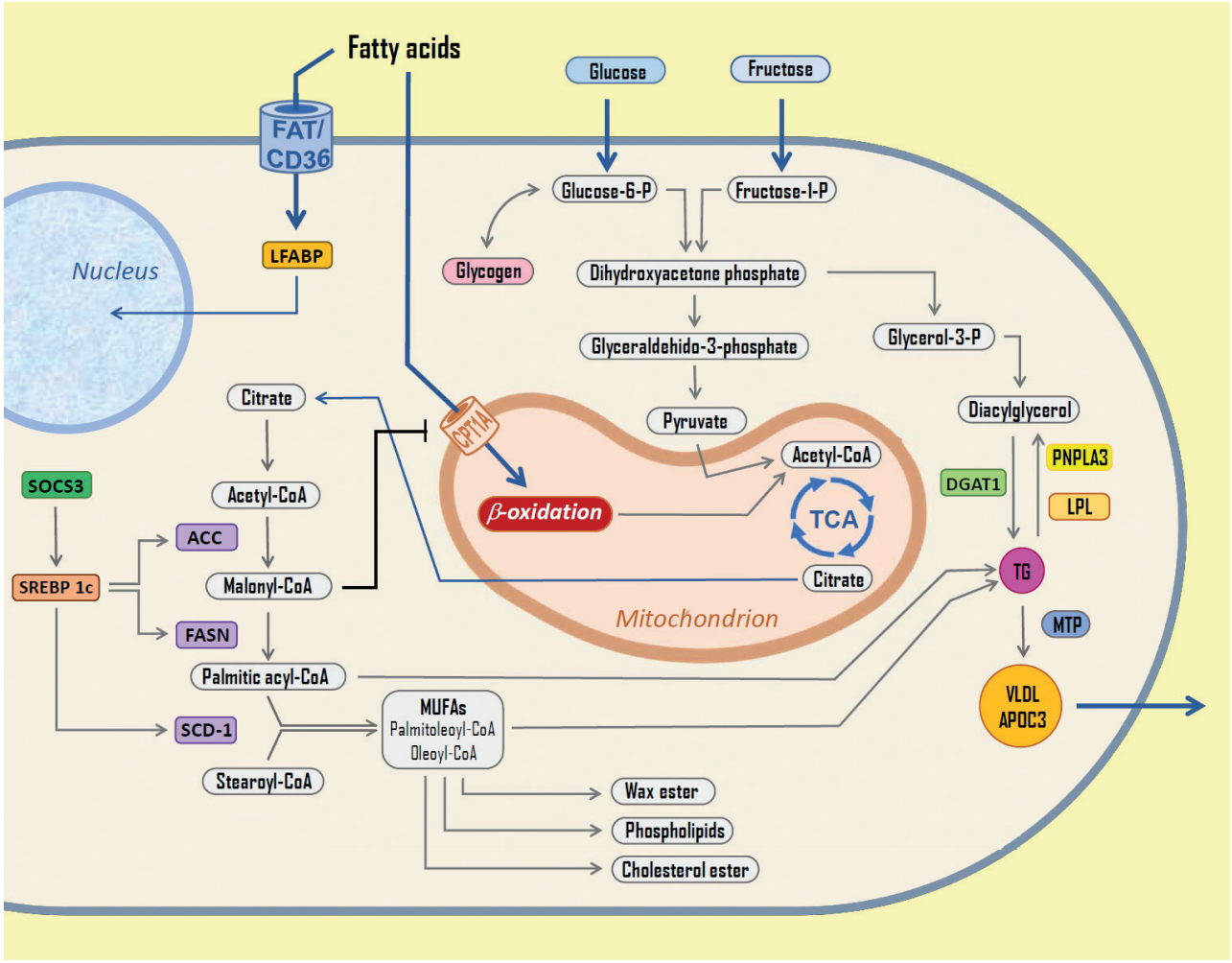

Figure 1. **Hepatic *de novo* lipogenesis** begins with the conversion of acetyl-CoA into malonyl-CoA by acetyl-CoA Carboxilase (ACC). Subsequently, malonyl-CoA becomes palmitic acyl-CoA. Three fatty acyl-CoAs are bound to a glycerol backbone to form one triglycerides (TG). ACC and fatty acid synthase (FASN) are induced by the sterol regulatory element binding protein 1c (SREBP-1c). Malonyl-CoA inhibits carnitine palmitoyltransferase 1 (CPT1A), which transports long chain FA into mitochondria for subsequent β-oxidation. Glucose and fructose are taken up and may be metabolized to pyruvate for energy production in the mitochondria, or to glycerol-3-phosphate for TG synthesis. To form TG, glycerol-3-phosphate is transformed to form diacylglycerol, which is converted to TG by the action of diacylglycerol acyltransferase (DGAT1). Subsequently, the microsomal TG transfer protein (MTP) transfers TG to apolipoprotein b, a key enzyme in the secretion of very low density lipoprotein (VLDL) and chylomicrons. Apolipoprotein C3 (APOC3) is a component of VLDL that inhibits LPL, which is an enzyme that hydrolyzes TG of chylomicrons and VLDL TG. Patatin-like phospholipase domain-containing protein 3 (PNPLA3) is a protein that has phospholipase and triglyceride hydrolase activity. FFAs are taken up into cells by passive diffusion and by FA transporters such as fatty acid translocase (FAT/CD36) ; the liver FA binding protein (LFABP) binds to long-chain FA and transports them to the nucleus. Stearoyl-CoA desaturase (SCD-1) converts palmitoyl and stearoyl CoA in monounsaturated fatty acids (MUFAs), SCD-1 is regulated by SREBP-1c, leptin and liver X receptor (LXR). The figure was created according to the information described in the article.

Non-alcoholic fatty liver disease (NAFLD)

The diagnosis of NAFLD is based on the presence of a percentage of hepatic fat accumulation higher than 5%, with no secondary causes (11). It is the most frequent and common hepatic disorder in Western industrialized countries ; the main risk factors are central obesity, type 2 diabetes mellitus (T2DM), dyslipidemia, MetS (9-12) and cardiovascular disease (CVD). Furthermore, NAFLD is predicted to become the most common indication for liver transplantation by 2030 (11).

The worldwide prevalence of NAFLD ranges from 6.3% to 33%, with a median of 20% (9). In the United States, the prevalence ranges from 10 to 46% (13-15), and according to the National Health and Nutrition Examination Survey (NHANES), it has increased from 5.5% between 1988 and 1994, to 9.8% between 1999 and 2004, to 11% between 2005 and 2008 (14) and to 30% between 2011 and 2012 (16).

In Europe, the prevalence reported in 2015 was 24% (17), an increase of 20% compared to 2005 (18). NAFLD poes a significant public health burden in the USA and Europe (with 64 and 52 million people respectively) (19). In Europe, the Belgian Association for the Study of the Liver (BASL) prepared a guide for the proper management of NAFLD patients (20).

There are data to suggest that hypothyroidism, hypopituitarism, hypogonadism, sleep apnea and polycystic ovary syndrome are important risk factors for NAFLD that are independent of obesity (9). One of the most predisposing factors for NAFLD is MetS. Marchesini *et al.* reported that out of 304 NAFLD patients, and 163 (54%) biopsies, a total of 120 patients (73.6%) were classified as NASH and 43 (26.38%) as NAFL. Furthermore, MetS was present in 88% of NASH patients and in 67% of patients with NAFL, and this association was statistically significant (21). Not only has metabolism been associated with fatty liver disease, but also with congenital errors of metabolism (Wilson's disease, abetalipoproteinemia), surgical procedures (including jejunum-ileum circuit), small intestinal resection, and biliopancreatic diversion), and drug intake (amiodarone, glucocorticoids, tamoxifen and others) (13).

Hepatic steatosis is present in 7% of normal-weight people with normal levels of liver enzymes (10). The association between obesity and NAFLD, and the dramatic increase in the global prevalence of obesity and MetS, suggest that the prevalence of NAFLD will continue to increase. A recent study showed that less than 4% of patients with NAFL progressed to advanced fibrosis ; in the case of NASH, 15 to 25% progressed to cirrhosis (6). In another article, Byrne, C., *et al.* mentioned that 40-50% of patients with NASH progress to fibrosis, while 30-40% of patients with NAFL progress to NASH (11).

Histology is needed for the diagnosis of steatosis, which is why liver biopsy remains the gold standard. However, steatosis can also be detected with liver ultrasound, magnetic resonance imaging (MRI) with proton density fat fraction (PDFF), controlled attenuation parameter (CAP) or computed tomography ; whenever imaging tools are not available, the fatty liver index (FLI) and the NAFLD Liver Fat score can help diagnose steatosis in large-scale epidemiological studies. These are simple indices ; the first is calculated through a formula based on body mass index (BMI), waist circumference, triglycerides (TG) (22) levels, and gamma glutamyl transferase (GGT), while the second considers the presence of MetS, DM2, fasting serum insulin, and the aspartate aminotransferase (AST)/ alanine aminotransferase (ALT) (23) ratio. It is necessary to exclude alcohol consumption, as well as secondary causes such as medication, parenteral nutrition,

Table 1. — **Possible new biomarkers for non-alcoholic fatty liver diseases**

Enzyme	Main location	Function	Regulators	Effect	Reference
Apoc3	Liver and small intestine	Inhibits lipoprotein lipase and hepatic lipase	Insulin (inhibitor) PPAR-a (inhibitor)	Its expression reduces lipolysis and increases VLDL and TG	(33, 34, 39)
MTP	Liver, small intestine, kidney, and heart	Transfers lipids to APOB for subsequent intrahepatic VLDL elimination	Cholesterol (stimulant) Insulin (inhibitor)	Decreased function causes steatosis.	(42, 106)
FAT/ CD36	Adipocytes, myocyte, and hepatocytes	Intracellular transport of FA	Insulin (stimulant)	Steatosis	(43, 47, 107)
FASN	Adipocytes, liver, and lung	Converts malonyl-CoA into palmitate	Insulin and citrate (stimulant)	Increases FA synthesis	(52, 55, 56)
DGAT1	Adipocytes, small intestine and, liver	Converts acetyl-CoA + diacylglycerol into triacylglycerol	Glucose (stimulant)	Increases de *novo* FA synthesis and TG accumulation	(57, 60)
ACC1	Adipocytes, lung, and brain	Carboxylation of acetyl CoA to malonyl CoA	SREBP-1c and insulin (stimulant) AMPK (inhibitor)	Increases *de novo* FA synthesis	(62)
LFABP1	Liver and colon	Transports LCFA into the nucleus; antioxidant	PPAR- α (stimulant)	Decreases FA oxidation and promotes hypertriglyceridemia	(68, 69, 71)
SREBP-1c	Adipocytes, liver, and kidney	Regulates glucose metabolism, FA synthesis, and stimulates FASN and ACC	Insulin (stimulant)	Increases FA synthesis	(79, 80)
PNPLA3	Liver, adipocytes, and kidney	Phospholipase activity and triglyceride hydrolase	Glucose, insulin, Oleate, and palmitate (stimulant)	Increases the risk of NAFLD	(25, 27, 29)
SCD-1	Adipocytes brain, heart, liver, and lungs	Conversion of saturated FA into MUFAs	SREBP-1c, LXR, leptin	Increased expression in NAFLD	(83, 86, 87, 108)
SOCS3	Adipocytes and lungs	Negative regulator of cytokines signaling by inhibition of JAK/ STAT pathway	IL6, insulin, leptin, and TNF- α (stimulants)	Increases DNL by induction of SREBP-1c, FAS and APOB; leptin resistance	(95-97)

Apolipoprotein C3 **(APOC3)**, peroxisome proliferator-activated receptor **(PPAR)**, very low-density lipoprotein **(VLDL)**, triglycerides **(TG)**, Microsomal triglyceride transfer protein **(MTP),** apolipoprotein B **(APOB)**, Fatty acid translocase CD36 **(FAT/CD36)**, fatty acids **(FA)**, Fatty acid synthase **(FASN)**, Diacylglycerol acyltransferase 1 **(DGTA1)**, Acetyl-CoA carboxylase **(ACC1)**, AMP-activated protein kinase **(AMPK)**, Liver fatty acid binding protein 1 **(LFABP-1)**, long chain fatty acids **(LCFA)**, sterol regulatory element binding protein isoform 1c **(SREBP-1c)** Patatin-like phospholipase domain-containing protein 3 **(PNPLA3)**, non-alcoholic fatty liver disease **(NAFLD),** Stearoyl -CoA desaturase **(SCD-1)**, monounsaturated fatty acids **(MUFAs)**, nuclear receptor liver X receptor **(LXR)**, Suppressor of cytokine signaling 3 **(SOCS3), de novo lipogenesis (DNL),** janus kinase **(JAK)**, signal transducer and activators of transcription **(STAT)**, tumor necrosis factor **(TNF).**

viral and genetic diseases. Hepatocellular carcinoma (HCC) is a complication of cirrhosis, but cases of HCC in non-cirrhotic NAFLD have also been described or suspected. The majority of deaths in NAFLD patients are related to CVD, and NAFLD increases the risk of CVD (24). Regarding treatment, lifestyle modifications are crucial. Several drugs, not specifically licensed for the treatment of NASH but with potential benefits based on their mode of action, have been tested, such as metformin, ursodeoxycholic acid, fibrates (the latter two are in clinical trials) and statins. These drugs have not been tested properly, and should therefore be used to treat dyslipidaemias, not only for treating NAFLD, at least until more data becomes available. Few drugs have shown efficacy in the treatment of NASH, but several are currently being used, such as glucagon-like-peptide analogues or incretin, liraglutide and pioglitazone (20).

Proteins involved in the transport chain and regulation of lipid metabolism

Patatin-like phospholipase domain-containing protein 3 (PNPLA3)

The PNPLA3 gene codifies for adiponutrin, a transmembrane protein that is expressed in adipose tissue and liver. Its functions are unclear but the purified protein is believed to have phospholipase, triglyceride hydrolase, and retinol palmitate esterase activity (25). The promoter activity of PNPLA3 is upregulated by glucose concentrations in a dose dependent manner (26).

Romeo *et al.* identified that the substitution of isoleucine for methionine at position 148 of the PNPLA3 protein (I148M) (SNP rs738409 [G]) is associated with NAFLD and hepatic inflammation. This substitution is more common in Hispanic people (27). Yaron Rotman *et al.* found that this polymorphism increases steatosis, portal and lobular inflammation, and is associated with the progression of disease (28). It has also been reported that the presence of this polymorphism significantly increases the risk of NAFLD by 27-fold (29). Furthermore, this association has been identified in NAFLD patients without MetS (30), the association with other liver diseases has been reported by Trepo (31).

A recent study showed that those obese patients with NAFLD carriers of the PNPLA3 148M variant had higher risk of prediabetes, MetS and insulin resistance (32).

Table 1 shows the characteristics of the proteins involved in the transport chain and regulation of lipid metabolism, including their location in different tissues, their function, their regulators and their effect.

Apolipoprotein C3 (APOC3)

APOC3 gene encodes a small glycoprotein of 79 amino acids, and 8.8 kDa, which resides on chromosome 11q23. Is a component of TG (22) rich lipoproteins, expressed primarily in the liver and small intestine (33). It inhibits the activity of lipoprotein lipase, which causes an increase in plasmatic TG by decreasing the lipolysis rate of TG (34). It also inhibits hepatic lipase, an enzyme involved in the conversion of very-low-density lipoproteins (VLDL) to low-density and intermediate-density lipoproteins, as well as in the remodeling of high-density lipoproteins (HDL). APOC3 hinders the clearance of TG-rich lipoproteins from the circulation, causing the accumulation of atherogenic VLDL and chylomicron remnants (35).

Polymorphisms rs2854116 and rs2854117 of APOC3 are found in Indian people and in other ethnicities that don't have the typical risk factors. These polymorphisms significantly increase APOC3 expression, which hinders the clearance of TG and leads to its accumulation (36). However, another study found no association between APOC3 and hepatic steatosis, NAFLD prevalence or hypertriglyceridemia (37). Kozlitina *et al.* also did not find an association between APOC3 and hepatic fat content in African Americans, European Americans or Hispanics (38). These findings suggest that there are ethnical or geographical variations in the susceptibility to NAFLD.

Microsomal triglyceride transfer protein (MTP)

MTP protein is a heterodimeric complex, it possess a unique large subunit of 97,000 kDa, is expressed in the liver and small intestine, and it is responsible for transferring TG to apolipoprotein b, which is a key factor in the secretion of VLDL and chylomicrons (39). Patients with abetalipoproteinemia (who have mutations in the coding region of the MTP gene) develop hepatic steatosis at earlier stages of life (40). However, it has been shown that patients infected with hepatitis C virus (HCV), especially with genotype 3, show reduced MTP activity and mRNA levels, as well as a high degree of steatosis (41). A meta-analysis involving 636 patients with NAFLD and 918 controls reported that the MTP -493G/T polymorphism increases the risk of developing NAFLD (42). The frequency of polymorphisms in the MTP gene (genotype -493 GG) was similar between Japanese (60.7%), French (55.7%) and Brazilian (59.3%) patients (43).

Fatty acid translocase CD36 (FAT/CD36)

Long chain FA can diffuse rapidly across phospholipid bilayers, but there is now evidence that their integral or membrane-associated proteins facilitate their uptake. One of the most important is FAT/CD36 (44) which is a transmembrane glycoprotein of 88 kDa (GP88), known as FA translocase (FAT), platelet GPIV (CD36 was identified as a glycoprotein IV on human platelet membranes), and scavenger receptor class B type 2 (SR-B2), is expressed on the cell surface in multiple cell types, including dendritic cells (DCs), microvascular endothelial cells (MVECs), retinal epithelial cells,

monocytes, adipocytes, platelets, enterocytes, microglial cells, and podocytes (45), it plays essential roles in lipid homeostasis, angiogenesis, immune response, adhesion, and metastasis in cancer (46).

A study of patients with NAFL, NASH, and HCV infection found significantly higher levels of FAT/CD36 mRNA compared with controls, and its expression was higher in patients with insulin resistance. The study also found a positive correlation between hepatic FAT/CD36 expression and the histological grade of steatosis in patients with NASH, and in patients with HCV genotype 1 infection and fatty liver (47). Another study showed that patients with NASH and morbid obesity had elevated levels of FAT/CD36, which were associated with increased apoptosis (48). An increased expression of FAT/CD36 was also observed in morbidly obese patients with diabetes and dyslipidemia, compared to morbidly obese patients without such comorbidities (49). Other studies have shown that FAT/CD36 expression is also increased in patients with NAFLD (50,51).

Fatty acid synthase (FASN)

In normal conditions FASN converts excess carbohydrate into FAs that are then esterified to storage triacylglycerols (TAG), which, when necessary, provide energy through β-oxidation. FASN is a key enzyme in the synthesis of new FA. It catalyzes the last step in the formation of FA ; when acetyl- CoA carboxylase converts acetyl-coA into malonyl-CoA, FASN converts the latter into palmitate (52) . FASN is mainly expressed in the cytosol of healthy liver, adipose, brain, cycling endometrium, and lactating mammary gland cells ; in these tissues and organs, lipogenesis is a crucial physiological process (23,53,54).

Dorn *et al.* found that the expression of FASN mRNA was significantly higher in livers with steatosis than in healthy livers, and that the increased expression of FASN mRNA was correlated with NAFL but not with NASH (55). Another study showed that FASN and lipoprotein lipase (LPL) levels correlate significantly with the severity of steatosis (56). However, further research is needed because most of the studies that have found an association between FASN and NAFLD have been conducted in murine models.

Diacylglycerol acyltransferase 1 (DGTA1)

DGAT1 is a membrane-embedded protein required for the absorption of dietary FA and the storage of TAG in adipocytes, is currently classified into four types : DGAT1, DGAT2, WS/DGAT, and cytoplasmic DGAT3 (CytoDGAT). DGAT1 has a specific role in the esterification of exogenous FA. It catalyzes the last step in the formation of TG, which is the conversion of diacylglycerol into TAG (57). It is expressed in adipocytes in the small intestine, liver, and mammary glands (58,59). One study reported higher levels of DGAT1 mRNA in NAFLD patients (60). Furthermore, studies carried out in mice have observed that DGAT1 deficiency reduces the accumulation of intrahepatic TG and, therefore, the risk of developing NAFLD. These results suggested that inhibiting this enzyme could reduce the risk of developing NAFLD even in subjects consuming a high-fat diet (61) .

Acetyl-CoA carboxylase (ACC)

ACC exists as two isozymes, are encoded by separate genes (62) and display distinct cellular distributions, ACC1 is predominantly expressed in lipogenic tissues ; liver and adipose tissue, while ACC2 is predominantly expressed in the heart, skeletal muscle and liver (63-66). ACC participates in the *de novo* synthesis of FA, catalyzing the formation of malonyl-CoA from acetyl-CoA. It is expressed mainly in adipocytes, in the lungs and brain. Biopsies revealed that the expression of ACC1, and of its regulator SREBP-1c, were twice as high in NAFLD patients compared with patients with healthy livers (60). Increased levels of malonyl CoA were found in the hepatocytes of mice with mutations in ACC1 Ser 79 and ACC2 Ser212 ; as a consequence, they showed an increase in DNL and decreased FA oxidation (67).

Liver fatty acid binding protein 1 (LFABP-1)

Liver fatty acid-binding protein (LFABP)1 belongs to a family of 14-15 kDa intracellular lipid-binding proteins (iLBPs) that have 126-134 amino acids and the ability to bind long-chain FA and in some cases other small hydrophobic ligands. LFABP-1 is found in hepatocytes. It binds to long-chain FA (LCFAs) and transports them to the nucleus, where they regulate nuclear receptors, which is an important step in the transcription of genes that encode proteins involved in the metabolism of LCFA and glucose (68). It also works as a liver antioxidant, inactivating free radicals (69), and thus its depletion causes a decrease in the LCFA β-oxidation, which has repercussions on the accumulation of intrahepatic lipids (70). It has been reported that a polymorphism of LFABP-1, T94A, promotes hypertriglyceridemia and increases low-density lipoprotein levels (71,72). The expression of LFABP-1 is not regulated in patients with NAFLD (73, 74). Furthermore, LFABP-1 is a sensitive serum marker of acute hepatocellular damage in post-transplant patients (75), and of liver injury in patients with chronic HCV infection (76). Increased LFABP-1 levels have been found in NAFLD patients, correlated with higher BMI, hyperglycemia and increased levels of GGT, ALT, and AST. In view of these results, LFABP-1 has also been suggested as a marker of liver damage (22).

Sterol regulatory element binding protein (SREBP)

The regulatory mechanisms of DNL include transcription factors ; one of the most important is SREBP (77).

This protein has three isoforms : 1a, 1c, and 2. The SREBP-1c isoform, which predominates in the liver and in adipose tissue, stimulates the transcription of FASN and ACC (78). It is involved in the metabolism of FA and glucose, and is regulated by insulin. SREBP-2 regulates cholesterol biosynthesis and maintains cholesterol homeostasis (79). One study found that the expression of SREBP-1c, FASN, and ACC was significantly elevated in NAFLD patients (80). Furthermore, Kohjima *et al.* found an increased expression of SREBP-1c in NAFLD patients (60). SREBP-2 has also been associated with NAFLD ; the GG genotype and G carrier of the rs2228314 G>C polymorphism in the SREBP-2 gene are associated with a higher risk of disease in the Chinese population. Given these results, Ying Wang *et al.* proposed that the SREBP-2 rs2228314 G>C polymorphism may be a biomarker for NAFLD (81). Another study reported that patients with NAFL and NASH had SREBP-2 mRNA levels that were 7 and 3 times higher than controls. Moreover, since SREBP-2 regulates the transcription of hydroxymethylglutaryl-CoA, its levels were also 4 times higher in patients with NAFL and NASH (82).

Stearoyl -CoA desaturase (SCD-1)

SCD-1 is considered as rate-limiting enzyme in the biosynthesis of monounsaturated FA (MUFAs) from saturated fatty acyl-CoAs (83) that are either synthetized de novo or derived from the diet. SCD-1, in conjunction with NADH, the flavoprotein cytochrome b5 reductase and the electron acceptor cytochrome b5, as well as molecular oxygen, introduces a single double bond into its preferred substrates, palmitoyl- and stearoyl-CoA, which are then converted into palmitoleoyl- and oleoyl-CoA, respectively (84). These are the most abundant MUFAs and serve as substrates for the synthesis of various kinds of lipids, including phospholipids, TG, cholesterol esters, wax esters, and alkyldiacylglycerols. SCD-1 is located in the endoplasmic reticulum, where it undergoes a rapid turnover in response to a variety of nutritional and hormonal signals (85). Its expression is induced by nuclear SREBP-1c and liver-X-receptor activation (86). Furthermore, it was ranked as the most potently leptin-regulated gene, which suggests that the downregulation of the expression and activity of SCD-1 plays a major role in leptin-mediated depletion of hepatic lipids (87). It has been reported that SCD-1 deficiency reduces lipid synthesis and enhances lipid oxidation, thermogenesis and insulin sensitivity in liver, muscle and adipose tissue, while the pharmacological inhibition of SCD-1 improves hepatic lipid accumulation and metabolic disorders, (88-90), liver injury, hepatocellular degeneration and inflammation in experimental NASH models (91). Another study with liver samples from patients with NAFLD and ob/ob mice (which have a recessive mutation in leptin and are hyperphagic, obese, hyperinsulinemic, and hyperglycemic) showed an increased expression of the SCD-1 gene and protein. Moreover, the authors reported that berberin (inhibitor of SCD-1) induced a decrease in SCD-1 expression mediated by the phosphorylation of SREBP-1c, and reduced the binding of SREBP-1c to the SRE motif within the scd-1 promoter (92).

Suppressor of cytokine signaling 3 (SOCS3)

SOCS3 is a member of the SOCS family, which can directly inhibit the activity of JAK kinase and phosphorylation of its downstream STAT protein, and block the activation and transduction of janus kinase-signal transducer and activators of transcription (JAK-STAT) signaling pathway (93). SOCS proteins increase the synthesis of FA by upregulating the expression of SREBP-1c, presumably through suppression of STAT3 phosphorylation and persistent hyperinsulinemia (94). There is evidence that insulin stimulation has an additive effect with SOCS3 overexpression, inducing the expression of SREBP-1c, FAS, and apolipoprotein B (95). Overexpression of SOCS3 leads to leptin-resistance, which can contribute to obesity (96). There is also evidence that shows that interleukin 6 (IL6) and TNF-α induce hepatic expression of SOCS3 (97). One study found an increase in SOCS3 mRNA in NAFL and NASH patients compared to controls. The highest levels of SOCS3 mRNA were observed in patients with HCV infection, regardless of the presence of steatosis. Increased hepatic TNF-α and IL-6 levels were also found in those patients (98).

Figure 1 summarizes the functions and interactions of proteins during metabolism and the transport of lipids.

Therapeutic potential of selected proteins in NAFLD/NASH management

Inhibitors or agonists of some of the proteins mentioned have been proposed for NAFLD/NASH management, and several are in clinical trials. Aramchol, inhibitor of SCD-1, is obtained by conjugating cholic acid (bile acid) and arachidonic acid. In a study in phase 2, it was administered to NAFLD patients over 3 months, achieving 70 to 83% inhibition of SCD-1 and showing a decrease of liver fat content in a dose-dependent manner (99). Among the inhibitors of ACC1/2, firsocostat (GS0976), an allosteric inhibitor, was administered for 3 months to non-cirrhotic patients with NASH, and was associated with significant reductions of hepatic fat content (≥30%), reduced serum levels of TIMP1 (endogenous regulator of matrix metalloproteinases that promotes liver fibrosis) and liver biochemistry (100). PF-05221304, another ACC1/2 inhibitor, inhibited DNL in primary human hepatocytes, stimulated FA oxidation and reduced TG accumulation ; furthermore, it inhibited DNL in lean rats and normalized steatosis (101), while in healthy individuals it achieved approximately 70% DNL inhibition in a dose-dependent manner (102). Fibrates are peroxisome proliferator-activated receptor α (PPAR-α)

agonists that decrease serum TG levels and increase HDL-cholesterol levels (103). In NAFLD patients, fibrates are a safe and effective treatment for atherogenic dyslipidemia, especially for patients with MetS and or/T2DM (104). It has been reported that bezafibrate reduced hepatic inflammation, fibrosis, suppressed expression of inflammatory cytokines and profibrogenic genes in cultures of stimulated stellate cells, in NASH patients, fibrates had not effect (105).

Conclusions

The prevalence of NAFLD has been increasing worldwide, and there is evidence of the existence of unregulated enzymes that are involved in the *de novo* synthesis of FA, transcription factors, and FA transport proteins, likely increasing the risk of NAFLD. Some of these enzymes have been correlated with the severity of the disease or proposed as possible biomarkers. Further research on the expression of these enzymes in humans is needed, since most related studies have been conducted in mice. It would also be important to have more data from different geographic regions in order to determine the association between these enzymes and NAFLD prevalence. It must be pointed out, though, that existing studies have opened the way to research on therapy targets that might regulate the expression level of these biomarkers and possibly decrease liver steatosis.

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Conflicts of interest

All authors are requested to disclose any potential conflict of interest including any financial activities, additional affiliations, personal or other relationships with other people or organizations that could influence, or be perceived to influence, their work, such as employment, consultancies, stock ownership, honoraria, patent applications/registrations, grants or other funding.

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