Universidade de Lisboa

Faculdade de Farmácia

Treatment options and combined therapies for metabolic associated fatty liver disease

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Monografia orientada pelo Professor Doutor Rui Castro,
Professor Auxiliar.

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Resumo

Com o aumento da prevalência de obesidade, especialmente em países desenvolvidos, surge uma série de consequências metabólicas nas quais se inclui o fígado gordo não alcoólico. O fígado gordo não alcoólico caracteriza-se por uma acumulação de gordura intra-hepática excessiva (superior a 5% do volume do fígado) frequentemente associada a resistência à insulina.

O fígado gordo não alcoólico é atualmente considerado a doença hepática com maior prevalência a nível mundial. Porém, até o momento, não existe nenhuma terapêutica farmacológica aprovada pelas autoridades reguladoras do medicamento. Com o intuito de facilitar o diagnóstico e assim aumentar a procura de novos fármacos, foi recentemente sugerido a alteração na nomenclatura da doença, de fígado gordo não alcoólico, para fígado gordo metabólico.

Com esta monografia pretende-se sintetizar a informação existente relativamente à heterogeneidade da doença e, em especial, à sua abordagem farmacológica. A abordagem terapêutica inicial consiste na melhoria e controlo dos distúrbios metabólicos associados à síndrome metabólica, com alteração do estilo de vida, que envolve modificação da dieta, prática regular de exercício físico e perda de peso. No entanto, quando estes objetivos não são atingidos ou revelam ser insuficientes será necessário conjugá-los com terapêutica farmacológica que contrarie os mecanismos fisiopatológicos da doença.

Considerando a sua prevalência e os fatores associados à progressão da doença torna-se imperativo que se investigue com maior detalhe a fisiopatologia do fígado gordo não alcoólico, realizando também a estratificação de risco de cada doente de modo a ser possível direcionar eficazmente a terapêutica, descobrir novos alvos terapêuticos e adaptar o tratamento a cada indivíduo.

Palavras-chave: fígado gordo não alcoólico, esteatose; metabolismo lipídico; modificação do estilo de vida; opções terapêuticas.

Abstract

With the increasing prevalence of obesity, especially in developed countries, a series of metabolic consequences arise, including non-alcoholic fatty liver disease (NAFLD). NAFLD is characterized by excessive intrahepatic fat accumulation (greater than 5% of the liver volume) often associated with insulin resistance and that can progress to non-alcoholic steatohepatitis.

NAFLD is currently considered the most prevalent liver disease worldwide. However, until now, there is no pharmacological therapy approved by the National Authority of Medicines. To facilitate the diagnosis and thus increase the research of new drugs, it was recently suggested a change in the disease nomenclature, from NAFLD to metabolic fatty liver disease (MAFLD).

This monograph is intended to synthesize the existing information regarding the heterogeneity of the disease and, in particular, its pharmacological approach. The initial therapeutic approach, based on improvement and control of metabolic disorders associated with the metabolic syndrome, consists in lifestyle changes, involving dietary modification, regular physical exercise and weight loss. However, when these goals are not achieved or prove to be insufficient, it may be necessary to combine them with pharmacological therapy that counteracts the pathophysiological mechanisms of the disease.

Considering the prevalence of NAFLD and the factors associated with the disease progression, it is imperative to investigate the pathophysiology of the disease in greater detail, making sure to perform risk stratification on each patient, so that it is possible to effectively target the therapy, discover new therapeutic targets and adapt the treatment to each individual.

Keywords: non-alcoholic fatty liver disease; steatosis; lipid metabolism; lifestyle modification; therapeutic options.

Abbreviations

ACC - Acetyl Coenzyme A carboxylase

CCR – C-C Chemokine receptors

CVD - cardiovascular disease

DNL - *De novo* lipogenesis

DPP – Dipeptidyl peptidase

FFAs - Free fatty acids

FGF - Fibroblast growth factors

FXR - Farnesoid X receptor

GLP – Glucagon like peptide

HDL-C - High Density Lipoprotein Cholesterol

LXR - Liver X receptor

MAFLD - Metabolic-associated fatty liver disease

MeS - Metabolic Syndrome

NAFLD - Non-alcoholic fatty liver disease

NASH - Non-alcoholic steatohepatitis

NEFAs - Non-esterified fatty acids

OCA - Obeticholic Acid

PNPLA3 - Patatin-like phospholipase domain-containing protein 3

PPAR - Peroxisome proliferator-activated receptor

PUFAs - Polyunsaturated fatty acids

SCD - Stearoyl-CoA desaturase

SGTL- Sodium glucose cotransporter

T2DM - Type 2 diabetes mellitus

THR – Thyroid hormone receptor

VLDLs - Very low–density lipoproteins

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1 Introduction

The association between fat accumulation in the liver and development of hepatic injury and scarring was identified more than 50 years ago. However, it was first recognized as a distinct entity by Jurgen Ludwig and colleagues in 1980, who described a condition mimicking alcoholic hepatitis histologically, in the absence of significant alcohol consumption (1, 2). They named this condition non-alcoholic steatohepatitis (NASH). NASH is now recognized to be part of a histological spectrum of a disease that was later named non-alcoholic fatty liver disease (NAFLD) (1).

NAFLD, once believed to be an innocuous condition, has now become the most common cause of chronic liver disease in many countries worldwide (3). Its prevalence has increased to more than 30% of adults in developed countries and its incidence is still rising (4).

NAFLD is characterized by pathologic fat accumulation in the liver with >5% of hepatocytes containing visible intracellular triglycerides (TGs) - or steatosis affecting at least 5% of the liver volume or weight, in the absence of significant alcohol consumption and other specific causes of fatty liver disease, including hepatitis C (5).

The majority of patients with NAFLD have simple steatosis but in up to one third of patients, NAFLD progresses to its more severe form - NASH - which is characterized by inflammation, hepatocellular ballooning degeneration and fibrosis (4, 6). Whereas steatosis is not associated with an increase in liver-related morbidity or mortality, NASH can progress to more-severe stages such as cirrhosis and hepatocellular carcinoma, eventually leading to liver failure and liver transplantation (7).

Accumulated research has highlighted that NAFLD is a heterogeneous condition often associated with the most common clinical features of metabolic syndrome, such as central obesity, type 2 diabetes mellitus, dyslipidemia and arterial hypertension (2, 6).

Since the initial descriptions, there have been major conceptual advances in our understanding of the complex pathophysiological mechanisms of this common liver disease (8). To reflect the evolved understanding of the disease, an international panel

of experts in a consensus statement recommended a change in name from non-alcoholic fatty liver disease (NAFLD) to metabolic-associated fatty liver disease (MAFLD) (9).

This change goes far beyond a mere semantic revision and may be the first step towards a better identification of this common and burdensome metabolic liver disease for improved health promotion, case identification, patient awareness, ongoing clinical trials, and health services delivery (8, 10). By dissociating the disease from the word 'alcoholic', experts found the new name less pejorative and predicted that patients might prefer a 'specific' diagnosis denoting a cause rather than a 'non' diagnosis defined by the absence of a cause (11).

As the change in nomenclature and diagnostic criteria for NAFLD has not yet been accepted by major scientific societies; and given that NAFLD and MAFLD are not precisely the same disease, the definition and terminology for clinical practice and clinical trials requires further deliberation and consensus (7, 12).

Although there has been steady progress in clarifying the pathogenesis of NAFLD, identifying therapeutic targets and advancing drug development, there are significant unmet challenges, and no specific pharmacological agent is yet approved for this condition (13).

2 Materials and methods

The sources of information were obtained using the search engine Pubmed. The research material was acquired by using the terms: “Metabolic fatty liver disease”; “Non-alcoholic fatty liver disease”; “History of NAFLD”; “Scoring system in NAFLD”; “NAFLD pathogenesis”; and “Management of NAFLD”, among others. Only scientific articles published in the last 10 years and written in English were included.

3 Non-alcoholic fatty liver disease (NAFLD)

3.1 Epidemiology

The public health importance of NAFLD stems from its multifaceted impact on morbidity, mortality and health care utilization globally. NAFLD, and particularly, NASH and fibrosis, is associated with an excess all-cause mortality and liver-related mortality in the general population (14).

NAFLD has become the most common liver disorder worldwide, with a global prevalence of roughly 25% in the adult population (15). Estimates of NAFLD prevalence changes according to the population studied (for example, studies in patients with different ethnicities, genders and comorbidities) and the sensitivity of the modality used for diagnosis (16).

Highest prevalence rates, mostly ultrasound based, have been reported from Middle East and South American countries (14). Roughly 60% of those people subjected to liver biopsy presented with NASH. In accordance with its metabolic nature, 42% of NAFLD subjects had metabolic syndrome (MetS); 69%, hyperlipidemia; 51%, obesity; 39%, hypertension; and 22%, diabetes (17).

Despite relatively robust data about the prevalence of NAFLD, the data about the incidence of NAFLD is quite limited due to the longitudinal nature of such studies, as well as the non-availability of a non-invasive biomarker- that would detect NAFLD when used repetitively and reproductively (14, 18).

Overall, the epidemiological trends of NAFLD parallels the changes in prevalence of obesity, diabetes, hypertension and other diseases that accompany this social transition (14).

3.2 Sources of Heterogeneity

The heterogeneity in NAFLD clinical presentation and disease progression is likely influenced by multiple factors, including age, gender, hormonal status, ethnicity, diet, alcohol consumption, smoking, genetic susceptibility, the microbiota, and metabolic status. Hence, the outcome will reflect the balance of these diverse inputs, each interacting with the other and modifying the ultimate manifestations and clinical course (19).

3.2.1 Age and Gender

The liver is a complex metabolic organ that is essential for maintaining whole body homeostasis via regulation of energy metabolism, xenobiotic and endobiotic clearance, and molecular biosynthesis. As such, age-related fluctuations in liver function contribute to systemic susceptibility to age-related diseases (20).

As age increases, so does the prevalence of NAFLD and NAFLD-related fibrosis (18). With aging, considerable changes occur in the liver, these include a decrease in hepatic blood flow, hepatic volume, and liver function; a reduction in bile acid synthesis and alterations in cholesterol metabolism; as well as a reduction in mitochondrial number with subsequent increases in oxidative respiration. Additionally, aging is accompanied by changes in body composition, including a decrease in muscle mass, an increase in abdominal adiposity and ectopic fat deposition, with higher insulin resistance and prevalence of the metabolic syndrome (19).

In addition, NAFLD is also a sexually dimorphic disease. Epidemiological data corroborates a higher prevalence in men than women; however, prevalence of NAFLD in menopausal women is comparable with that of age-matched men, and two-fold higher than in pre-menopause (5).

The disparities between sexes may be explained by estrogens physiologic effects. Estrogens have been shown to confer protection from NAFLD in menopausal women receiving hormone replacement therapy. Estrogens promote the gynoid phenotype of body fat distribution, limiting visceral fat accumulation, and stimulating subcutaneous fat depots. They also trigger sex-specific immune responses and have a role in modulating inflammation and tumorigenesis in the liver (5).

3.2.2 Ethnicity

Ethnicity plays a significant - yet complex - role in the prevalence of NAFLD (5). While NAFLD prevalence is disproportionally lower among African American patients and higher among Hispanic patients compared to white populations, these discrepancies are less marked in high-risk cohorts (T2DM and obesity) and the rates of advanced fibrosis do not seem to differ significantly between ethnicities. This indicates that ethnicity may play a comparatively greater role in determining NAFLD prevalence rather than severity (21).

The lower prevalence of NAFLD among African Americans comparing to Hispanics, despite the higher prevalence of obesity amongst non-Hispanics, brings into focus the complexities in NAFLD pathogenesis, implicating the role of genetic as well as epigenetic influences operating through diet, lifestyle and other environmental factors (14).

On this regard, researchers have proposed that age and levels of triglycerides and serpin family E member 1 (PAI-1, a marker of fibrosis) are only associated with NAFLD in Hispanic patients, whereas serum levels of adiponectin are associated with NAFLD in African Americans (18).

3.2.3 Alcohol consumption

Usually, low levels of alcohol intake, possibly even lower than the diagnostic cut-offs of NAFLD, are not associated with advanced fibrosis in epidemiological studies and may even be protective. However, data are far from solid. The protective effects are largely derived from retrospective analyses, all based on self-assessed methods and subject to several selection biases. In prospective analyses, even modest alcohol intake has been associated with possible disease progression, less disease improvement and, more significantly, cancer development (22, 23).

With that said, the effect of alcohol abuse on liver disease evolution likely has a dose-response, rather than a J-shaped association, with a synergistic detrimental effect with the presence of metabolic syndrome (19).

3.2.4 Dietary Intake, Gut Microbiota, and Bile Acids

The abrupt increase in NAFLD in the past 25 years in the developed world has been largely attributed to a diet that is rich in fructose, sucrose and saturated fats; and an increase in sedentary lifestyle. An additional risk factor for NAFLD may be the evolution of the human microbiota, reflecting both a changing diet as well as the widespread use of antibiotics in farm animals and the indiscriminate prescription of antibiotics to humans (13).

The collective data suggests that gut microbiota may play a significant role in the pathogenesis of obesity. As such, it would be logical to think that gut microbiota also plays a role in the development of NAFLD and NASH (24).

The first clue on the role of the microbiota in the pathogenesis of obesity came from studies by Backhed et al. They compared body weight gain in germ free mice and conventionally raised mice - and found that the latter gained more weight, with increased adipose tissue and body fat percentage, which could not be explained by distinct diet intake (24).

According to evidence from other studies, the gut microbiota may contribute to the pathogenesis of NAFLD through several mechanisms, including (1) increased production and absorption of gut short-chain fatty acids (SCFAs); (2) altered dietary choline metabolism by the microbiota; (3) altered bile acid pool by the microbiota; (4) increased delivery of microbiota derived ethanol to liver; (5) gut permeability alterations and release of endotoxin; and (6) interaction between specific diet and microbiota (25) (Figure 1).

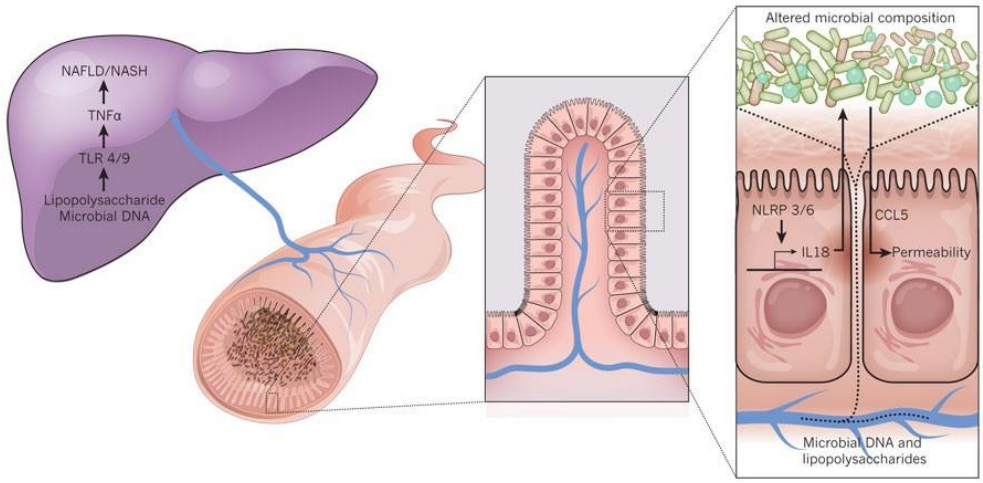

Figure 1- Dysbiosis as an influencer for NAFLD. Alterations of the intestinal microbiota composition and barrier function resulting in an increased permeation of

bacterial endotoxin have been suggested to be critically involved in the onset and progression of NAFLD. In the context of an impaired epithelial barrier and altered gut microbiota, lipopolysaccharide and bacterial DNA activate tool like receptors (TLR), TLR4 and 9, leading to increased tumour-necrosis-factor-α (TNFα) secretion which in turn leads to inflammation and development of NASH. Adapted from (26).

3.2.5 Obesity and Metabolic Status

Obesity is considered a key player in the development of NAFLD, and most patients with NAFLD are either obese or overweight. However, NAFLD has also been reported in lean subjects. “Lean” NAFLD represents a subpopulation of patients with fatty liver and normal body mass index. These patients are usually insulin resistant and have low HDL-C and higher triglyceride concentrations when compared to lean healthy controls (27).

The severity of fatty liver in the morbidly obese (BMI> 40 kg/m2) is influenced by the degree of impaired glycemic status and the adipose tissue distribution. Hepatic steatosis is strongly associated with visceral adiposity (measured as waist circumference), as visceral adipose tissue (VAT) is more lipolitically active on a per unit weight basis than subcutaneous fat (28).

An adipocyte-like function has been attributed to hepatocytes, when the capacity of adipose tissue to store excess energy is diminished, which occurs in common obesity or conditions lacking adipose tissue such as lipodystrophies. In these cases, hepatocytes store the extra lipids, mainly in the form of triglycerides, leading to simple steatosis. More specifically, excess circulating free fatty acids (FFAs) availability resulting from accelerated lipolysis and reduced fatty acid uptake in subcutaneous adipose tissue could lead to ectopic fat accumulation (for instance, in the liver and skeletal muscle) and, subsequently, to multi-organ insulin resistance (IR) (29).

3.2.6 Genetic Susceptibility

At this time, at least 5 common variants in different genes have been associated with NAFLD, namely patatin-like phospholipase domain-containing protein 3 (PNPLA3), transmembrane 6 superfamily member 2 (TM6SF2), glucokinase regulator (GCKR), MBOAT7, and hydroxysteroid 17-beta dehydrogenase-13 (HSD17B13) (19).

The most strongly associated genetic variant with NASH is a single-nucleotide polymorphism (I148M) in the gene for PNPLA3 (30). PNPLA3 has hydrolytic activity towards triacylglycerols, diacylglycerol and monoacylglycerol, and the I148M substitution causes a loss of function in the enzyme. This genetic variant is associated with higher liver lipid content, greater NASH activity, and increased risk of liver fibrosis and development of hepatocellular carcinoma (31).

Different PNPLA3 gene alleles have been shown to either confer susceptibility (rs738409[G], encoding I148M, Hispanics), or protection from NAFLD (rs6006460[T], encoding S453I, African American populations). Importantly, the presence of the mutant I148M seems to increase NAFLD risk, specifically in the context of body weight gain (1).

Another widely validated genetic variant in NAFLD is the rs58542926 variant (c.449 G>A) within the TM6SF2 gene. Considering TM6SF2 gene ability to modulate triglyceride transport out of the liver throw the VLDL secretion pathway, people with TM6SF2 E167K variant may display increased hepatic triglyceride content (25, 32).

3.3 Pathogenesis

3.3.1 "Multiple parallel-hit model"

The pathophysiologic mechanism for the development and progression of NAFLD was firstly hypothesized by Day and James who proposed the "two-hit model" (33). According to this, hepatic accumulation of lipids secondary to sedentary lifestyle, high fat diet, obesity, and insulin resistance (IR), would act as the first hit, sensitizing the liver to further factors acting as a 'second hit'. The 'second hit' would activate inflammatory cascades and fibrogenesis (34).

This first hypothesis has been subsequently revised in a "multiple parallel-hit model", which states that multiple etiological and pathogenic factors (including lipotoxicity, proinflammatory cytokines, increased oxidative stress, mitochondrial dysfunction, genetic or environmental susceptibilities) act in a parallel and somehow synergic way on a genetically predisposed subject, to cause NAFLD thus defining the spectrum of the disease phenotype (Table 1) (15, 35).

Table 1. Contribution factors to the pathogenesis of NAFLD and NASH. Adapted from (36)

FACTORS	OBSERVATION
Obesity	Hyperinsulinemia, Insulin resistance
Apoptosis	ER stress and Oxidative stress
Immune and inflammatory pathways	Activation of macrophages, Iron metabolism
Intestinal bacteria	Small intestine bacterial overgrowth, Intestinal mucosa barrier malfunction and intestinal microbiota
Single nucleotide polymorphism	PNPLA3, TM6SF2
Epigenetic alterations	MicroRNA, DNA methylation

3.3.2 Hepatic lipid homeostasis

The liver constitutes an essential organ in lipid metabolism. As a central regulator of lipid homeostasis, the liver is responsible for orchestrating the synthesis of new fatty acids, their export and subsequent redistribution to other tissues, as well as their utilization as energy substrates (37).

Liver lipid levels are regulated by the interplay between the delivery of lipids to the liver (mainly from plasmatic non-esterified fatty acids (NEFAs), de novo lipogenesis (DNL) and dietary fats) and their hepatic uptake, synthesis, oxidation, and secretion within very low–density lipoproteins (VLDLs) (Figure 2).

Dietary fats are absorbed in the intestinal lumen. The liver is essential for enterocyte hydrolysed lipid absorption via bile acids (BAs); once absorbed, lipids are esterified and packaged into nascent chylomicrons, and released into the circulation via the lymphatic system. Once in the circulation, nascent chylomicrons mature by gaining apolipoprotein E (apoE) and apolipoprotein C2 (apoC2); gain of apoC2 activates lipoprotein lipase, hydrolysing triacylglycerol (TAGs) into glycerol and FAs; and FAs are partially taken up by adipose tissue with the remainder transported in chylomicron remnants and taken up by the liver after binding with the apoE receptor (38).

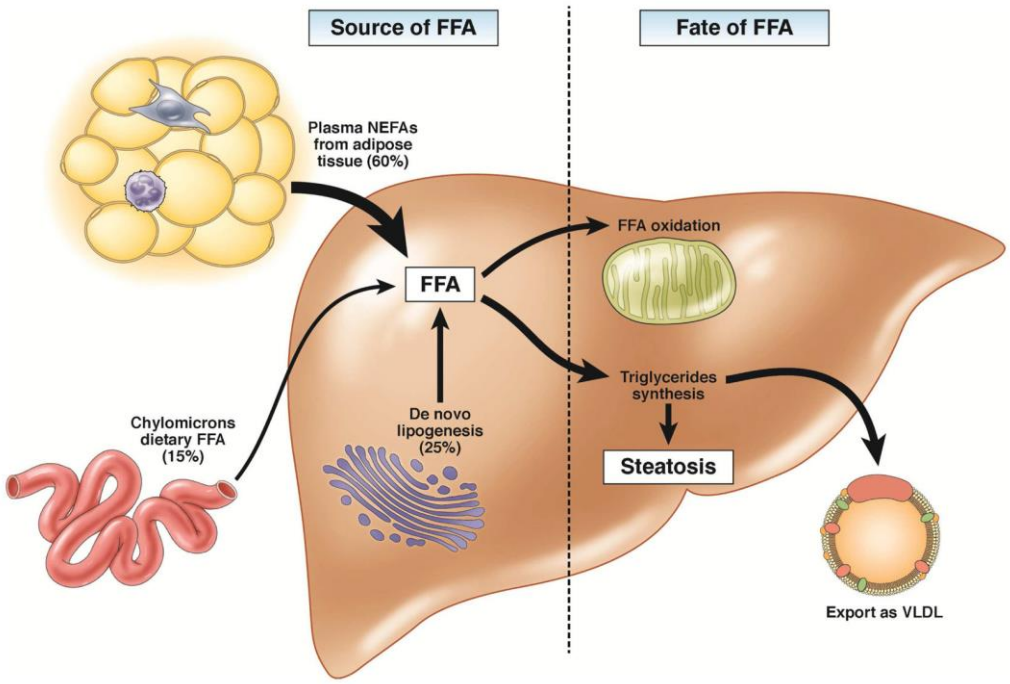

Figure 2- Dysregulation of lipid homeostasis. The main source of free fatty acid (FFAs) are plasmatic non-esterified fatty acids (NEFAs), followed by *de novo* lipogenesis (DNL) and dietary fats. While NEFAs arises from the lipolysis of the adipose tissue, *de novo* lipogenesis is a process in which hepatocytes convert excess carbohydrates, especially fructose, into fatty acids (35, 39). Once in the hepatocyte, fatty acids are further processed to form TAGs for storage, oxidized by mitochondria to create energy and ketones, added to lipoproteins (apoB) for secretion as VLDL, or used to synthesize phospholipids, depending on ongoing metabolic requirements (38). When these FFA disposal mechanisms are overwhelmed, triglycerides (TGs) start accumulating as lipid droplets in the hepatocytes (steatosis). Adapted from (40).

In the setting of carbohydrate and fatty acid substrate overload or impairment of the pathways of fatty acid disposal or, most likely a combination of both, fatty acids may promote the generation of lipotoxic species (e.g., diacylglycerols [DAGs], ceramides, lysophosphatidyl choline species [LPCs]) (41). Lipotoxicity might produce endoplasmic reticulum stress, oxidative stress and mitochondrial dysfunction, resulting in inflammation and hepatocyte degeneration (ballooning), which are the defining characteristics of NASH (42).

Over time, hepatocyte death, inflammation and immune cell activation might promote hepatic stellate cell activation. Stellate cells differentiate into fibrogenic myofibroblasts that migrate to sites of hepatic injury and are the major drivers of fibrosis. Detectable fibrosis is present in approximately 41% of patients with NASH and 22% of individuals with advanced fibrosis go on to develop hepatic scarring, referred to as cirrhosis. Lastly, about 2% of patients with cirrhosis will probably develop hepatocellular carcinoma within 3 years (Figure 3) (42).

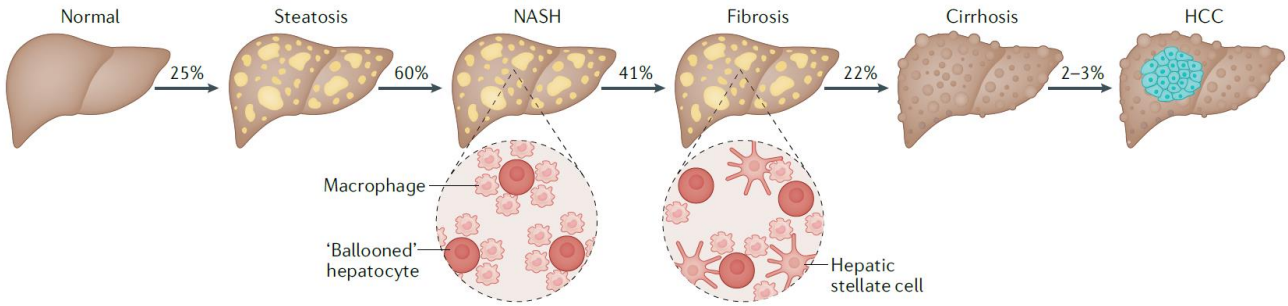

Figure 3- Natural pathophysiological progression of NAFLD. Adapted from (42)

3.4 Clinical Manifestations

NAFLD is a phenotypically polymorphic disease which, owing to its systemic nature, has a variable clinical presentation, a multitude of potentially associated disease and a rich spectrum of laboratory features (43). It tends to remain asymptomatic until progression to end-stage liver disease and decompensation with ascites, hepatic encephalopathy and variceal haemorrhage occurs. While hepatomegaly and central adiposity are frequent, there are no pathognomonic examination findings in NAFLD. If symptoms are present, they are of non-specific nature, such as fatigue, mild right upper quadrant tenderness or epigastric fullness (21).

Because liver is central for the whole-body metabolism, NAFLD leads to changes in cell transcriptional status that may cause a perturbation in energy metabolism, contributing to the development and progression of many chronic diseases, including atherosclerosis and type 2 diabetes mellitus (6).

3.5 Diagnosis

The diagnosis of NAFLD requires the presence of hepatic steatosis by imaging or histology and the absence of significant alcohol consumption, other competing etiologies for steatosis, or coexisting causes of chronic liver disease (44).

Investigation of NAFLD is usually initiated in response to elevated liver transaminases, especially in metabolically predisposed patients (45). However, serum transaminase levels alone are inadequate for detecting fibrosis and identifying which patients will have a benign versus progressive disease course. Indeed, patients at all stages of disease can have normal transaminases (46).

Since the prevalence of NAFLD in those with T2DM and obesity is substantial and most patients with NAFLD have normal liver biochemistry, there is an argument for suspecting NAFLD in all such patients and conducting risk stratification (21).

Once NAFLD is suspected, evidence of hepatic steatosis is necessary to satisfy diagnostic criteria. Quantification of hepatic steatosis is prognostically insignificant, and steatosis often regresses as fibrosis progresses (21). To determine the presence of hepatic steatosis, ultrasound has been recommended as the first-line diagnostic test, since it offers 60–94% sensitivity and 66–97% specificity for hepatic steatosis, is readily available and has a low cost (44). Despite observer dependency, ultrasound, as well as computed tomography (CT) and magnetic resonance imaging (MRI) robustly diagnoses moderate and severe steatosis and provides additional hepatobiliary information (47).

The diagnosis is then further investigated by assessing the presence of liver fibrosis and ultimately performing a liver biopsy in selected individuals (48). Liver biopsy is essential for the diagnosis of NASH and is the only procedure that reliably differentiates NAFL (simple steatosis) from NASH, despite limitations due to sampling variability (47). Nonetheless, it is important to consider that liver biopsy is an invasive procedure that can be painful, has a risk of post biopsy bleeding (up to 2%) and might convey a sampling error, due to only about 1/50.000th of the liver tissue being analysed, while NAFLD is often not equally distributed throughout the liver (49).

Thus, diagnostic decisions should be made individually based on the specific case, the available diagnostic tools and the potential harms and benefits of the different diagnostic tools (49). Effective clinical NASH treatment can only be achieved when fibrosis progression is prevented and/or fibrosis is improved (50).

An ability to identify which patients are at greatest risk for progressing to cirrhosis is essential for targeting therapeutic interventions (51). NASH, particularly when associated with advanced fibrosis, should be identified in patients at risk (age >50 years, type 2 diabetes mellitus [T2DM] or Metabolic Syndrome), because of its prognostic implications (47).

3.5.1 Staging of liver disease

Staging of liver disease can be determined by both invasive and non-invasive techniques, yet the gold standard is still represented by histological examination from liver biopsy. Simple steatosis is characterized by microvesicular accumulation of TGs in hepatocytes, whereas steatohepatitis comprises signs of hepatocellular injury, mitochondrial changes, cell ballooning, and fibrosis (5).

Given that fibrosis is the strongest predictor of outcome, risk stratification based on fibrosis severity can determine who would benefit from liver-directed therapeutic interventions (21). Three important pathological classifications have been proposed for NAFLD: Matteoni's classification, Brunt's classification, and the NALFD activity score (NAS) (52).

In 1999, Matteoni et al presented the first diagnostic criteria to categorize NAFLD into four different subtypes: NAFLD type 1 with fatty liver alone; type 2 with fatty liver plus lobular inflammation; type 3 with fatty liver plus ballooning degeneration; and type 4 with fat accumulation, ballooning degeneration, and either Mallory-Denk bodies or fibrosis (53). They confirmed the benign clinical course of patients with type 1 or 2 NAFLD and the progressive clinical course of patients who had either type 3 or 4 NAFLD. As a result of these differences, these authors defined type 1 and type 2 histological forms of NAFLD as "non-NASH," and type 3 and type 4 as NASH (52).

In the same year as Matteoni's classification system was published, Brunt et al proposed a semiquantitative grading and staging system for NASH. This classification was applicable only to NASH and not to the entire spectrum of NAFLD (52).

Brunt et al classified the necro-inflammatory grades of NASH as grade 1 (mild), grade 2 (moderate), and grade 3 (severe) based on the degree of hepatocellular steatosis, ballooning and disarray, and inflammation (intralobular and portal). At the same time, they proposed a scoring system for staging based on the location and extent of fibrosis: stage 1, zone 3 perisinusoidal fibrosis; stage 2, portal fibrosis with the abovementioned stage 1; stage 3, bridging fibrosis in addition to stage 2; and stage 4, cirrhosis [Table 2] (54).

Later on, in 2005, the NASH Clinical Research Network Pathology Committee created and validated a histological scoring system based on Brunt's classification - NAS - as a semiquantitative instrument by which to judge treatment responses or disease progression (Table 2) (52).

NAS is the unweighted sum of semiquantitative scores for steatosis (0-3), lobular inflammation (0-3), and hepatocellular ballooning (0-2). The magnitude of NAS elevation is closely linked to disease progression in a retrospective population study, and subjects with a high NAS, experience greater fibrosis progression over time (55). Indeed, it was found that most biopsies with a total NAS $\geq$5 were associated with the diagnosis of definite steatohepatitis, thus supporting the NAS activity score. Nevertheless, and although the likelihood of NASH increases with NAS, there is a wide grey zone (NAS 3-4) in which NASH may or may not be present (56).

Table 2- Brunt System for Grading and Staging of Steatohepatitis. Adapted from (57)

ACTIVITY GRADE					FIBROSIS STAGE	
GRADE	Steatosis	Ballooning	Lobular Inflamation	Portal Inflamation	STAGE	Fibrosis
MILD	Involves up to 2/3drs	Occasional, zone 3	Scattered, mild acute and chronic	None or mild	**0**	None
MODERATE	Any degree	Obvious, zone 3	Mild associated with ballooning	Mild to moderate	**1**	Perisinusoidal or periportal fibrosis
SEVERE	Usually more than 2/3drs	Marked, mainly zone 3	Mild to moderate	Mild to moderate	**2**	Perisinusoidal fibrosis with portal or periportal involvement
					3	Bridging fibrosis
					4	Cirrhosis

3.6 Prognosis

NASH is characterized by histologic evidence of progressive hepatocellular injury (ballooning) which can progress to cirrhosis and its complications including hepatocellular carcinoma with eventual need for liver transplant (58). Long-term mortality studies in NAFLD patients during 15 years of follow-up show a 26% risk of death in these patients, 34–69% higher than in the general population of the same age and sex (58).

Although NASH is linked to an increased risk of hepatocellular carcinoma and cirrhosis and has now become the leading cause of liver failure-related transplantation, the majority of patients with NASH will ultimately die as a result of complications of type 2 diabetes mellitus (T2DM) and cardiometabolic diseases (42).

About 90% of NAFLD patients present at least one feature of the metabolic syndrome, while the fully symptomatic metabolic syndrome (diagnosed in 33% patients) is a significant risk factor for the progression from NAFL into NASH. In addition, co-existence of other medical conditions, such as hypothyroidism, hypogonadism, obstructive sleep apnea, polycystic ovary syndrome, and celiac disease, may be associated with or promote the progress of NAFLD into NASH (59).

The overall mortality of NAFLD patients is significantly increased because of both cardiovascular and liver-related complications; and the mortality of NASH patients is higher than of those patients with NAFL (4).

3.7 Management

Management of NAFLD and NASH has become a major challenge to healthcare systems as a consequence of the increasing rates of obesity worldwide (60). Proper dietary and pharmacological measures are essential for preventing NAFLD progression (59).

3.7.1 Non-pharmacological therapy

3.7.1.1 Lifestyle intervention

Lifestyle intervention is the fundamental and, currently, the sole treatment of NAFLD, as no drugs are approved by regulatory agencies (61). The goals of treatment include weight reduction and prevention of metabolic syndrome, and NAFLD progression (59).

Given the high prevalence of overweight/obesity and diabetes in NAFLD patients, attention should be paid to the glycemic index (IG) and the energy value of products (59). Favourable effects were observed in patients on a Mediterranean diet which consists of eating primarily unrefined cereals, vegetables and fresh fruit, olive

oil, and nuts; eating fish, white meat and legumes in moderation; limiting red meat, processed meats and sweets; and drinking wine in moderation (27).

Caloric restriction and exercise are proven to improve liver histology. Even a relatively short period of caloric restriction (28 days) has been shown to markedly improve liver steatosis in a cohort of highly motivated living liver lobe donors whose initial biopsies showed they were not suitable to donate (62).

When it comes to exercise, results from a recent Chinese study supported the current physical activity guidelines (150 min of moderate-intensity activity per week) for the management of NAFLD, and proved that moderate and vigorous-moderate exercise programmes have similar effects on intrahepatic triglyceride content in patients with NAFLD (63).

3.7.1.2 Bariatric surgery

In patients unresponsive to lifestyle changes and pharmacotherapy, bariatric surgery is an option for reducing weight and metabolic complications, with stable results in the long-term (47).

Bariatric surgery very effectively promotes weight loss and its maintenance; the effects on body weight largely exceed the 10% weight loss target associated with clearance of liver fat, resolution of NASH, and reversal of fibrosis. Accordingly, surgery is a possible treatment to reduce the burden of NASH in patients who meet the agreed criteria for the management of obesity (BMI ≥40 or BMI ≥35 with comorbidities) (61).

Very recently, bariatric/metabolic endoscopy has been proposed to facilitate rapid and large weight loss, particularly in type 2 diabetes. These procedures include endoscopic sleeve gastroplasty, endoscopic small bowel bypass, and duodenal mucosal resurfacing. Although apparently safe and effective in the short term, much more data on histological outcomes and adverse events are needed for their extensive clinical application (61).

3.7.1.3 Liver transplantation

Liver transplantation is currently the only recourse for people with advanced NASH presenting with late-stage cirrhosis and/or liver cancer. However, it is not a cure for NASH as it does not address the underlying cause. As long as people exhibit metabolic risk factors, the risk of recurrent NASH remains even after transplantation (64).

Besides, some individuals with NASH may not be eligible for transplantation due to comorbidities related to metabolic syndrome, such as morbid obesity or coexistent CVD (64).

3.7.2 Pharmacological therapy

Hepatic steatosis is a consequence of an imbalance in TG production or uptake into the liver and clearance or removal. Therefore, altering the balance of hepatic TG accumulation and removal by either (or both) reducing fat production or promoting fat clearance is likewise expected to reduce steatosis (65).

As such, and although there is no single drug approved for the treatment of NAFLD and NASH, it is possible to reduce steatosis by directly modulating lipid metabolism within the liver; inhibiting fructose metabolism; altering delivery of free fatty acids from the adipose to the liver by targeting insulin resistance and/or adipose metabolism; modulating glycemia; and altering pleiotropic metabolic pathways simultaneously (65).

With that said, and according to the European Association for the Study of the Liver (EASL) guidelines, pharmacological therapy should be reserved for: progressive NASH (bridging fibrosis and cirrhosis); early stage NASH at high risk for disease progression (age > 50 years, metabolic syndrome, diabetes mellitus or increased ALT); and active NASH with high necro-inflammatory activities (66).

3.7.2.1 Vitamin supplementation

Vitamin supplementation was found to be essential for NAFLD management. Vitamins with antioxidant properties, such as vitamin E and C, were found to decrease ALT and AST serum activity, as well as decrease lipoatrophy and lobular hepatitis without affecting liver fibrosis (59). Vitamin E is one of the most potent micronutrient antioxidants. The antioxidant activity of vitamin E is attributed to the hydroxyl group

in the tocochromanol aromatic ring, which donates hydrogen to neutralize free radicals and ROS (67).

Vitamin E has been assessed in several experimental models of NAFLD. In mice with NASH, dietary α-tocopherol supplementation attenuated LPS-induced liver injury and suppressed methionine-choline-deficient (MCD) diet-induced oxidative stress and inflammation-related pathologies, such as steatosis and necroinflammation (67).

In the PIVENs trial, a randomized control trial examining the benefit of pioglitazone or vitamin E versus placebo in non-diabetic NASH patients, patients treated with 800 IU of vitamin E daily for 96 weeks demonstrated reduced steatosis and inflammation. The use of vitamin E is reserved for biopsy-proven NASH in non-diabetic patients (68).

3.7.2.2 Anti-hyperglycemic agents

3.7.2.2.1 Metformin

Metformin, firstly introduced in the 1950s is currently the recommended first-line agent for the management of diabetes and tends to lower blood glucose through several mechanisms that may be beneficial in patients with NAFLD (69).

The effectiveness of metformin as an antidiabetic drug is explained by its ability to lower blood glucose by decreasing gluconeogenesis in the liver, stimulating glucose uptake in the muscle, and increasing fatty acid oxidation in adipose tissue (70).

The exact mechanism of action of metformin is unclear, but it appears to involve an increased activation of adenosine monophosphate activated protein kinase (AMPK) and/or an alteration of the hepatocellular cytosolic/mitochondrial redox state, consequently reducing hepatic gluconeogenesis (71).

Despite its beneficial effects on body weight reduction, metformin was not associated with improvement of liver histology in a 6-month RCT examining participants with NAFLD. Importantly, retrospective studies have suggested a decline in the rates of hepatocellular carcinoma in metformin-treated humans, but no RCTs demonstrating the effect of metformin on hepatocellular carcinoma have been published (72).

With that said, because of its metabolic effects and its safety profile, metformin remains a promising drug in NAFLD therapy, especially in patients that meet the diagnostic criteria of metabolic syndrome (70).

3.7.2.2.2 Peroxisome proliferator-activator receptors (PPARs) agonists

Agonists of the nuclear transcription factor peroxisome proliferator-activated receptor (PPAR) are ligand-dependent transcription factors that form heterodimers with the retinoid X receptors (RXR), bind to specific regions in the DNA, and finally regulate the transcription of target genes related to lipid and glucose metabolism, inflammatory processes, and cellular differentiation (Figure 4) (73, 74).

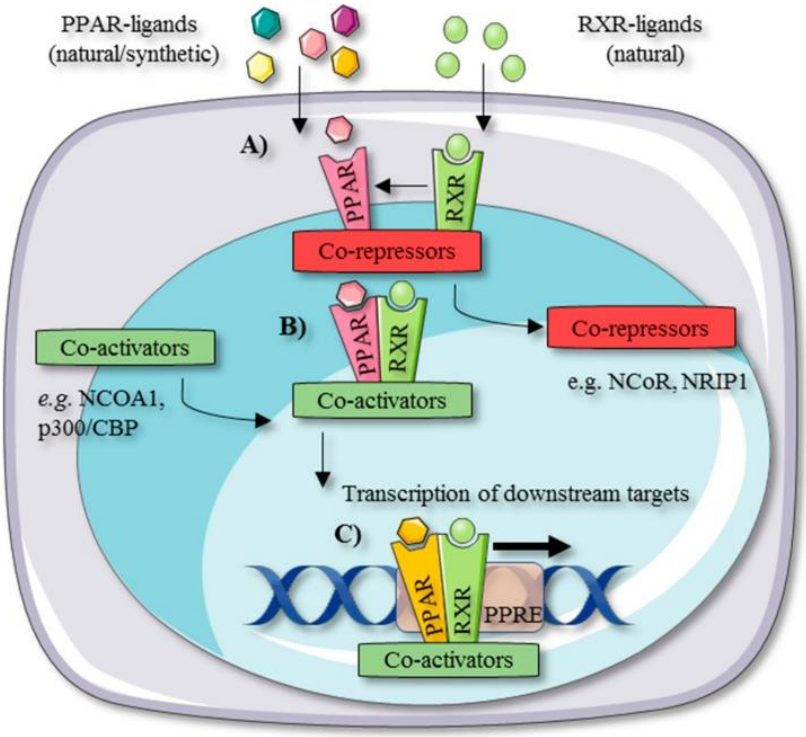

Figure 4- Mechanism of PPAR activation and downstream gene transcription. Adapted from (73)

In mammals, three different isoforms of PPARs have been described so far: PPAR-α, PPAR-β/δ and PPAR-γ (75). Despite sharing high sequence homology and key functions, the PPAR isotypes are encoded by different genes, which are located on different chromosomes, and are more or less specifically expressed in the body (73).

3.7.2.2.2.1 PPAR-α agonists

PPARα, the first member to be cloned among the PPAR isotypes, is expressed in various types of cells in the skeletal muscle, heart, liver, kidney and other metabolically active tissues. PPARα activation was found to increase circulating levels of high-density lipoprotein cholesterol (HDL-C) and decrease serum levels of triglycerides, free fatty acids and apolipoprotein, which improves the overall serum lipid profile and finally exerts positive effects on inflammation and insulin resistance (76).

PPARα agonists, such as fibrates, are extensively used in the treatment of hypertriglyceridemia but have been shown to have no significant benefit in NAFLD, probably because of the receptor's extensive distribution in organs outside the liver (77).

3.7.2.2.2.2 PPAR-β/δ agonists

The second isoform of the PPAR family, PPAR-δ, is ubiquitously expressed and has been implicated in lipid metabolism and energy homeostasis of various organs, including the liver (78). Because of its presence in macrophages, PPAR-β has the additional effect of decreasing macrophage and Kupffer cell activation and increasing fatty acid oxidation (77).

In preclinical models of NASH, PPARδ agonists enhanced hepatic lipid oxidation and insulin sensitivity and reduced steatosis, inflammation and fibrogenesis. MBX-8025, a potent selective PPARδ modulator (EC50 = 2 nm), reduced liver enzyme levels, inflammatory marker levels, insulin resistance and atherogenic dyslipidaemia in overweight patients with dyslipidaemia (78).

3.7.2.2.2.3 PPAR-γ agonists

The third isotype of the PPAR family, PPARγ, is mainly expressed by adipocytes, pancreatic β-cells and immune cells (including macrophages, Kupffer cells and hepatic stellate cells (HSCs)) and is found at lower levels in hepatocytes and skeletal myocytes (78).

PPARγ agonists, such as pioglitazone, are insulin sensitizers acting mainly in adipose tissues, but they are also known to attenuate inflammation and monocyte or macrophage infiltration in the liver and downregulate the pro-inflammatory functions of macrophages in vitro (79). In fact, the best evidence for an improvement of histologically proven NAFLD has been reported for pioglitazone, which demonstrated

reductions in hepatic steatosis and lobular inflammation, without significant effects on fibrosis scores after treatment for 96 weeks (72).

Unfortunately, the use of pioglitazone is limited by its adverse effect profile, which includes weight gain, osteopenia, increased fracture risk, fluid retention, congestive heart failure and bladder cancer. These adverse effects have largely relegated pioglitazone to a second-line treatment, whereby careful consideration of its risk–benefit profile is warranted before starting treatment (80).

3.7.2.2.2.4 Dual PPAR agonists

Dual PPAR agonists or partial agonists, e.g., dual α/γ, α/δ or β/δ were developed with the aim of achieving the TG-lowering and HDL-raising effects of PPAR-α activators as well as the insulin-sensitizing and antihyperglycemic effects of TZDs with a single drug. Such combination of effects would be ideal for the treatment of T2D, MetS and NAFLD, which all share as common features atherogenic dyslipidemia and insulin resistance (75).

The most studied dual PPAR agonist is elafibranor (GFT-505) which is a dual PPARα/δ agonist, aiming to combine the beneficial effects of activating the two receptors. Animal data demonstrate a beneficial effect of elafibranor on serum triglycerides, cholesterol and high-density lipoprotein (HDL), and a reduction in hepatic fat that is mediated, at least in part, by a non-PPARα-dependent mechanism (81).

3.7.2.2.3 Sodium glucose cotransporter (SGLT)-2 inhibitors (SGLT2i)

SGLT-2 inhibitors, one of the newest classes of medications approved for treatment of type 2 diabetes, decrease glucose through a unique mechanism. Normally, the kidneys filter about 180 g of serum glucose per day, with most of it being reabsorbed through glucose transporters (passive transporters) and SGLTs (active transporters that use the sodium gradient). In patients with diabetes, when serum glucose typically exceeds 180 mg/dL, the kidney threshold for reabsorption is exceeded, and glucose begins to be excreted in the urine. By inhibiting SGLT-2, these agents decrease renal

reabsorption of glucose and increase its excretion, therefore improving glycemic control (69).

The main SGLT2 inhibitors include by canagliflozin, dapagliflozin, and empagliflozin, used as second-line treatment of T2DM in association with metformin (82).

Small studies have shown the effect of dapagliflozin in patients with NAFLD and T2DM. In particular, dapagliflozin showed a beneficial effect in patients with NAFLD, although it is still not clear whether the reduction in body weight or visceral adipose tissue caused by dapagliflozin associates with a decrease in liver steatosis or fibrosis (83).

SGLT2 inhibitors have an overall satisfactory safety profile, but some concerns are the risk of genitourinary tract infections and candida vulvovaginitis, bone fractures (particularly with canagliflozin), and normoglycemic ketoacidosis, mainly in the context of low body weight, severely impaired insulin secretion, and low carbohydrate intake (72).

3.7.2.2.4 Glucagon-like peptide 1 (GLP-1) analogues

Glucagon-like peptide 1 (GLP-1) is a hormone that belongs to the incretin group of proteins that is secreted in the distal ileum and proximal colon by L cells in response to meal ingestion. Besides stimulating the pancreas to cause beta cell proliferation and enhancing insulin biosynthesis, GLP-1 also interacts with receptors in other parts of the GI tract and in the lung, kidney, and CNS (77).

GLP-1 analogues, including exenatide, liraglutide, lixisenatide, dulaglutide, albiglutide and semaglutide, are mainly anti-diabetic medications. By activating the GLP receptors, GLP-1 analogues delay gastric emptying, decrease appetite, enhance liver glucose uptake and peripheral insulin sensitivity and increase postprandial satiety and fullness (29, 77).

The mechanism(s) by which GLP1 improves hepatic steatosis, liver inflammation, and injury remain unclear. Although somewhat controversial, the liver is not likely a direct target of GLP1 actions because most studies have not been able to detect expression of the GLP1 receptors in hepatocytes. It is possible that improved

steatosis seen with GLP1-based therapies is secondary to weight loss, improved glycemic control and effects on inflammation or the gut microbiota. Regardless, a treatment that targets multiple components of metabolic derangement present in NASH, including body weight, glycemia, hepatic steatosis and inflammation, is highly desirable (65).

The most compelling evidence to date for the potential of the GLP-1 mechanism in NASH arises from the LEAN (Liraglutide Efficacy and Action in NASH) trial. The GLP-1R agonist liraglutide (dose ¼ 1.8 mg given once daily for 48 weeks to overweight and obese subjects with a diagnosis of NASH) resulted in increased resolution of NASH and importantly, reduced progression of fibrosis, compared with the placebo group (65).

3.7.2.2.5 Dipeptidyl peptidase-4 (DPP-4) inhibitors

DPP4, also known as adenosine deaminase binding protein or cluster of differentiation 26 (CD26), is a serine exopeptidase able to inactivate various oligopeptides through the removal of N-terminal dipeptides (17).

Individuals with NAFLD and insulin resistance have elevated plasma DPP-4 activity, which is consistent with lower GLP1 and GIP levels in the blood of these individuals. Consistent with human studies, liver-specific overexpression of DPP-4 impairs whole-body glucose tolerance in high-fat-fed mice, effects that are linked to reduced circulating GLP-1. In agreement with these observations, genetic ablation or administration of oral DPP-4 inhibitors such as vildagliptin or sitagliptin improves both hepatic steatosis and glucose tolerance, further highlighting the systemic and autocrine/paracrine actions of DPP-4 (84).

3.7.2.3 Modulators of bile acid and metabolism

3.7.2.3.1 Farnesoid X receptor agonists

Farnesoid X receptor (FXR) is a ligand-activated transcription factor involved in the control of bile acid (BA) synthesis and enterohepatic circulation. Bile acids are natural ligands of the FXRs and regulate expression of the gene encoding for CYP7A1, the rate limiting enzyme in bile acid synthesis (9). FXR activation reduces bile acid

synthesis by inhibiting the conversion of cholesterol to bile acids, and it holds anti-inflammatory and antifibrogenic activity (83).

In addition to its principal effect on bile acid homeostasis, FXR signalling has several pleiotropic functions on various metabolic pathways. It has been demonstrated that FXR activation lowers plasma glucose (represses gluconeogenesis), free fatty acids (enhancing β oxidation of FFAs via PPAR-α), TGs (repressing TG synthesis and promoting TG clearance via Apo-CII) and improves insulin sensitivity (9).

This class of drugs probably has the largest body of evidence in NASH to date with short-term results from a trial of obeticholic acid (already licenced for the treatment of primary biliary cholangitis) recently demonstrating some benefit over placebo. As a class, FXR agonists are associated with pruritus and appear to increase serum low-density lipoproteins (46).

3.7.2.3.1.1 Obeticholic Acid (OCA)

Obeticholic acid (OCA), the first drug in this class under investigation for NASH, is derived from the primary human bile acid, chenodeoxycholic acid, which is a natural FXR agonist. As a result of synthetic modification, OCA stimulates FXR activity 100-fold more intensely than chenodeoxycholic acid (83).

A recent randomized, double-blinded placebo-controlled trial of non-diabetic NASH patients demonstrated improvement in histologic NASH in 45% of patients who received obeticholic acid versus 21% of placebo patients. In addition, patients who received obeticholic acid had improvement of fibrosis compared with placebo (68).

3.7.2.3.2 Liver X receptor alpha (LXR-α) inhibitors

Liver X receptors (LXRs), belonging to the nuclear receptor's superfamily, are activated by specific oxidized forms of cholesterol and intermediate products of the cholesterol biosynthetic pathway (85). When activated, LXR stimulates fatty acid synthesis (lipogenesis) in hepatocytes and intensifies production of triglyceride rich large VLDL particles in the liver (86).

By inhibiting LXRs, oltipraz, an LXR isoform, exerts antisteatotic effects. It activates adenosine monophosphate activated protein kinase (AMPK) and inactivates S6K1, affecting LXR-α thus reducing lipogenesis and increasing lipid oxidation (87).

Results from a phase 2 study in patients with a liver fat content of >20% and elevated liver enzymes has shown that oltipraz is capable of reducing liver fat content in a dose-dependent manner. However, the absolute changes in insulin resistance and the levels of liver enzymes, lipids, and cytokines were not significantly different among the placebo group and the oltipraz receiving group (83).

3.7.2.3.3 FGF analogues

Fibroblast growth factors (FGF) are a group of signalling proteins involved in cell proliferation and in glucose regulation and lipid metabolism. FGF21, in particular, is largely produced by the liver and is mainly responsible for the regulation of sugar intake via hypothalamic signalling and stimulation of adipocyte glucose uptake and mitochondrial function (88, 89).

Pegbelfermin, also known as BMS-986036, is a pegylated FGF21 analogue already tested in a phase IIa 16-week trial in patients with NASH. A significant decrease in absolute hepatic fat fraction was found in patients given 10mg pegbelfermin daily (-6.8% versus -1.3% in those receiving placebo; P=0.0004) or 20mg weekly (-5.2% versus -1.3%, respectively; P=0.008) (90).

Fibroblast growth factor 19 (FGF19) on the other hand, is responsible for regulating bile acid synthesis and glucose homeostasis (83). Bile acids stimulate- via FXR- the ileal transcription of FGF19, which decreases bile acid synthesis; levels of FGF19 are reduced in NAFLD and metabolic syndrome (89).

Aldafermin (NGM282) is an engineered FGF19 analogue evaluated in a study of 82 patients (37% with T2DM) with biopsy proven NASH with NAS ≥4 and fibrosis stages 1–3. At 12 weeks, 74% of patients receiving 3mg daily and 79% of those receiving 6mg daily achieved at least a 5% reduction in absolute liver fat content from baseline. The study met the primary end point of a statistically significant reduction in liver fat content that was 5% higher than in those receiving the placebo (90).

3.7.2.4 Novel modulators of lipid metabolism

3.7.2.4.1 Thyroid hormone receptor (THR)-β agonists

Thyroid hormones regulate many processes involved in hepatic triglyceride and cholesterol metabolism to decrease serum cholesterol and intrahepatic lipid content. The thyroid hormone functions as a ligand to its two receptors, thyroid hormone receptor-α (THR-α) and thyroid hormone receptor-β (THR-β). Although both isoforms are expressed in most tissues, THR-β is the major form expressed in the liver, whereas THR-α is highly expressed in the heart and bone (90). THR-β is responsible for regulating specific metabolic pathways in the liver, often impaired in NAFLD (61).

Resmetirom and VK2809 are two orally effective agonists of THR that are liver-directed with a severalfold higher selectivity for THR-β (90).

Resmetirom (MGL-3196) is a once daily, oral, highly selective agonist of THR-β specifically acting in the liver, without systemic effects (mediated through THR-α in the heart and bone). The mechanism by which resmetirom reduces hepatic fat in NASH is probably dependent on the restoration of normal mitochondrial function and increased β oxidation (61). MGL-3196 administration to healthy volunteers with mildly elevated cholesterol was well tolerated with reductions in total cholesterol, LDL and TG levels observed at doses >50 mg (65).

3.7.2.4.2 Acetyl Coenzyme A Carboxylase (ACC) inhibitors

Acetyl-CoA carboxylase is an enzyme responsible for the conversion of acetyl-coenzyme A (acetyl-CoA) to malonyl-CoA, the rate-limiting step in *de novo lipogenesis*. ACC has two isoforms. The ACC1 isoform catalyses the formation of malonyl-CoA, the main substrate for fatty acid biosynthesis in the cytosol. ACC2 is located in the mitochondria, where malonyl-CoA serves as a potent allosteric inhibitor of carnitine palmitoyl-transferase (CPT) 1, the carrier protein of fatty acids into mitochondria for β-oxidation. Inhibition of ACC1 and ACC2 would be expected to reduce DNL and enhance mitochondrial β-oxidation, respectively, supporting ACC inhibition as a therapeutic target in NASH (83).

3.7.2.4.3 Stearoyl-CoA desaturase (SCD) 1 inhibitors

Stearoyl-CoA desaturase 1 (SCD1) is a fatty acid desaturase, highly expressed in lipogenic tissues, such as adipose and liver, that converts saturated fatty acids, such as palmitate and stearate, to monounsaturated fatty acids. By inhibiting SCD1, aramchol, reduced hepatic oxidative stress, inflammation and fibrosis in a mouse model of NASH and decreased levels of lipids in the liver, liver enzymes and HbA1c in patients with NAFLD (42).

3.7.2.5 Lipid-altering agents

To limit the risk of cardiovascular events, NAFLD patients with dyslipidemia should be treated with statins, ezetimibe or omega-3 polyunsaturated fatty acids (PUFAs); however, these drugs have shown no significant effect on liver histopathology. Several novel compounds interfering with lipid metabolism are being tested for their efficacy in NAFLD treatment (59).

Despite patients with T2D and NAFLD being at a higher risk of CVD compared with patients with diabetes alone, statins are under-prescribed in NASH mainly due to concerns regarding their safety in patients with deranged liver function tests (LFTs) (91).

However, the use of statins in patients with NAFLD or NASH is associated with a reduction in transaminase plasma levels and improvements in steatosis and the liver necro-inflammatory grade. Some evidence suggests that patients with NAFLD have a higher 10-year risk of cardiovascular disease than patients without NAFLD. In the GREACE study, a post hoc analysis showed that statin therapy is safe and can improve the plasma levels of liver enzymes and reduce cardiovascular events in patients with mild abnormal liver tests attributed to NAFLD (92).

Ezetimibe, on the other hand, exerts its function by inhibiting cholesterol absorption from the intestinal lumen into enterocytes. The molecular target of ezetimibe is the sterol transporter Niemann-Pick C1-like 1 protein (NPC1L1). Human NPC1L1 is abundantly expressed in the liver and may facilitate the hepatic accumulation of cholesterol. In the context of hyperlipidemia, ezetimibe is usually given in combination with other hypolipidemic drugs which leads to complementary results in terms of cardiovascular disease risk factors due to the different mechanisms of action (93).

3.7.2.6 Anti-fibrotic agents

Even if effective treatments for NASH are found, some patients will continue to present with advanced fibrosis or cirrhosis and thus effective antifibrotic agents may always be needed. Effective anti-NASH drugs will likely have indirect antifibrotic effects by eliminating the stimulus for fibrogenesis, but some drugs have been designed to be directly antifibrotic (e.g., the galectin-3 inhibitor MD-02) or increasing extracellular matrix turnover (e.g. simtuzumab) and are currently in clinical trials (51).

3.7.2.6.1 Chemokine receptors (CCR) 2 and 5 antagonists

CCR2 (C-C motif chemokine receptor 2) and CCR5 have been implicated in the pathogenesis of NAFLD through their promotion of local macrophage infiltration and fibrogenesis (94). The ligand of CCR2, C-C chemokine ligand 2 (CCL2) is secreted by Kupffer cells when hepatocytes are injured, causing recruitment of monocytes in the liver and maturation of monocytes into macrophages. The macrophages then secrete cytokines, which activate hepatic stellate cells that stimulate collagen and promote hepatic fibrosis. CCR2 and CCR5 promote activation and migration of Kupffer cells and hepatic stellate cells and increase inflammatory cells (95).

Cenicriviroc (CVC) is an oral dual antagonist of C-C motif chemokine receptor CCR types 2 and 5, which prevents macrophage trafficking and efficiently inhibits monocyte infiltration. CCR2 antagonism by CVC is expected to reduce the recruitment, migration, and infiltration of proinflammatory monocytes and macrophages at the site of liver injury. CVC-mediated antagonism of CCR5 is expected to additionally impair the migration, activation, and proliferation of collagen-producing activated hepatic stellate cells/myofibroblasts (88)

4 Conclusions

NAFLD currently represents one of the commonest sources of liver disease in the Western world and the growing levels of obesity, diabetes and metabolic syndrome will ensure that it remains a major cause of morbidity and mortality.

One of the biggest problems with NAFLD is that it hardly manifests specific and easy to identify symptoms which makes the diagnosis of the disease frequently incidental. Once diagnosed it is important for the patient to be aware of the prognosis of the disease and to be encouraged to improve its lifestyle starting with a change in diet.

Current treatment strategies for NASH focus on improving components of the metabolic syndrome, such as obesity and IR, with no liver-specific agents yet being available. However, modulation of any of the multiple mechanisms involved in NASH pathogenesis could provide valuable targets to prevent the development of fibrosis and its associated complications (Figure 5). This knowledge, and the significant advances that continue to be made in our understanding of the pathogenesis of NASH, are required to develop novel therapeutic strategies for this increasingly critical condition.

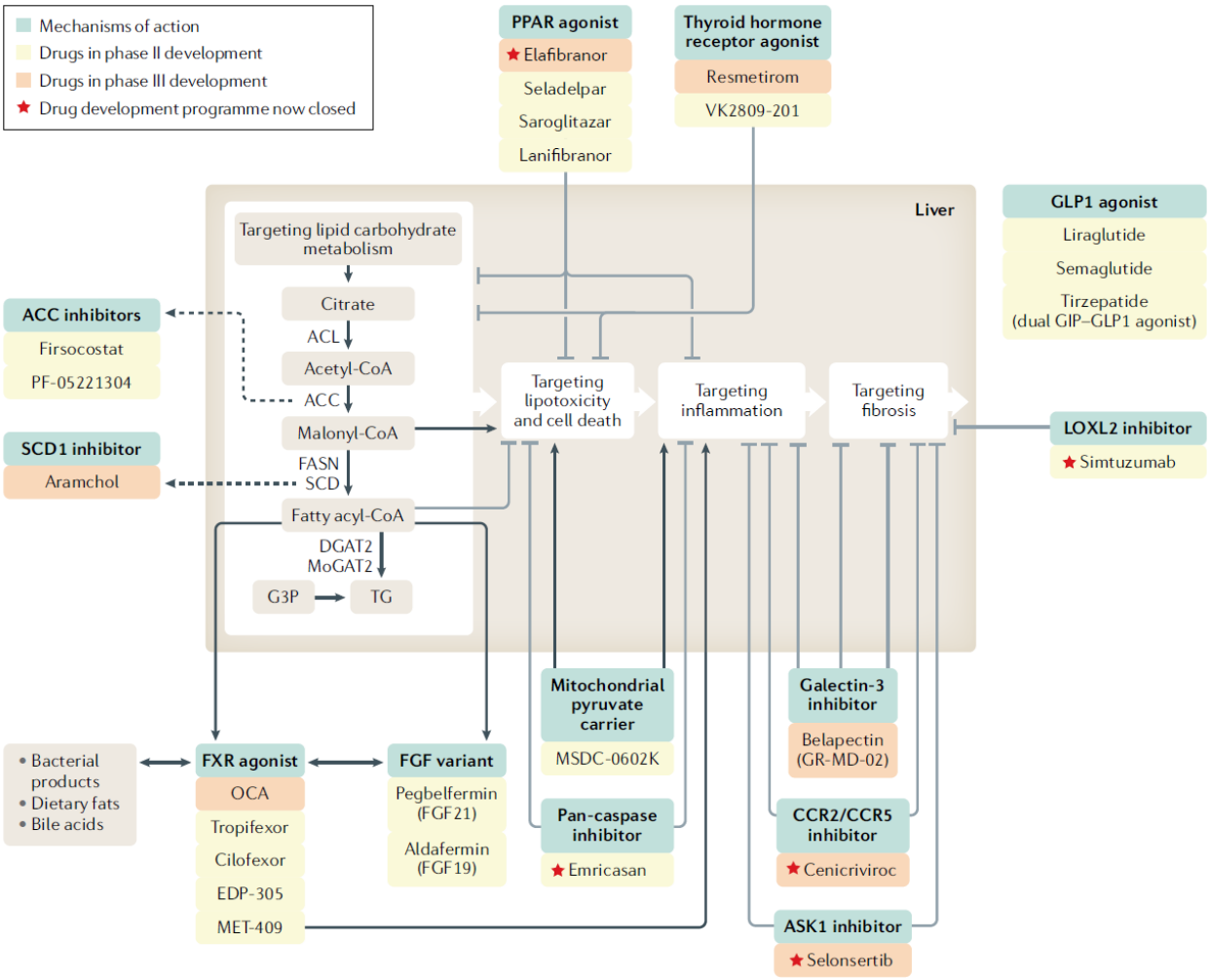

Figure 5- Mechanism of action of NASH drugs currently under development. Several drugs are now in phase II and phase III development. The target population for these studies are patients with intermediate and advanced fibrosis (F2-F4 fibrosis) which are at greatest risk of overall and disease specific mortality. ACC, acetyl CoA carboxylase; ACL, ATP-citrate lyase; CCR, CC-chemokine receptor; DGAT2, diacylglycerol O-acyltransferase 2; FASN, fatty acid synthase; FGF, fibroblast growth factor; FXR, farnesoid X receptor; G3P, glycerol-3-phosphate; GLP1, glucagon-like peptide 1; MoGAT2, monoacylglycerol O-acyltransferase 2; OCA, obeticholic acid; PPAR, peroxisome proliferator-activated receptor; SCD, stearoyl-CoA desaturase; TG, triglyceride. Adapted from (90).

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