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LEADING ARTICLE

Nonalcoholic Fatty Liver Disease: Current Issues and Novel Treatment Approaches

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Abstract Nonalcoholic fatty liver disease (NAFLD) is considered the most common liver disorder in the Western world. It is commonly associated with insulin resistance, obesity, dyslipidaemia, type 2 diabetes mellitus (T2DM) and cardiovascular disease. Nonalcoholic steatohepatitis (NASH) is characterized by steatosis with necroinflammation and eventual fibrosis, which can lead to end-stage liver disease and hepatocellular carcinoma. Its pathogenesis is complex, and involves a state of 'lipotoxicity' in which insulin resistance, with increased free fatty acid release from adipose tissue to the liver, play a key role in the onset of a 'lipotoxic liver disease' and its progression to NASH. The diagnosis of NASH is challenging, as most affected patients are symptom free and the role of routine screening is not clearly established. A complete medical history is important to rule out other causes of fatty liver disease (alcohol abuse, medications, other). Plasma aminotransferase levels and liver ultrasound are helpful in the diagnosis of NAFLD/NASH, but a liver biopsy is often required for a definitive diagnosis. However, there is an active search for plasma biomarkers and imaging techniques that may non-invasively aid in the diagnosis. The treatment of NASH requires a multifaceted approach. The goal is to reverse obesity-associated lipotoxicity and insulin resistance via lifestyle intervention. Although there is no pharmacological agent approved for the treatment of NAFLD, vitamin E (in patients without T2DM) and the thiazolidinedione pioglitazone (in patients with and without T2DM) have shown the most consistent results in randomized controlled trials. This review concentrates on our current understanding of the disease, with a focus on the existing therapeutic approaches and potential future pharmacological developments for NAFLD and NASH.

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1 Introduction

Nonalcoholic fatty liver disease (NAFLD) represents a broad spectrum of disorders defined by accumulation of fat in the liver. It ranges from simple hepatic steatosis through lobular inflammation (nonalcoholic steatohepatitis or NASH) to variable degrees of fibrosis, cirrhosis and even hepatocellular carcinoma [1, 2]. There is an increasing awareness of and interest in NAFLD because it is considered the leading cause of abnormal liver aminotransferase levels and chronic liver disease. The prevalence of NAFLD and NASH are escalating given the worldwide epidemic of obesity and their strong association with the metabolic syndrome (MetS). The biological mechanisms underlying fatty liver disease occurrence and progression to NASH and its co-morbidities remain incompletely understood. However, there is increasing recognition about the role of fatty acids in promoting liver injury through lipotoxicity [3–6]. Fatty liver infiltration and lipid-induced mitochondrial dysfunction and oxidative stress to the hepatocyte are believed to be major factors behind the progression of the disease from simple fatty infiltration of the liver to hepatocellular damage, inflammation and progressive liver

disease [3, 7–11]. Treatment of NASH will require the reduction of insulin resistance and obesity-associated metabolic damage through lifestyle interventions that induce weight loss or through pharmacotherapy with insulin sensitizers (pioglitazone) and/or reversal of oxidative stress (vitamin E), all of which have shown improvements in patients with NAFLD [12–14].

This article reviews our current understanding about NAFLD, with a particular focus on existing and future therapeutic options, trying to piece together our incomplete knowledge about how to approach and manage these complex patients.

2 Nonalcoholic Fatty Liver Disease (NAFLD) and Nonalcoholic Steatohepatitis (NASH)

2.1 Prevalence of NAFLD in the General Population and High-Risk Groups

The prevalence of NAFLD is estimated to be between 30 % and 50 % [15–18], depending on the methods used for screening, and as high as 90 % in morbidly obese subjects [17, 19, 20]. In the US, using the most sensitive technique to diagnose NAFLD, magnetic resonance imaging and spectroscopy (MRS), the prevalence of fatty liver has been reported to be 34 % [15]. The metabolic environment plays a key role in the pathogenesis of the disease, as NAFLD is frequently associated with components of the MetS such as obesity, dyslipidaemia, insulin resistance and type 2 diabetes mellitus (T2DM) [21, 22]. Moreover, patients with cryptogenic cirrhosis due to NASH who become liver transplant recipients frequently develop NASH in the new liver [23]. However, not all subjects with the MetS develop fatty liver, and the majority of subjects with hepatic steatosis do not develop NASH [15]. Therefore, the development of NASH is a consequence of both genetically and environmentally determined factors.

The prevalence of abnormal glucose metabolism in patients with NAFLD ranges from 9 % to 31 % if based only on self-reported diagnosis or the fasting plasma glucose concentration [24–26]. However, when patients with NAFLD have systematically been screened for impaired glucose tolerance (IGT) or T2DM with an oral glucose tolerance test (OGTT), the rate of abnormal glucose metabolism is much higher [27–29]. In our experience, when obese patients believed to have normal glucose metabolism are thoroughly screened with an OGTT, the prevalence of impaired fasting glucose (IFG) or IGT, and of newly diagnosed T2DM, is about three-fold higher in patients with fatty liver than in those without the disease [30].

Ethnic variations have also been suggested in patients with NAFLD. Hispanics have been reported to have a higher prevalence rate for NAFLD than African Americans or Caucasians [15, 18, 24, 31]. However, in these studies, Hispanics had a higher prevalence of well known risk factors for NAFLD, such as obesity, insulin resistance and T2DM. When Hispanics and Caucasians are matched for adiposity (body mass index [BMI], total body fat by dual-energy x-ray absorptiometry [DXA]), the severity of NASH is no different [32], suggesting that previously described ethnic differences were more a reflection of the unfavourable metabolic risk of Hispanics in these studies [15, 18, 24, 31].

NAFLD has also been recognized among children, reflecting rising paediatric obesity rates. In the US, the prevalence of elevated plasma aminotransferase levels in obese adolescents was reported as 10 %, and higher in the Hispanic population [33]. This likely underestimates the true prevalence of NAFLD in children, as few paediatric studies include imaging or biopsies; yet, NAFLD can be present with normal liver enzymes [34]. A retrospective hospital-based cohort study has demonstrated that children with NAFLD have a significantly shorter long-term survival and a 13.8-fold higher risk of dying or requiring liver transplantation than the age- and sex-matched general population [35].

2.2 Natural History of NAFLD

NAFLD presents with a broad clinical spectrum that ranges from asymptomatic patients with normal liver aminotransferase levels to those with steatohepatitis and cirrhosis with complications of organ failure or hepatocellular carcinoma. It is believed that 40 % of patients who have NAFLD may go on to develop NASH [36–38], although the natural history is not well established. There are few studies that focus on the natural history of NAFLD [13, 39–44]. The need for paired liver biopsies has been a major limitation and a determinant of the restricted number of studies available. Despite other shortcomings such as their retrospective nature, variable length of follow-up and inclusion of individuals with advanced disease at baseline, among others, the available studies have provided useful information about the progression of the disease over time.

Figure 1 shows the most relevant studies indicating that NASH may progress in a significant number of patients [13, 39–41, 43–45]. Indeed, NASH is now recognized as a major cause of cryptogenic cirrhosis [24, 43, 46, 47]. In studies that included patients with 4 years or more of follow-up, progression of fibrosis occurred in 32–41 % of patients [39, 40, 43]. Duration is critical when establishing the risk of disease progression, with more severe NASH and advanced fibrosis reported in studies with longer

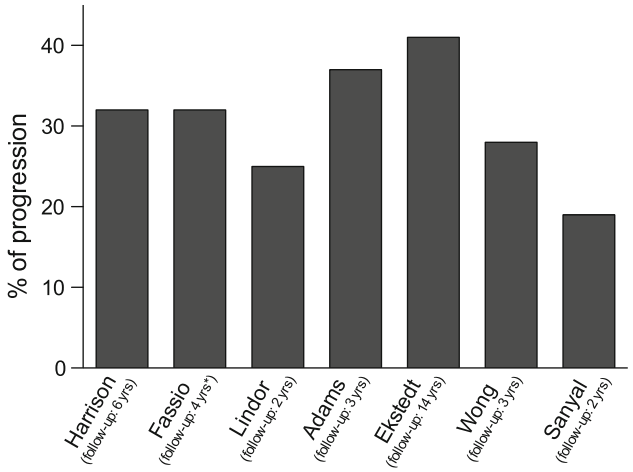

Fig. 1 Progression of nonalcoholic steatohepatitis evaluated in studies with paired liver biopsies. Follow-up reported as mean years unless otherwise noted. * follow-up reported as median years

follow-up [43]. Recognition of factors associated with disease progression has been a challenge, although the presence of obesity, diabetes and the initial stage of fibrosis are major determinants of poor prognosis [40, 42–44]. In general, while very high aminotransferase levels suggest the presence of NASH, there is poor correlation between plasma levels of liver enzymes and histology [39, 43], and most controlled studies have found them of limited value in monitoring treatment [40–42, 44]. Furthermore, there are many reports of advanced liver disease and unanticipated cirrhosis in NASH with normal plasma liver aminotransferase levels [24, 48–50].

Long-term mortality in NASH is a highly controversial area and, as yet, there is no definitive answer. The long-term liver-related prognosis is closely correlated with severity of steatohepatitis and, in particular, of fibrosis. As shown in Fig. 2, compared with the general population, patients with simple steatosis have a good long-term prognosis. Overall mortality is not increased and patients

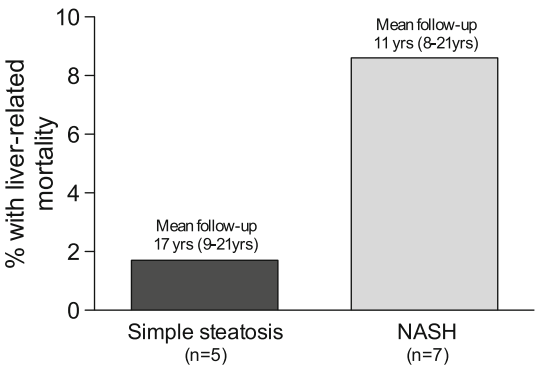

Fig. 2 Long-term liver-related mortality in patients with nonalcoholic fatty liver disease. *n* number of studies included

rarely died of liver-related complications [42, 43, 51, 52, 162–165]. However, these data come from only a few studies and they carry limitations such as small sample size, lack of standardized follow-up and variable duration of follow-up.

In contrast, patients with NASH have approximately a three-fold increase in liver-related mortality (from 1.7 % to 8.6 %; Fig. 2). Indeed, some studies have suggested that patients with NASH have higher overall mortality rates than patients with simple steatosis [43, 51, 52]. Soderberg et al. [52] reported, in a study with a mean duration of 21 years, that there was a clear difference in the overall survival of subjects with NAFLD when divided among those with only fatty liver or subjects with fat and any type of inflammation, ballooning or fibrosis. These studies suggest that a subgroup of patients with NAFLD may be at risk of disease progression to more severe disease and higher liver-related mortality. However, the challenge remains in the early diagnosis of these individuals and identification of long-term strategies to halt liver damage [53].

2.3 Role of Type 2 Diabetes Mellitus (T2DM): Prevalence in NAFLD and NASH

Whether liver steatosis is a cause or a consequence of the metabolic imbalances in insulin sensitivity is poorly understood. Dysfunctional fat releases excessive amounts of free fatty acids (FFA), leading to ectopic fat deposition in tissues that are poorly adapted to triglyceride (TG) accumulation such as liver, muscle and pancreatic β cells [54, 55]. This may lead from peripheral (muscle) insulin resistance to NASH (hepatocyte lipotoxicity) and T2DM (pancreatic β-cell lipotoxicity). Therefore, NAFLD and T2DM have a very close relationship as they share pathogenic defects, and may promote or exacerbate each other. In the general population, elevated plasma liver aminotransferase levels are associated with a greater risk of having T2DM [56, 57]. On the other hand, it is believed that the majority of patients with diabetes have a fatty liver and that as many as 50 % may have NASH [58]. In addition, T2DM worsens liver disease, although the underlying mechanisms remain unclear [59, 60]. Several studies have reported that the presence of T2DM is associated with a two- to four-fold increase in serious liver disease [37, 43], cirrhosis and hepatocellular carcinoma [38, 47, 59, 61, 62]. In patients with NAFLD, the presence of T2DM is associated with worse insulin resistance at the level of the liver and adipose tissue [32, 63] and more severe liver histology (NAFLD activity score [NAS] and fibrosis) [24, 32, 43, 46]. Screening of patients with T2DM for NAFLD may also be useful in implementing early intervention in NAFLD and to prevent long-term complications of T2DM. A recent study by Ortiz-Lopez et al. [30], using the

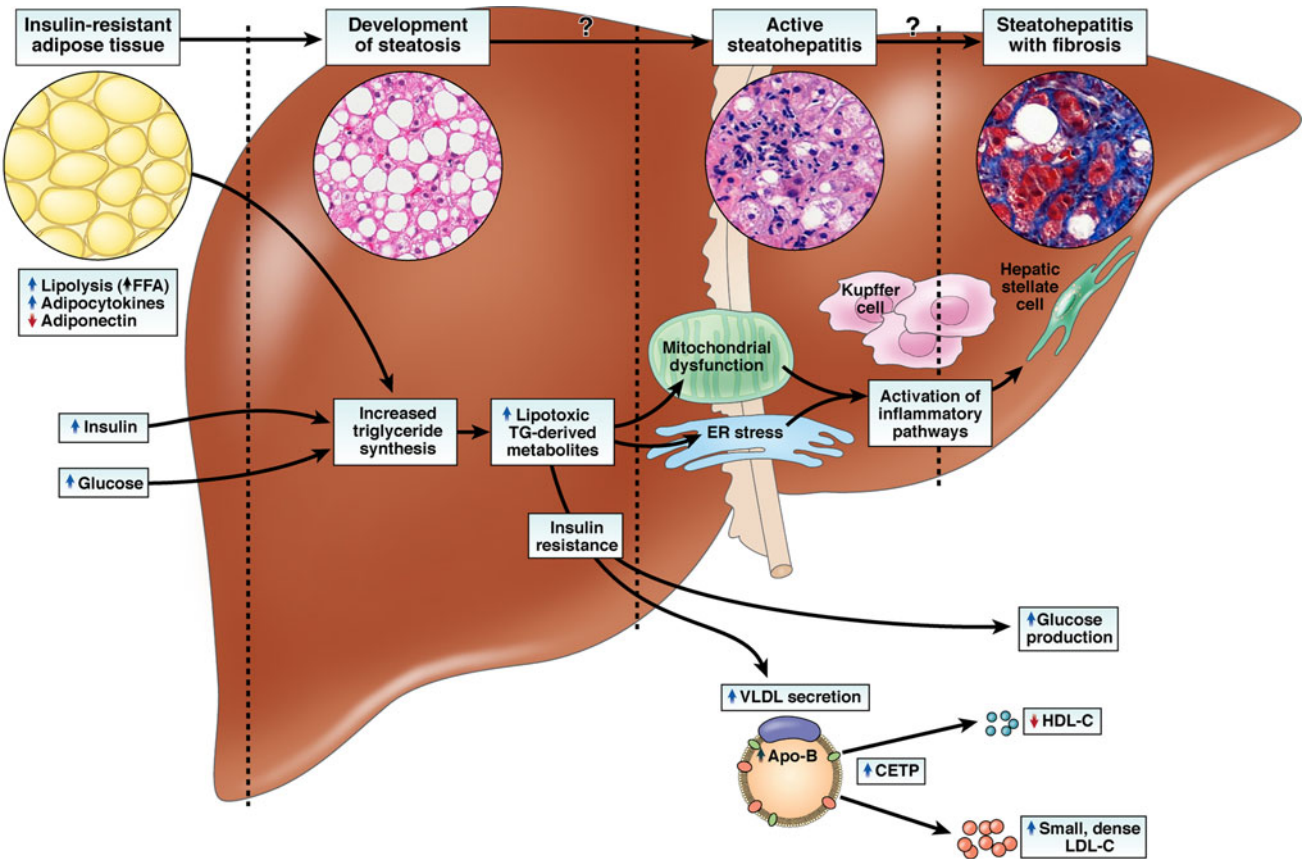

Fig. 3 Pathogenesis of nonalcoholic steatohepatitis (see text for details). Reproduced from Cusi [6], with permission from Elsevier. *Apo-B* apolipoprotein B, *ER* endoplasmic reticulum, *CETP* cholesterylester transfer protein, *FFA* free fatty acid, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *TG* triglyceride, *VLDL* very low-density lipoprotein

gold-standard OGTT found that T2DM was three-fold higher in obese patients with NAFLD than in patients without fatty liver (83 % vs. 35 %; $p < 0.0001$).

3 Pathogenesis

The pathogenesis of NAFLD and its transition to NASH involve complex interactions between alterations in nutrient metabolism, hormonal deregulation and onset of inflammation in multiple organ systems (Fig. 3). Insulin resistance is a key component of NAFLD that favours high rates of FFA flux to the liver from increased adipose tissue lipolysis [6, 64], suggesting that the lipotoxic environment created by insulin-resistant adipose tissue may mediate disease progression in the liver. When we compared 187 middle-aged obese patients with biopsy-proven NASH with controls well matched for obesity measured by DXA but without NAFLD, the severity of adipose tissue insulin resistance maintained a close association with metabolic and histological damage in patients with NASH [50]. This suggests that it is not only the amount of fat, but also its degree of dysfunction and insulin resistance that accounts more for the development of NASH. Whole body insulin resistance and the accompanying hyperinsulinaemia results in a concomitant increase in hepatic lipogenic gene expression, primarily mediated by sterol regulatory element-binding protein (SREBP)-1c and carbohydrate response element binding protein (ChREBP), thus promoting increased hepatic TG synthesis [5, 65]. Even in the presence of high rates of TG synthesis [5] and saturated very low-density lipoprotein (VLDL) export [66], hepatic mitochondrial tricarboxylic acid cycle (TCA) activity, which is the dominant route of fat oxidation in humans, continues unabated in NAFLD subjects [67]. An overactive TCA cycle has the potential to overload the hepatic mitochondrial electron transport chain (ETC) with reducing equivalents and thus accelerate the production of reactive oxygen species (ROS). In fact, defects in mitochondrial morphology [68], ETC [69] and ATP production [70, 71] have been documented in patients with NASH along with high levels of ROS and other mediators of inflammation [65]. Hepatocellular lipid accumulation, together with high ROS production, in turn results in a cascade of events leading to higher rates of lipid peroxidation, formation of cytotoxic aldehydes and production of proinflammatory

cytokines, resulting in DNA damage and eventual cell death [65]. This spectrum of mitochondrial alterations constitutes what is known as 'mitochondrial dysfunction', which is now believed to be a major determinant in the pathogenesis of NAFLD. At the same time, FFA overload on the mitochondria also results in diversion of a small portion of FFA from the normal oxidative routes (β-oxidation and TCA cycle) to synthesis of potentially toxic lipid intermediates such as ceramides and diacylglycerol [72, 73]. These lipid byproducts are potent inhibitors of insulin signalling in multiple tissues, including the liver [74, 75] and further, also elicit multiple inflammatory pathways (e.g. c-Jun N-terminal kinase, IKK/nuclear factor κB, TLR4) [6, 76]. Even though we do not fully understand the mechanisms mediating the transition of simple liver steatosis (NAFLD) to NASH and ultimately cirrhosis, several of the above mechanisms may act singly or in combination to aid in this transformation.

4 Diagnosis

The diagnosis of NAFLD involves liver fat accumulation by imaging or histology, in the absence of significant alcohol consumption and other co-existing causes for chronic liver disease, such as viral hepatitis, Wilson's disease and medications, among others [53]. NAFLD is frequently underdiagnosed, as most of the time it is asymptomatic. Several non-invasive methods are currently used for diagnosis of NAFLD (Table 1). In clinical practice, plasma liver aminotransferase levels and ultrasound are the most common diagnostic techniques. Both are widely available, easy to perform and have low cost; however, their sensitivity for fatty liver is less than that of MRS [77]. Computed tomography (CT) provides a semi-quantitative method and can be used to diagnose moderate to severe hepatic steatosis, but has relatively low sensitivity and is not usually used to this end [78]. MRS remains as the gold-standard technique with a high sensitivity and specificity for steatosis (>5.5 % intrahepatic TG content considered diagnostic for NAFLD), involves no radiation and allows quantification of liver steatosis, but it has limited availability and requires expensive hardware and software, making each test costly. In recent years, transient elastography has been proposed for assessing liver fibrosis. It is easy to use but operator dependent and a high BMI reduces the accuracy of the study [79]. Neither MRS nor transient elastography are US FDA approved and their availability is currently limited to research settings.

Significant effort is on-going to find a non-invasive method for diagnosing NAFLD. Combinations of clinical and plasma scoring systems based on clinical and plasma biochemical measurements have been suggested, with variable degrees of success [80]. Recently, caspase-cleaved cytokeratin (CK)-18 fragments have been studied as a promising biomarker in steatohepatitis [81]. In NASH, there is significant caspase activation and hepatocyte cell death by apoptosis [82] with release of CK-18 fragments into the bloodstream, which allows their measurement. Several studies have demonstrated significant elevation of this protein in steatohepatitis when compared with patients with liver steatosis without NASH [81–85]. When we analysed CK-18 fragments, we found that in patients with NAFLD levels were clearly elevated compared with those

Table 1 Non-invasive diagnostic methods for nonalcoholic fatty liver disease

Method	Pros	Cons
AST/ALT	Low cost, safe, widely available	LFAT unknown, low sensitivity
Ultrasonography	Low cost, safe, widely available, no radiation	LFAT not quantifiable, ↓ accuracy in obesity, operator-dependent, ↓ sensitivity if LFAT <30 %
Computed tomography	Widely available	LFAT not quantifiable, high cost, radiation exposure
MRS	Quantification of LFAT, good correlation with histology, no radiation exposure	High equipment cost, expensive testing, limited availability (research only)
Transient elastography	Ease of use, XL probe for obesity available	High equipment cost, needs greater validation, ↓ accuracy in obesity, limited availability (research only)
Combined clinical and plasma scoring systems	Most routinely available, acceptable accuracy for diagnosing, potential clinical utility	Need for greater validation, limited for staging fibrosis
Plasma CK-18	Potential for differentiation of NAFLD/NASH with or without fibrosis, may be combined with other markers, commercially available	Need for greater validation, limited value in borderline or moderate disease, moderate cost

ALT alanine aminotransferase, *AST* aspartate aminotransferase, *CK-18* cytokeratine-18, *LFAT* liver fat, *MRS* magnetic resonance imaging and spectroscopy, *XL* extra large, ↓ indicates decreased

without a fatty liver and also in those with NASH versus those with simple steatosis [86]. However, it was less accurate in grading necroinflammation or staging fibrosis in patients with NASH. Further investigation is warranted to confirm the role of these plasma biomarkers in the diagnosis and management of patients with NAFLD/NASH.

Unfortunately, liver biopsy is still the gold standard for diagnosing and distinguishing between the broad range of forms of NAFLD. However, it is often not recommended in patients with NAFLD because of its invasive nature, potential complications, and the lack of well established long-term treatments. Nevertheless, liver biopsy should be considered in those patients with fatty liver who are at increased risk of having NASH and advanced stages of fibrosis [53].

5 Treatment of NAFLD and NASH

5.1 Lifestyle Intervention

At present, there is no approved drug for the treatment of NASH, and lifestyle modification remains the standard of care to reduce steatosis and plasma liver aminotransferase levels in patients with NAFLD [87–89]. However, most studies have been small and of short duration. Table 2 summarizes the available randomized controlled trials (RCTs) on the effect of different types of diets and exercise programmes on liver fat content assessed by MRS [90–99]. With an isolated exception [92], these changes decreased liver steatosis by 10–51 %, although the duration and intensity of the interventions has varied among these trials. Overall, reduction of liver fat correlates closely with the amount of weight loss. A decrease in body weight of 7–10 % has been associated with a 42–51 % reduction in liver fat in patients with NAFLD [91, 93, 95, 97]. Caloric restriction by means of a low-carbohydrate diet may be particularly beneficial, even in the absence of increased physical activity [94].

Several trials have failed to induce significant weight loss, and studies with less than 5 % reductions in weight have reported a minimal impact on liver fat content, lipids and overall metabolic parameters in NAFLD [92, 96, 98, 99]. This is consistent with the challenge to achieve success in the clinical setting and highlights the importance of alternative therapies for weight loss in NAFLD. The use of orlistat, a pancreatic lipase inhibitor, in NASH may help patients to lose weight but has produced inconsistent results regarding liver histology [100–102]. On the other hand, bariatric surgery (roux-en-Y gastric bypass or laparoscopic adjustable gastric banding) has shown improvement in metabolic and histologic abnormalities in patients with steatohepatitis. However, changes in fibrosis have been less predictable, with some studies even reporting increased fibrosis over time [103, 104]. Taken together, the current evidence suggests an urgent need for long-term, controlled studies that can address the best lifestyle approach for patients with fatty liver and NASH.

5.2 Management of Dyslipidaemia in Patients with NAFLD

5.2.1 Statins

NAFLD is strongly related with obesity and dyslipidaemia and carries a high risk of cardiovascular disease (CVD). It is well established that statins (HMG-CoA reductase inhibitors) have beneficial effects in primary and secondary prevention of CVD [105], and people with T2DM may benefit the most [106]. However, their role in patients with NAFLD

Table 2 Effect of lifestyle intervention in randomized controlled trials in obese patients with nonalcoholic fatty liver disease diagnosed by magnetic resonance imaging and spectroscopy

Author (year)	n	Main intervention (weeks)	Weight loss (%)	LFAT after intervention (%)	*p*-Value
Tamura et al. [90] (2005)	14[a]	Diet + exercise (2)	2	↓ 28	0.03
Larson-Meyer et al. [91] (2006)	46	Diet + exercise (24)	10	↓ 29	<0.01
Kantartzis et al. [93] (2009)	50	Diet + exercise (36)	NR	↓ 35	<0.0001
Shah et al. [95] (2009)	18	Diet + exercise (24)	10	↓ 50	0.02
Lazo et al. [97] (2010)	96[a]	Diet + exercise (48)	8	↓ 51	0.04
Kirk et al. [94] (2009)	12	Low-carbohydrate diet (11)	7	↓ 42	<0.05
Johnson et al. [96] (2009)	19	Aerobic exercise alone (4)	⇔	↓ 21	<0.05
Sullivan et al. [99] (2012)	18	Aerobic exercise alone (16)	⇔	↓ 10	0.04
Shojaee-Moradie et al. [92] (2007)	15	Moderate exercise alone (6)	⇔	⇔	NS
Hallsworth et al. [98] (2011)	19	Resistance exercise alone (8)	⇔	↓ 13	0.01

[a] Patients with type 2 diabetes mellitus

LFAT liver fat, *NR* not reported, *NS* not significant, ↓ indicates decreased, ⇔ indicates no change

Table 3 Effect of statin therapy on liver aminotransferase levels, steatosis and overall histology in patients with nonalcoholic fatty liver disease

Author (year)	n	Design	Duration (months)	Statin	Endpoint	Results
Lewis et al. [111] (2007)	204	RCT	6	Pravastatin	LFTs	⇔
Athyros et al. [115] (2010)	437	Post hoc	36	Variable	LFTs	↓
Maroni et al. [117] (2011)	43	Retrospective	5	Variable	LFTs	⇔
Hatzitolios et al. [100] (2004)	28	Open label	6	Atorvastatin	US	↓
Gomez-Dominguez et al. [108] (2006)	22	Open label	12	Atorvastatin	US	↓
Athyros et al. [109] (2006)	186	Open label	14	Atorvastatin	US	↓
Kiyici et al. [107] (2003)	27	Open label	6	Atorvastatin	CT	↓
Foster et al. [116] (2011)	80	Post hoc	42	Atorvastatin	CT	↓
Browning [110] (2006)	54	Post hoc	NR	Variable	MRS	⇔
Georgescu and Georgescu [118] (2007)	10	Open label	9	Atorvastatin	Liver biopsy	↓ Steatosis
Ekstedt et al. [112] (2007)	17	Retrospective	72	Variable	Liver biopsy	↓ Steatosis
Nelson et al. [113] (2009)	16	RCT	12	Simvastatin	Liver biopsy	⇔
Kimura et al. [114] (2010)	22	Open label	12	Atorvastatin	Liver biopsy	↓ Steatosis, N-I

CT liver computed tomography, *LFTs* liver aminotransferases, *MRS* liver magnetic resonance imaging and spectroscopy, *N-I* necroinflammation, *NR* not reported, *RCT* randomized controlled trial, *US* liver ultrasonography, ↓ indicates decreased, ⇔ indicates no change

is unclear, especially when plasma liver aminotransferase levels are elevated. Table 3 summarizes studies that have evaluated the use of statins in patients with NAFLD using plasma aminotransferase levels, imaging techniques or biopsy to diagnose the disease [100, 107–117]. Patients with NAFLD, with or without NASH, receiving statins do not appear to carry a higher risk of hepatotoxicity. Moreover, some of these studies reported a significant reduction of plasma AST and ALT levels [100, 107, 108, 114, 118]. However, it is unclear whether this effect was due to statin therapy or was the result of concomitant weight loss from dietary modification. The anti-inflammatory and antioxidant properties of statins have been proposed as the mechanism for such hepatic improvements, as they can decrease plasma levels of multiple cytokines (tumour necrosis factor [TNF]-α, interleukin-6 and high-sensitivity C-reactive protein) that are associated with advanced disease in NASH. In a recent review of the literature, Bril et al. [119] concluded that statins were safe overall in this population. This interpretation is consistent with current guidelines that recommend the use of statins to treat lipid abnormalities in patients with NAFLD and NASH [53].

5.2.2 Fibrates

Patients with NAFLD frequently have alterations in plasma TG and high-density lipoprotein cholesterol (HDL-C) concentrations, making them natural candidates for fibrate therapy in combination with statins. Current evidence supports the use of fibrates (fenofibrate) with statins if plasma TG is elevated (>200 mg/dL) and HDL-C is low [120]. In this study, liver fat was not measured, but liver aminotransferase levels were unchanged. The effect of fibrates in patients with NAFLD and NASH has been assessed by several studies [109, 121–124]. While plasma liver enzymes may, on occasion, decrease, these studies have been consistent about a lack of liver histological improvement. In apparent discordance, one study in 186 non-diabetic patients with NAFLD treated with atorvastatin and fenofibrate showed an improvement in liver aminotransferase levels and steatosis measured by ultrasound [109], but the result was most likely linked to a greater than 10 % mean weight reduction in the statin-treated group. In summary, in patients with NAFLD, fibrates appear to be a safe option when added to statins, although they have a minimal or no impact on liver fat content.

5.3 Pharmacological Treatment of NASH

5.3.1 Metformin

Metformin has been used for more than 5 decades across the world although its exact mechanism of action remains elusive. It improves insulin resistance primarily at the level of the liver, and to a lesser extent, skeletal muscle [125]. Several studies have reported reductions in plasma aminotransferase levels with metformin [64, 65, 126–128]. However, improvement of liver steatosis, inflammation and fibrosis has been reported in only a few small studies [65, 128, 129] with more recent studies, including three RCTs [66, 68, 130], finding no benefit [131]. Metformin still has clinical value in patients with NASH as it controls hyperglycaemia (common in these patients) and may reduce CVD in patients with T2DM [132].

Of note, a number of recent epidemiological studies have suggested a role for metformin in the prevention of

hepatocellular carcinoma [133–135], as well as for statins [136–138]. These observations may not take into account many confounders and require confirmation in future controlled prospective studies.

5.3.2 Thiazolidinediones

Thiazolidinediones (TZDs) are peroxisomal proliferator-activated receptor-γ (PPAR-γ) agonists, a class of nuclear transcription factors that are very abundant in adipose tissue. In patients with NASH, they reduce subclinical inflammation, improve adipose tissue and hepatic insulin sensitivity, and restore liver histology [69, 131]. Several relatively small RCTs have demonstrated the efficacy of TZDs in patients with steatohepatitis [12–14, 70, 139]. In the only study in patients with prediabetes or T2DM and NASH [12], pioglitazone (45 mg/day) significantly diminished insulin resistance at the level of the liver, adipose tissue and muscle and improved liver steatosis, necroinflammation and hepatocellular ballooning when compared with placebo. The NAS improved in 73 % of patients treated with pioglitazone compared with 24 % in the placebo group ($p < 0.001$). Two RTCs in patients with NASH and without T2DM later confirmed these findings [13, 14]. In the largest of these studies, 247 subjects were randomized to vitamin E, pioglitazone or placebo [13]. They found histological improvement in liver steatosis and inflammation but not fibrosis after pioglitazone treatment. Unfortunately, the studies have been of relatively short duration (6–24 months) and confirmation about their long-term benefit is needed. Moreover, the long-term safety of TZDs (heart failure, bone loss and bladder cancer) has been under much debate. Regarding bladder cancer, the FDA currently recommends avoidance of pioglitazone if active bladder cancer is present, and caution if there is prior history of the disease. On the other hand, pioglitazone has been shown to reduce CVD in patients with T2DM [6]. Recent guidelines from the American Association for the Study of Liver Diseases, American College of Gastroenterology and the American Gastroenterological Association [53] recommend the use of pioglitazone in patients with biopsy-proven NASH, but more studies are needed to assess efficacy and safety of the drug in the long term.

5.3.3 Vitamin E

Vitamin E is an antioxidant, and its mechanism of action in patients with NASH is believed to reduce hepatocyte oxidative stress [128, 139, 140]. Early small studies of short duration in patients with NAFLD/NASH reported inconsistent results [128, 139–143]. A more recent 2-year RCT in non-diabetic patients with biopsy-proven NASH [13] reported a significant difference in the response to the primary histological endpoint for patients receiving vitamin E compared with placebo-treated patients (43 % vs. 19 %; $p = 0.001$). The primary histological endpoint was an improvement of ≥2 grades in the NAS, with at least 1 point improvement in hepatocellular ballooning and 1 point in either the lobular inflammation or steatosis score, with no worsening of fibrosis. Using this endpoint, only 34 % of patients on pioglitazone improved. However, more subjects in the pioglitazone group lacked hepatocellular ballooning at study entry (a component of the primary endpoint and thus classified as non-responders) and did not have a post-treatment liver biopsy (therefore considered by default as non-responders), both negatively affecting the efficacy comparisons of pioglitazone. Moreover, there were no statistical differences between both treatments when vitamin E and pioglitazone patients were matched for baseline histology (i.e. ballooning) or when histological outcomes were examined under a variety of sensitivity analysis scenarios [13]. More recently, in a 2-year study, vitamin E has been shown to have modest benefit in children and adolescents [144]. In summary, vitamin E is recommended because it appears safe, is relatively inexpensive and may be beneficial in patients with NASH without T2DM, but its long-term value and indication in patients with T2DM remains to be established.

5.3.4 Pentoxifylline

Pentoxifylline is a methylxanthine product that inhibits TNF-α, a well known proinflammatory cytokine. It reduces blood viscosity and platelet aggregation and increases blood cell flexibility [145]. Several studies with pentoxifylline have yielded negative results [118, 146–150]. However, a modest improvement has been reported in a recent small RCT [150]. In this study, 10 out of 26 patients receiving pentoxifylline (38.5 %) experienced a ≥2 grade improvement in the NAS compared with 4 out of 29 receiving placebo (13.8 %; $p = 0.036$), with marginal benefits on fibrosis. Of note, improvement occurred only in one third of patients and treatment did not improve ballooning, a key histological component in NASH.

5.4 Incretin-Mimetics

The incretin-mimetics such as once-weekly exenatide [151, 152] and liraglutide [153] have generated great interest because of their potential to reduce hepatic steatosis in patients with NAFLD, but studies have been small and did not examine liver histology. Addition of twice-daily exenatide to bedtime basal insulin improves liver steatosis in patients with well controlled T2DM [154]. This may be either due to a direct effect on liver signalling pathways as glucagon-like peptide (GLP)-1 receptors may be present in

human hepatocytes [155, 156], or indirectly by means of weight loss with reversal of lipotoxicity and reduction of hyperglycaemia. These studies report that activation of GLP-1 receptors improve hepatic PPARα and PPARγ, Akt and adenosine monophosphate-activated protein kinase (AMPK) phosphorylation and activity in patients with NASH [151, 152]. In mice models of obesity, exendin-4 has been documented to normalize liver aminotransferase levels and steatosis by improving GLP-1-mediated signalling pathways [157]. For instance in HepG2 and Huh7 cells, GLP-1 analogues may improve phosphorylation of 3-phosphoinositide-dependent kinase (PDK)-1, AKT and protein kinase C (PKC)-zeta [155], autophagy and endoplasmic reticulum stress [158]. The role of oral dipeptidyl peptidase (DPP)-IV inhibitors has not been explored, but these agents are being widely used for the treatment of T2DM. Clearly, more work is needed to fully establish the role of GLP-1 agonists in the management of NAFLD.

5.5 New Pharmacological Agents

A number of agents are being tested for the treatment of NASH (www.ClinicalTrials.gov), including novel insulin sensitizers such as resveratrol (a polyphenol found in grape skins that may act as an antioxidant), fenretinide (a synthetic retinoid that may inhibit the biosynthesis of lipotoxic ceramides that antagonize insulin action), the insulin-sensitizer farnesoid X receptor (FXR) ligand obeticholic acid (OCA), or novel AMPK activators (such as oltipraz) that ameliorate insulin resistance and fatty acid synthesis through activation of the AMPK-S6K1 pathway and inhibition of liver X receptor (LXR)-γ (a nuclear hormone receptor) and SREBP-1c (the sterol regulatory element-binding protein-1c gene). Other approaches include the restoration of normal gut flora with the antibacterial rifaximin (altered gut microbiota in obese patients may lead to hepatic insulin resistance and NASH), using 'hepatoprotective' agents such as silymarin (milk thistle), or the administration of polyunsaturated fatty acids (PUFA). Taken together, these trials are encouraging, as combined therapeutic approaches may become available in the future, in the same way as current treatments for dyslipidaemia, hypertension and T2DM.

Two new medications have been recently approved by the FDA for weight loss and may hold promise for the management of patients with NAFLD. Lorcaserin is a selective serotonin 2C receptor agonist [159], while the other agent is a combination of the sympathomimetic amine phentermine plus the antiepileptic topiramate [160]. Both drugs act as appetite suppressants. In the main clinical trials leading to their approval, the ~5 % (lorcaserin) to ~10 % (phentermine plus extended-release topiramate) weight loss achieved would be expected to have positive effects on hepatic steatosis and necroinflammation in patients with NAFLD. Controlled trials will establish their clinical value in the future.

6 Conclusion and Future Directions

NAFLD is a condition with potentially severe outcomes. The presence of obesity, insulin resistance and diabetes are major factors associated with an increased risk of progression to NASH, end-stage liver failure and hepatocellular carcinoma. The best approach for the management of NAFLD remains controversial due to our incomplete understanding of the natural history of the disease. While many cases of NAFLD can be diagnosed by the use of plasma aminotransferase levels and imaging, a liver biopsy is still required to confirm the diagnosis and stage the disease. There is significant interest in developing clinically reliable plasma biomarkers [85] and/or genetic tests [2, 154] for the diagnosis of NAFLD. While these approaches hold great promise, they await further validation. More recently, metabolomics has emerged as a novel approach for discovering new biomarkers in NAFLD [161].

Lifestyle intervention remains the cornerstone of treatment in NAFLD but it is difficult to achieve and maintain. So far, TZDs and vitamin E are the most promising pharmacological therapies. Better understanding about the long-term safety and efficacy of TZDs (pioglitazone) is needed before they can be fully incorporated into clinical practice for the long-term management of patients with NASH. Vitamin E, being inexpensive and having been tested in non-diabetic patients, is usually recommended for this population, while pioglitazone is preferred in those with T2DM although it has been only tested in a short-term study [12]. On-going clinical trials may expand our current understanding of the disease and provide hope about finding safer and more effective agents in the future.

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