Drugs 2003; 63 (22): 2379-2394
0012-6667/03/0022-2379/$33.00/0

Advances in the Understanding and Treatment of Nonalcoholic Fatty Liver Disease

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is a well recognised form of chronic liver disease that has recently gained greater recognition. Originally described in the late 1950s, NAFLD is currently considered the leading cause of abnormal liver enzyme levels in the US, closely paralleling the increase in obesity and diabetes mellitus. NAFLD has a worldwide distribution, affecting both adults and children, and typically is seen in association with obesity, diabetes, hypertension and hypertriglyceridaemia. Most patients are asymptomatic and usually present with mild elevations in aminotransferases.

The natural history of NAFLD is not clearly defined but progression to cirrhosis and end-stage liver disease is well recognised in some patients. The accumulation of hepatic steatosis is thought to occur initially, primarily through hepatic and peripheral insulin resistance, which leads to altered glucose and free fatty acid metabolism. The progression from simple fatty liver to more severe forms of NAFLD (nonalcoholic steatohepatitis and cirrhosis) is much less clear but evidence suggests that oxidative stress may preferentially enhance proinflammatory cytokines, which leads to cellular adaptations and dysfunction followed by development of inflammation, necrosis and fibrosis.

Therapeutic modalities remain limited and are largely focused on correcting the underlying insulin resistance or reducing oxidative stress. However, at the present time, there are several limitations to the current potential therapies, mainly because of the lack of large-scale, prospective, randomised studies, as well as clearly defined histological endpoints. Ultimately, the future for potential therapeutic modalities to treat this disease are quite promising, but further research is needed to clearly demonstrate which therapy or therapies will be effective at eliminating fatty liver disease and its potential complications.

The histological findings of nonalcoholic fatty liver disease (NAFLD) have been known for quite some time. Only recently has there been an increased interest in this disease. As early as 1958, Westwater and Fainer[1] described this disease in a small cohort of patients with obesity. This was further characterised in 1975 by Peters et al.[2] in a group of patients undergoing gastric bypass surgery for obesity. The disease was also described in 1979 by Adler and Schaffner.[3] In 1980, Ludwig et al.[4]

evaluated the liver biopsies of 25 nonalcoholic, obese, diabetic patients and described a collection of histological findings that were similar to alcoholic liver disease but were seen in patients who denied alcohol use. He proposed the term nonalcoholic steatohepatitis (NASH) for this entity. Subsequently, it has become recognised that NAFLD incorporates a spectrum of disease that ranges from simple hepatic steatosis to NASH and finally cirrhosis (figure 1). Whether this condition originates as simple steatosis and progresses through NASH to cirrhosis is still a matter of debate. In fact, data suggest that hepatic steatosis alone on biopsy confers no greater risk of progression in disease[5,6] and, given this, it could be argued that the term 'NAFLD' be changed to 'NAFL'. However, it has become clear that if histological criteria exist for the diagnosis of NASH, a significant proportion of these patients may progress to fibrosis and cirrhosis, sometimes quite rapidly.[7-10] Willner et al.[11] showed that up to 50% of obese patients with cirrhosis developed complications of portal hypertension, requiring a liver transplant.[11] Additionally, evidence now suggests that a significant number of liver transplants performed for cryptogenic cirrhosis may be due to NASH.[12,13] Furthermore, recent studies report progression of NASH-related cirrhosis to hepatocellular carcinoma.[14-16] This is disturbing because it appears that the prevalence of NAFLD is increasing as obesity and diabetes mellitus increase in Western societies at an alarming rate.[17,18] In fact, the most recent US National Health and Nutrition Education Survey (NHANES) reveals that 30.5% of Americans are obese, as defined by a body mass index of >30,[19] up from 22.9% in NHANES III (1988–1994).[20]

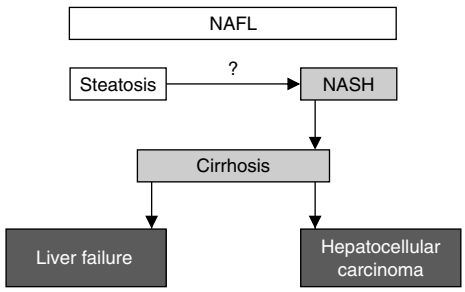

Fig. 1. The term nonalcoholic fatty liver (NAFL) is used broadly to encompass all aspects of fatty liver from simple steatosis to nonalcoholic steatohepatitis (NASH) and potential cirrhosis with its possible complications.

NAFLD is associated with clinical conditions such as obesity, diabetes and hyperlipidaemia, which have a similar pathophysiological foundation: insulin resistance.[21] To date, however, the complete pathogenesis of NAFLD remains elusive and proven therapeutic modalities remain quite limited. Many investigators are actively pursuing promising treatment options that are aimed at improving the underlying insulin resistance, with a goal of improvement in steatohepatitis. These findings will need to be prospectively randomised with adequate statistical power to show a clear benefit.

1. Epidemiology

The true incidence and prevalence of NAFLD are not known, in part because the precise diagnosis requires a liver biopsy and consensus on histological criteria is lacking. However, NAFLD does have a worldwide distribution. Japan and Germany report a prevalence of 14%.[22,23] Recent data from the NHANES III trial reveal that 24% of American adults (30 million) have elevated serum aminotransferase activity with negative serologies for viral hepatitis, serum transferrin saturation <50% and no significant history of alcohol consumption, suggesting that these patients are likely to have NAFLD,[24] as corroborated by others.[25] Furthermore, limited data suggest that NASH occurs in approximately 3% of the general population,[26] rising to 69% in patients with morbid obesity.[27] In fact, 42% of patients with obesity have been shown to have significant hepatic fibrosis at the time of liver biopsy.[28] However, Bacon et al.[9] have demonstrated that this disease can occur in lean, nondiabetic patients as well.

2. Clinical Presentation

NAFLD occurs in both children and adults.[29-32] Children are generally diagnosed around 12 years of age, whereas adults typically present in the fourth to fifth decade of life.[21] Although previously thought to affect women more often,[4,7,8,33] recent data suggest a predominance in men.[34-36] However, it ap-

Table I. Patient demographics and characteristics of patients with non-alcoholic fatty liver disease

Study	n	Mean age (y)	Sex (% male)	Dyslipidaemia (%)	DM (%)	Obesity (%)	Hypertension (%)	ALT (U/L)	AST (U/L)
Ludwig et al.[4]	20	54	35	67	25	90	15	38	72
Powell et al.[8]	42	49	17	62	36	95	ND	96	70
Bacon et al.[9]	33	47	58	21	21	39	18	ND	ND
Angulo et al.[39]	144	51	33	27	28	60	ND	82	63
Harrison and Hayashi[35]	102	51	57	74	42	73	58	89	63
Chitturi et al.[36]	93	49	60	ND	29	57	ND	90	53

ALT = alanine aminotransferase; **AST** = aspartate aminotransferase; **DM** = diabetes mellitus; **n** = number of patients; **ND** = no data.

pears that women may have a greater risk of progression to the advanced stages of disease.[35,36] This disease can be seen in a broad array of ethnic groups but appears to be more prevalent in Caucasians and Hispanics,[35,37] although this is still controversial[24] and may reflect ascertainment bias. NAFLD does appear to be less frequent among African Americans.[38]

Most patients with NAFLD are asymptomatic. A small minority of patients present with fatigue or a vague, nondescript pain or discomfort in the upper right quadrant. Typically, serum aminotransferases are only mildly elevated, with ALT predominating (table I)[4,8,9,35,36,39] and rarely exceeding three times the upper limit of normal. In addition, some patients may have normal serum transaminases or only intermittent elevations on repeated measurements. Alkaline phosphatase is occasionally elevated but usually less than twice the upper limit of normal.[40]

2.1 Disease Associations

Characteristically, NAFLD is associated with several common clinical diseases (table I). These include hypertension, adult-onset diabetes, hyperlipidaemia and obesity, which comprise the metabolic syndrome (or syndrome X).[41,42] The underlying link between these disease processes appears to be insulin resistance.[42] Recent studies have elegantly demonstrated that NAFLD is associated with insulin resistance.[43-46] In fact, as insulin resistance increases so does the severity of NAFLD.[43,47] These data suggest that NAFLD may represent the hepatic manifestation of the metabolic syndrome.

The association of iron overload and NASH is not uncommon. Serum iron indices, including ferritin, are often abnormal in patients with NASH. In fact, several studies have demonstrated abnormal ferritin levels in 40–58% of patients.[9,36,39] Given the relatively common association of increased serum iron indices with NASH, a number of studies have evaluated the prevalence of mutations in the haemochromatosis (*HFE*) gene in this disease.[39,48-50] The data are conflicting, with two studies[48,49] supporting an association and two studies [39,50]refuting an association. Both studies supporting an association of *HFE* mutations and iron overload in patients with NASH were performed at centres with significant iron-overload referral practices. George et al.[48] showed the prevalence of C282Y mutations to be 31% in this population. Bonkovsky et al.[49] found that NASH patients with any mutation in *HFE* had elevated serum iron indices and significantly more stainable hepatic iron, but normal hepatic iron levels. Although Younossi et al.[50] did not perform *HFE* analysis, they found no relationship between hepatic iron accumulation and aggressive histology in patients with NASH. Furthermore, although Angulo et al.[39] cited increased transferrin saturation, correlating with severity of fibrosis, this association was no longer present when controlling for age, sex, obesity, diabetes and AST : ALT ratio. Additionally, no patients had increased hepatic iron levels.

Using a different approach, Mendler et al.[51] evaluated 161 patients with unexplained hepatic iron overload, as defined by a liver iron level of >36 μmol/g dry liver weight or a histological iron score

of ≥3. Forty-three patients were compound heterozygotes (C282Y/H63D). A total of 45 patients had histological evidence of NASH, with a mean ferritin level of 698 μg/L and, of these, only four patients were compound heterozygotes (C282Y/H63D). Incidentally, 94% of all iron-loaded patients were noted to have some component of the insulin resistance syndrome.[51] This is intriguing, because a recent study by Facchini et al.[52] showed that in 17 patients with NAFLD, normal iron indices and no *HFE* mutations who underwent phlebotomy to near iron deficiency, serum ALT levels significantly improved, and fasting and glucose-stimulated plasma insulin levels improved by 40–50%.[52]

3. Diagnosis

When considering the diagnosis of patients with NAFLD, a thorough history and physical examination should be performed with careful attention to the amount of alcohol the patient consumes. Occasionally, it is quite helpful to enroll the help of close family members or friends to determine an accurate alcohol history. Most investigators in the field of NAFLD consider that patients consuming <20 g/day of alcohol may be considered for this diagnosis.[21]

Laboratory investigations should include tests for hepatitis B and C viruses, as well as a fasting iron panel, antinuclear antibody, antismooth muscle antibody, antimitochondrial antibody and thyroid function testing. If the patient is <40 years of age, then serum ceruloplasmin levels should also be included.

Once the above investigations have been performed and are determined to be negative or not significant, the possibility that the patient has NAFLD becomes much greater. Imaging studies, looking for fatty liver, have been advocated by some but, unfortunately, the positive predictive value of the two most readily available imaging modalities, ultrasound and computed tomography scan, are not ideal.[53,54] Furthermore, no imaging study to date has been able to distinguish between simple fatty liver and NASH or NASH with fibrosis.

Ultimately, to definitively diagnose NASH, histological assessment is required. The histological features of NASH are essentially the same as those found in alcoholic liver disease. Steatosis is necessary for the diagnosis and is most commonly macrovesicular in nature, although some microvesicular steatosis can be seen. There is a mixed inflammatory infiltrate found within the hepatic lobule, predominantly in zone 3, with associated ballooning degeneration and necrosis of hepatocytes. Mallory's hyaline, although not as well formed or as numerous as that seen in alcoholic steatohepatitis, may also be found. Glycogenated nuclei are often seen.[55] Fibrosis, when seen, characteristically initiates in the perivenular and perisinusoidal region. Recent data suggest that at the time of initial biopsy up to 30–40% of patients with NASH have advanced fibrosis,[7,9] and cirrhosis may be found in 10–15% of patients.[7-9,34]

In addition to these findings, which are readily apparent on light microscopy, recent studies have suggested that significant changes may be seen within hepatocyte mitochondria, as visualised with electron microscopy.[44,56] In up to 10% of hepatocytes, mega-mitochondria can be found with characteristic para-crystalline inclusions. Interestingly, these inclusions are not seen in patients whose disease has progressed to cirrhosis.

The decision to perform a liver biopsy on patients with suspected NAFLD is the subject of much debate. Advocates of performing liver biopsies point to the ability to prognosticate patients based on biopsy findings of simple steatosis, which has a low likelihood of progression to advanced disease, and NASH with or without advanced fibrosis, which is more likely to progress to cirrhosis over time. Those clinicians who oppose liver biopsy in patients referred for elevated serum aminotransferases point out the potential risks of the procedure and the current lack of therapeutic modalities to treat the disease at this time.

Given this debate, as well as recent studies showing that a large number of obese patients with NASH and advanced fibrosis have normal serum aminotransferases (as reviewed by Garcia-Monson et al.[27]), recent efforts have centred around development of noninvasive tools to assist the clinician in determining the probability of NASH and severe

Table II. Predictors of significant fibrosis in patients with nonalcoholic steatohepatitis (NASH)[a]

Study	n	Mean age (y)	Sex (% female)	Mean BMI	HSS system used	stage 0–2 (%)	stage 3–4 (%)	Predictors of fibrosis
Angulo et al.[39]	144	50.5	67	31.2	0–4, Brunt	73	27	Age, obesity, DM
Marceau et al.[47]	93[b]	36	80	47	0–5, Metavir	88	12	Age, steatosis, FBS, WHR, BMI, DM
Garcia-Monzon et al.[27]	32	41	65	50.5	0–4, Brunt	84	16	Age, steatosis, inflammation grade
Ratziu et al.[34]	93	49	34	29.1	0–4, Metavir	84	16	Age, BMI, ALT, triglycerides, inflammation grade
Dixon et al.[28]	26	44	58	47.2	0–4, Brunt	59	41	HTN, ALT, C-peptide, Homa % B
Chitturi et al.[36]	93	49	40	32	0–4, Brunt	67	33	Women, DM, inflammation grade
Harrison and Hayashi[35]	102	51.3	43	33.9	0–4, Brunt	81	19	BMI, AST : ALT ratio, HbA_{1C}

a Several studies have evaluated the clinical and biochemical characteristics of patients with NASH in an attempt to identify the noninvasive predictors of fibrosis. The study populations are varied and different fibrosis scoring systems are used. Interestingly, severe fibrosis at initial liver biopsies ranged from 12% to 41%.

b With histological evaluation.

ALT = alanine aminotransferase; **AST** = aspartate aminotransferase; **BMI** = body mass index; **DM** = diabetes mellitus; **FBS** = fasting blood sugar; **Homa % B** = homeostasis model assessment, a validated method of estimating insulin resistance and β-islet cell function; **HbA_{1C}** = glycosylated haemoglobin; **HSS** = histological scoring system; **HTN** = hypertension; **n** = number of patients; **WHR** = waist : hip ratio.

fibrosis in patients with suspected NAFLD. Dixon et al.[28] evaluated a cohort of patients with morbid obesity undergoing bariatric surgery and found three clinical variables; hypertension, an elevated ALT (>40 U/L) and insulin resistance (>5.0) [HAIR], which when combined (HAIR score) yielded a sensitivity of 80% and a specificity of 85% for the diagnosis of NASH. This study has yet to be validated either by others or in a prospective fashion. In addition, several studies have retrospectively evaluated independent predictors of severe hepatic fibrosis in patients with NASH (table II),[27,28,34-36,39,47] with varying results. Furthermore, imaging modalities such as magnetic resonance spectroscopy and serological marker analysis are currently being investigated for their ability to assist in the detection of NASH and advanced hepatic fibrosis.

4. Pathogenesis

New developments in both animal models and human studies have allowed us to gain a better understanding of the pathogenesis of NAFLD and its progression to NASH and, potentially, cirrhosis. However, fully defined pathways remain elusive. The most prevailing theory since 1998, is the 'two hit' hypothesis proposed by Day and James.[57] The first 'hit' is the accumulation of fat within the liver, specifically fatty acids and triglycerides. This fatty accumulation within the liver leads to chronic oxidant stress, the so-called 'second hit', which subsequently makes the hepatocyte vulnerable to apoptosis or necrosis. Figure 2 illustrates the conceptual pathogenesis of hepatic steatosis. Figure 3 outlines the potential progression of steatosis to NASH and cirrhosis in NAFLD.

4.1 Pathogenesis of Hepatic Steatosis

The metabolism of dietary carbohydrates, proteins and fats is the result of a detailed orchestration of many molecular pathways that allow the use of ingested nutrients for energy requiring cellular processes or storage for later use. Hepatic steatosis is the accumulation of excess triglyceride within the liver that occurs as a result of increased circulating free fatty acids (FFAs) and the subsequent complex array of cellular interactions between skeletal muscle, adipose tissue and liver. Although these processes are currently being delineated, there is still much that is not understood fully. This section describes the mechanisms for the development of hepatic steatosis and then briefly discusses some of

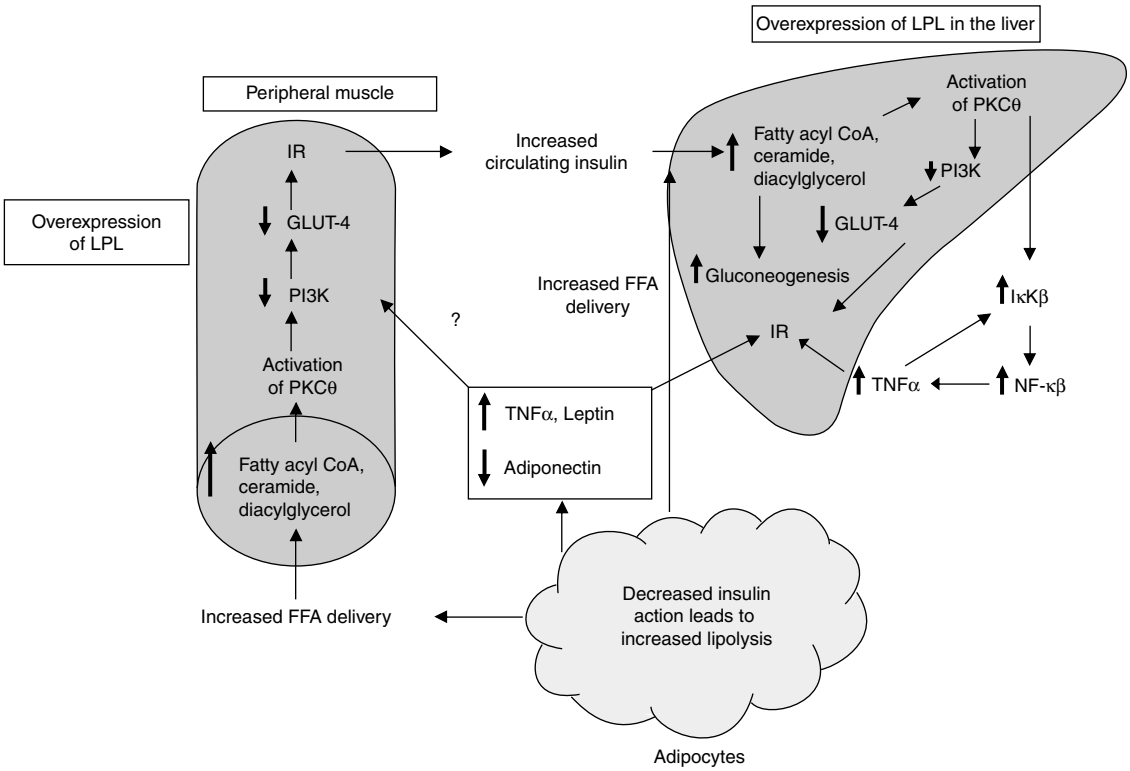

Fig. 2. This simplified model illustrates the potential contribution of adipocytes, peripheral muscle and the liver in the pathogenesis of insulin resistance and subsequent fatty liver development that has been proposed in the literature. This is certainly preliminary and a complete understanding of insulin resistance as a result of altered insulin signalling, and the interactions with adipokines such as leptin, adiponectin and tumour necrosis factor-α (TNFα) are not fully understood. **CoA** = coenzyme A; **FFA** = free fatty acid; **GLUT-4** = glucose transporter 4; **IκKβ** = inhibitor κ kinase β; **IR** = insulin resistance; **LPL** = lipoprotein lipase; **NF-κβ** = nuclear factor κβ; **PI3K** = phosphatidyl-3-hydroxy kinase; **PKCθ** = protein kinase C theta.

the potential hypotheses for the dysregulation of FFA metabolism.

The accumulation of FFAs and triglycerides within the liver is the result of two major processes. First, FFAs are either synthesised within the liver itself or transported to the liver bound to albumin after absorption from the intestinal lumen or through lipolysis of adipose tissue. Alternatively, FFAs can accumulate if hepatic lipogenesis is inhibited or the transport of triglycerides out of the liver, in the form of very low-density lipoprotein (VLDL), is impaired. The accumulated FFAs are then either oxidised via hepatocyte mitochondria, peroxisomes or microsomes, or esterified to triglycerides.

NAFLD is associated with altered glucose and FFA metabolism, in most cases as a result of hepatic and peripheral insulin resistance.[58-61] Insulin normally acts on myocytes, adipocytes and hepatocytes to regulate energy metabolism.[62-65] In muscle, insulin mediates glucose uptake via a signalling cascade that results in the translocation of glucose transporter-4 (GLUT-4) from intracellular pools to the cell surface.[64,65] Circulating insulin acts on adipocytes by inhibiting hormone-sensitive lipase, preventing the breakdown of triglycerides and release of FFAs.[63,64] In hepatocytes, insulin enhances glycogenesis and inhibits glycogenolysis and gluconeogenesis.[63] Alternatively, insulin resistance is characterised by increased circulating levels of FFAs as a result of increased lipolysis[61] and inefficient FFA oxidation by peripheral muscle and the liver. Additionally, lipogenesis, under the control of sterol reg-

ulatory element binding protein-1, remains sensitive to insulin, now chronically elevated secondary to the resistant state.[66] Subsequently, the increased FFA load within peripheral muscle and the liver results in impaired glucose metabolism due to altered insulin signalling. Moreover, the hyperinsulinaemia associated with insulin resistance leads to decreased synthesis of apolipoprotein (apo) B-100, a critical component of VLDL, thus decreasing triglyceride transport out of the cell.[67] Recently, it has been shown that patients with NASH have impaired hepatic synthesis of apoB-100,[68] which could contribute to enhanced triglyceride deposition within the liver.

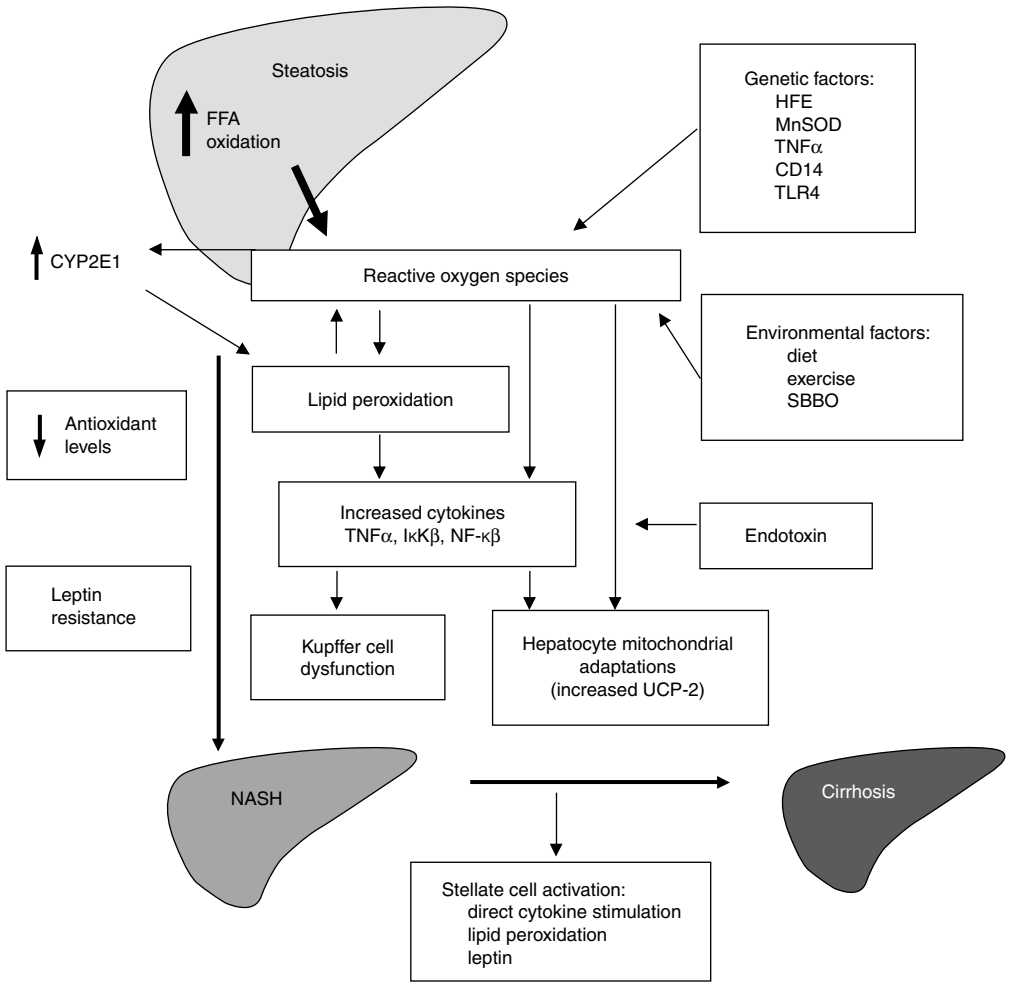

Fig. 3. The development of necroinflammatory activity and fibrosis in patients with nonalcoholic steatohepatitis (NASH) is quite complex and probably involves both genetic and environmental factors that predispose some patients with steatosis to progress in their disease. Conceptually, the generation of reactive oxygen species (ROS), through multiple mechanisms, leads to enhanced lipid peroxidation and depletion of the native antioxidant pool. Through the upregulation of proinflammatory cytokines, subsequent dysfunction of Kupffer cells and hepatocyte mitochondrial adaptations, it is thought that the characteristic histological features of NASH can develop. Concomitantly, quiescent hepatic stellate cells are activated via direct cytokine stimulation, lipid peroxidation products and leptin with resultant fibrogenesis. **CYP2E1** = cytochrome P450 isoenzyme 2E1; **FFA** = free fatty acid; **HFE** = haemochromatosis gene; **IκKβ** = inhibitor κ kinase β; **MnSOD** = mitochondrial superoxide dismutase; **NF-κβ** = nuclear factor κβ; **SBBO** = small bowel bacteria overgrowth; **TLR4** = toll-like receptor 4; **TNFα** = tumour necrosis factor-α; **UCP-2** = uncoupling protein-2.

Using animal models, much work has been done to explain the mechanisms responsible for the development of insulin resistance. Shulman and colleagues[69] have recently proposed that FFAs within muscle and the liver lead to overexpression of lipoprotein lipase. This results in decreased insulin activation of insulin-receptor substrate (IRS)-1 and IRS-2 in muscle and the liver respectively, despite high levels of insulin, thus leading to decreased phosphatidylinositol 3-kinase activity. Subsequently, glycogen synthase activity is decreased, GLUT-4-mediated glucose transport is decreased, gluconeogenesis continues and intracellular FFA-derived metabolites accumulate within muscle and the liver. These FFA-derived metabolites, such as fatty acyl coenzyme-A (CoA), ceramide and diacylglycerol, lead to the activation of protein kinase C-θ,[69] which has been shown to upregulate the transcription of the protein kinase inhibitor κ kinase β (IκKβ),[70] leading to further insulin resistance.[71]

Genetic factors may also influence the development of hepatic steatosis. Gene polymorphisms that result in decreased lipid export, such as apoE and microsomal triglyceride transfer protein (MTTP), have been associated with hepatic steatosis development.[72,73] Furthermore, MTTP-deficient mice exposed to lipopolysaccharide developed higher levels of lipid peroxides and subsequent hepatic inflammation compared with mice without MTTP deficiency.[74]

Leptin, a satiety hormone derived from adipocytes, is involved in energy regulation, modulating hypothalamic pathways that lead to decreased food intake and increased thermogenesis. This hormone may also have a direct peripheral effect on tissue regulation of glucose metabolism.[75] Leptin levels are elevated in obese patients,[76] and recent data suggest that leptin is also elevated in patients with NASH and that increasing leptin levels may correlate with NASH severity.[77] Given this background, it appears that leptin may be involved in the pathogenesis of both hepatic steatosis and NASH.

Evidence suggests that leptin is intimately involved with insulin signalling and regulation of glucose metabolism in muscle, adipose tissue and the liver.[75] In fact, lipodystrophic patients with almost no circulating leptin, as a result of the selective loss of visceral and subcutaneous fat, develop severe insulin resistance and hepatic steatosis.[78] Recently, Shulman and colleagues[58] demonstrated that leptin infusion in these patients markedly enhances insulin sensitivity within the liver and peripheral muscle, leading to dramatic decreases in both hepatic and muscle triglyceride levels. Additionally, Barzilai et al.[79] have shown that leptin infusion in rats decreases visceral fat and enhances the action of insulin on both peripheral glucose uptake and glycogen synthesis. The situation in patients with NAFLD appears to be contradictory to these findings, since NAFLD is characterised by increased leptin levels and insulin resistance. However, just as insulin resistance leads to increased circulating insulin levels, it has been suggested that resistance to leptin, both centrally and peripherally, may lead to increased circulating leptin in patients with NAFLD. This concept of leptin resistance has been evaluated by Sahu,[80] who recently demonstrated that chronic central leptin infusion in rats leads to resistance to the satiety effect of leptin, probably due to decreased gene expression of neuropeptide Y. Taken together, it is apparent that leptin and insulin have overlapping signalling pathways that are involved with hepatic steatosis but further study is needed in human subjects to clarify the interaction of these two hormones in NAFLD.

Other proposed regulators of energy metabolism include the adipokines, tumour necrosis factor-α (TNFα) and adiponectin, secreted by the adipocyte.[63] TNFα levels are increased in patients with NAFLD and has been shown to modulate insulin signalling within muscle and enhance insulin resistance.[81] Adiponectin mRNA expression is decreased in patients with NAFLD, and the replacement of this adipokine in mice reverses insulin resistance and decreases the triglyceride content of muscle and the liver.[63] Furthermore, it has been suggested that TNFα may repress the expression of adiponectin, potentiating more insulin resistance.[64]

4.2 Progression to Nonalcoholic Steatohepatitis

Much work has been done, mainly in animal models, in an effort to elucidate the mechanisms of disease progression from NAFLD to NASH, which occurs in up to 25% of patients.[40] It is now clear that multiple genetic and environmental factors may influence this process.

FFA levels are increased within the hepatocytes of patients with NAFLD, probably as a result of insulin[59] and leptin resistance.[75] Increased hepatic FFA levels can upregulate cytochrome P450 (CYP) 2E1 and CYP3A4, leading to enhanced generation of reactive oxygen species (ROS) and lipid peroxidation.[82-84] FFAs undergo mitochondrial β-oxidation with the generation of ROS.[85] Furthermore, as a result of the overload of FFA within the hepatocyte, oxidation also occurs through peroxisomal and microsomal pathways, generating further oxidative stress via the production of dicarboxylic acid derivatives, mediated through upregulation of the nuclear transcription factor peroxisomal proliferator-activated receptor (PPAR)-α.[86] Through the generation of ROS, redox-sensitive kinases are activated, resulting in the increased activity of IκKβ, which leads to induction of nuclear factor-κβ (NF-κβ).[71,87] Proinflammatory cytokines, such as TNFα[88] and interleukin (IL)-8, are generated and cellular adhesion molecules are synthesised, resulting in enhanced neutrophil chemotaxis and further mitochondrial ROS production[21] with eventual depletion of the natural antioxidant pool. Endotoxin, possibly via leptin modulation of macrophages,[89] has also been shown to increase inflammatory cytokines such as TNFα, with a resultant increase in neutrophil chemotaxis and further inflammation.[90] Clearly elevated levels of FFAs can have hepatotoxic effects but it remains to be seen if the generation of ROS from FFA oxidation is the instigator of cellular inflammation in NASH.

Research has recently focused attention on the hepatic mitochondria in NASH because FFAs and chronic oxidative stress may impair mitochondrial function.[85,91,92] In ob/ob mice, Diehl and colleagues[93] have shown that chronic oxidative stress leads to increased expression of uncoupling protein (UCP)-2, an inner mitochondrial membrane protein that partially depolarises the transmembrane electrochemical gradient and reduces the efficiency of mitochondrial oxidative phosphorylation, resulting in decreased adenosine triphosphate (ATP) production. Although the role of UCP-2 in hepatic steatosis is controversial,[94] it has been reported that patients with NASH have impaired hepatic ATP homeostasis,[95] which is suggestive of mitochondrial dysfunction.

Recent evidence suggests that Kupffer cells may be dysfunctional in animal models of NAFLD. Obesity has been shown to impair the function of Kupffer cells in an animal model.[96] Li et al.[97] have shown that Kupffer cells in ob/ob mice produce less IL-15 basally and more IL-12 after lipopolysaccharide stimulation. This is intriguing given that ob/ob mice are devoid of CD4+ natural killer (NK)+ T cells, the predominant liver lymphocyte, the viability of which is promoted by IL-15. This may increase the vulnerability to TNFα as CD4+NK+ T cells normally produce IL-10, an important cytokine that protects cells from the actions of TNFα.[98]

4.3 Development of Fibrosis

Fibrosis is a wound-healing response to liver injury and activated hepatic stellate cells are primarily responsible for fibrogenesis.[99] The process of stellate cell activation is initiated and perpetuated by a complex interplay of factors, including ROS, cytokines and products released from damaged hepatocytes.[100-102] All of these factors may play a role in the development of fibrosis in NASH. The cytokine transforming growth factor-β (TGFβ) has strong profibrogenic effects on stellate cells.[103] Although the exact mechanisms of hepatic fibrosis in NASH are not fully understood, it is known that leptin is required for fibrogenesis in animal models of NASH.[104] Evidence suggests that leptin is produced by activated hepatic stellate cells and interacts in a paracrine fashion with receptors on Kupffer cells and sinusoidal endothelial cells, leading to increased TGFβ production that may perpetuate fibrogenesis.[105,106]

5. Natural History

Simple steatosis, seen in approximately 20% of adults, has a relatively benign course and progression in disease severity occurs in only a small percentage of patients.[5] However, NASH has been demonstrated to progress to cirrhosis in 15% of patients over a mean follow-up interval of 3.7 years.[7-9] Recently, we showed that 32% of NASH patients with fibrosis will have a worse fibrosis score after 5.8 years and, in some patients, fibrosis progresses quite rapidly.[10] Matteoni et al.[6] have shown that the liver-related mortality for patients with NASH is similar to that for other types of liver disease and recent evidence suggests that some patients with NASH that progresses to cirrhosis will develop hepatocellular carcinoma.[14,15]

6. Treatment

The aims of treatment for NASH include regression of fibrosis, inflammation and steatosis. To date, there are no large, randomised clinical trials demonstrating efficacy in achieving all of these aims. Most published therapeutic studies are small, open-label, pilot trials and only a few have follow-up histological data (table III). Some therapies show promise but need to be studied in larger, randomised, placebo-controlled trials. Furthermore, these trials will need to have well defined and agreed upon histological endpoints.

6.1 Improving Insulin Resistance

Evidence suggests that improving insulin resistance will improve or eliminate hepatic steatosis. This has been shown conclusively in both the ob/ob mouse model[126] and in patients with lipodystrophy.[58] It is well known that both obesity and diabetes are intimately associated with insulin resistance.[21,24,40,58] Given that a majority of patients with NASH are either obese, diabetic or a combination of the two, it seems logical that potential therapeutic modalities should be aimed at improving insulin use.

6.1.1 Weight Loss

Historically, treatment for NASH has consisted of recommendations to control weight with diet and exercise, but there are few data to support the efficacy of weight loss in NASH. Several small studies have evaluated the effects on hepatic steatosis of both rapid weight loss through near starvation[127,128] and steady weight loss over many months.[107-109] Steady weight loss, with a goal weight reduction of ≥10%, appears to result in the most improvement in serum aminotransferase levels, and hepatic steatosis, underlying inflammation, necrosis and fibrosis. In fact, a 5–10% reduction in weight has been shown to result in a 30% reduction in visceral fat.[129] However, in two small studies, rapid weight loss through near starvation diets resulted in increased hepatic inflammation and fibrosis.[127,128]

Orlistat, a reversible inhibitor of gastric and pancreatic lipases, has been shown to result in a weight decrease of 5–10% with 6–12 months of therapy.[130] A small case series has shown that orlistat is effective at improving liver function tests, steatosis, inflammation and fibrosis with 6–9 months of therapy.[110] We recently completed a ten patient pilot trial demonstrating an improvement in fibrosis in four of the five patients who achieved a weight loss of ≥10%.[111]

6.1.2 Thiazolidinediones

Drugs in the thiazolidinedione class function as ligands for the PPAR-γ class of nuclear transcription factors in adipose tissue.[131] Data suggest that the thiazolidinediones upregulate specific protein kinases involved in decreasing fatty acid synthesis, resulting in improved insulin sensitivity within the adipocyte and skeletal muscle that ultimately leads to decreased plasma glucose and insulin levels.[132-134] Additionally, evidence suggests that the thiazolidinediones inhibit hepatic stellate cell activation and proliferation.[135] Two thiazolidinediones, pioglitazone and rosiglitazone, are currently on the market. Before its removal from the market, troglitazone was studied for NASH in a small pilot trial and demonstrated normalisation of aminotransferases and some histological improvement.[112] Neuschwander-Tetri et al.[113] have recently com-

Table III. Potential therapeutic modalities for patients with nonalcoholic fatty liver disease

Modality	n	Study design	Aminotransferases	Histology	Follow-up
Improved insulin resistance					
Weight loss					
Ueno et al.[107]	15	Open-label	Improved	Steatosis improved	None
Drenick et al.[108]	14	Open-label	Improved	Steatosis, inflammation, fibrosis improved	None
Palmar and Schaffner[109]	39	Retrospective review	Improved	No biopsies	None
Orlistat					
Harrison et al.[110]	3	Case series	Improved	Steatosis, inflammation, fibrosis improved	None
Harrison et al.[111]	10	Open-label	Improved	Steatosis, fibrosis improved	None
Thiazolidinediones					
Caldwell et al.[112]	10	Open-label	Improved	Mild inflammation improvement only	None
Neuschwander-Tetri et al.[113]	30	Open-label	Improved	Steatosis, inflammation, fibrosis improved	6 months; not yet published
Metformin					
Nair et al.[114]	25	Open-label	Improved	No biopsies	None
Marchesini et al.[115]	14	Open-label	Improved	No biopsies	None
Antioxidants					
Tocopherol/Ascorbic acid					
Harrison et al.[116]	49	Randomised, placebo-controlled	No improvement	Mild fibrosis improvement	None
Tocopherol					
Hasegawa et al.[117]	22	Open-label	Improved	Improved	None
Betaine hydrochloride					
Abdelmalek et al.[118]	8	Open-label	Improved	Improved	None
Improved dyslipidaemia					
Gemfibrozil					
Basaranoglu et al.[119]	46	Open-label, randomised	Improved	No biopsies	None
Clofibrate					
Laurin et al.[120]	16	Open-label	No improvement	No improvement	None
Atorvastatin					
Horlander et al.[121]	7	Open-label, dose-escalating	Improved	Steatosis, inflammation, fibrosis improved	None
Cytoprotective/anti-apoptotic					
Ursodeoxycholic acid					
Ceriani et al.[122]	31	Open-label, randomised	Improved	No biopsies	None
Laurin et al.[120]	24	Open-label	Improved	Steatosis improved	None
Guma et al.[123]	24	Open-label, randomised	Improved	No biopsies	None
Other					
Phlebotomy[a]					
Desai[124]	16	Open-label	Improved	No biopsies	None
Nitecki et al.[125]	9	Open-label	Improved	One biopsy, inflammation improved	None

a Pilot trials.

n = number of patients.

pleted a 1-year, open-label trial with rosiglitazone, with follow-up liver biopsy data. Improvement in aminotransferases and markers of insulin resistance correlated with histological improvement in steatosis, inflammation and fibrosis. Several other trials with pioglitazone and rosiglitazone are ongoing.

6.1.3 Metformin

Metformin has been shown to lower blood glucose levels by decreasing hepatic glucose production and increasing glucose utilisation within peripheral skeletal muscle.[136,137] Evidence suggests that metformin activates protein kinase signalling, resulting in improved insulin use. Using metformin in an animal model of NASH, Lin et al.[126] showed reversal of hepatic steatosis. Marchesini et al.[115] subsequently evaluated the effects of metformin 500mg three times daily for 4 months in 14 patients with NASH, and showed an improvement in aminotransferase levels and improved insulin sensitivity. Follow-up histology was not obtained. Similar improvement in aminotransferase levels was seen in another trial of 25 patients treated for 3–6 months with metformin 20 mg/kg/day.[114] Histological assessment of metformin-treated patients with NASH in this trial is anticipated.

6.2 Antioxidant Therapy

Chronic oxidative stress appears to be involved in the pathogenesis of NASH. Recently, it appeared that the administration of the antioxidant vitamin E may be beneficial in improving inflammation and fibrosis. Hasegawa et al.[117] showed improvement in both inflammation and fibrosis in five of nine patients with NAFLD who lost weight and then were treated with tocopherol (vitamin E) 300 mg/day for 6 months. We recently completed a double-blind, randomised, placebo-controlled trial of tocopherol 1000 U/day plus ascorbic acid (vitamin C) 1000 mg/day in a cohort of 49 patients treated for 6 months.[116] Follow-up liver biopsies showed a modest improvement in fibrosis in the vitamin group but no improvement in inflammation.

Betaine hydrochloride, also known as trimethylglycine, functions as a methyl donor in several key hepatic metabolic pathways involved in gene expression, membrane fluidity and generation of glutathione, a major hepatic antioxidant.[138] This nutritional supplement can be obtained from most local grocers and nutrition centres. It has been shown to increase the 2-year survival in some patients with alcoholic liver disease.[139] A small pilot trial of ten patients with histologically confirmed NASH were treated with betaine hydrochloride daily for 1 year.[118] Seven patients completed the trial and aminotransferase levels either normalised or improved in six of seven patients. Furthermore, six patients had follow-up liver biopsies demonstrating improvement in steatosis, inflammation and fibrosis.

6.3 Improving Dyslipidaemia

The association of hyperlipidaemia, in particular hypertriglyceridaemia, with NAFLD is well known. Treatment of this associated condition seems logical but it is unknown at the present time if treatment results in improvement in NAFLD. Gemfibrozil is the only lipid-lowering agent studied in a randomised clinical trial for NASH.[119] It improved serum ALT, but no follow-up liver biopsies were performed and thus there was no histopathological assessment of disease improvement. Atorvastatin, an HMG-CoA reductase inhibitor or statin, was recently evaluated in a small pilot trial of seven patients with histological evidence of NASH.[121] Lipid levels were reduced using titrated doses of atorvastatin. After 1 year of therapy, repeat liver biopsies showed improvement in steatosis, inflammation and fibrosis. A prospective, randomised trial of simvastatin is currently in progress.

6.4 Cytoprotective/Anti-Apoptotic Agents

Ursodeoxycholic acid, a hydrophilic bile acid, is frequently used in patients with chronic cholestatic liver disease. The precise mechanisms of action are not known, but data suggest that ursodeoxycholic acid stabilises hepatocellular membranes, reducing mitochondrial membrane damage and potentially decreasing cellular apoptosis during chronic oxidative stress.[140] Several small pilot trials in patients with NAFLD have demonstrated improvement in

aminotransferases,[120,122,123] but only one trial[120] obtained follow-up histological data, which demonstrated improvement in hepatic steatosis only.

6.5 Decreasing Hepatic Iron Levels

Two pilot studies, totalling 25 patients, have demonstrated the efficacy of phlebotomy in decreasing aminotransferase levels in patients with NASH.[124,125] Only one follow-up liver biopsy was performed in this cohort and it showed improvement in inflammation.[125]

7. Conclusion

In summary, data on the treatment of patients with NAFLD/NASH are sparse but positive preliminary data have been reported for some treatments. However, before embarking on widespread application of various treatment modalities, these potential therapies should be subjected to large, randomised, prospective trials, powered to give strong clinical and statistical significance. To this end, the US National Institutes of Health have recently established a clinical research network for NASH with a goal of initiating long-term, randomised, clinical trials with promising therapeutic modalities. It is likely that given the multiplicity of potential aetiological associations, treatment may be tailored to individuals based on their underlying clinical phenotype. Eventually, combined therapy may be evaluated. An example might be weight loss for patients who are overweight, using orlistat to augment weight reduction, a thiazolidinedione or metformin for improved insulin use, an antioxidant such as tocopherol or betaine hydrochloride, and a cytoprotective agent such as ursodeoxycholic acid.

Acknowledgements

This work was supported in part by an AASLD/Schering Advanced Hepatology Fellowship grant. The authors have no conflicts of interest that are directly relevant to the content of this manuscript.

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