# NON ALCOHOLIC FATTY LIVER DISEASE : FROM PATHOGENESIS TO PATIENT CARE

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## SUMMARY

1991Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease in Western countries. It encompasses a wide spectrum of liver lesions, from pure steatosis to end-stage liver disease with cirrhosis and hepatocellular carcinoma. Nonalcoholic steatohepatitis corresponds only to one stage of NAFLD. As NAFLD can be considered a liver manifestation of the metabolic syndrome, its prevalence is high in obese people and in patients who have type 2 diabetes—insulin resistance is one of the key elements of the pathogenesis of NAFLD. This disease is often asymptomatic in the absence of decompensated cirrhosis, but should be suspected in patients with elevated aminotransferase levels or radiological evidence of a fatty liver or hepatomegaly. Liver fibrosis is associated with age over 50 years, obesity, diabetes and high triglyceride levels. Liver biopsy is the only way to assess the histologic features of necrotic inflammation and fibrosis that define nonalcoholic steatohepatitis and to determine its probable prognosis. The prognosis is good for pure steatosis, whereas the presence of necrotic inflammation is associated with a significant risk of progression to cirrhosis and, possibly, hepatocellular carcinoma. Lifestyle changes, such as dietary modifications and exercise, are recommended. To date, there have been very few randomized, placebo-controlled trials of drug treatments for NAFLD.

## INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is being diagnosed with increasing frequency, and can progress to end-stage liver disease. NAFLD is seen in obese patients in the absence of significant alcohol use.1 Steatohepatitis corresponds to a stage within the spectrum of NAFLD.(2) Epidemiological studies have estimated that 20% (range 15–39%) of obese patients with steatosis go on to develop nonalcoholic steatohepatitis (NASH), fibrosis and cirrhosis,(3) which makes NAFLD one of the three principal causes of cirrhosis, together with viral hepatitis and alcohol abuse.(2,3) The mechanisms responsible for the shift from steatosis to steatohepatitis are not fully under-stood. The presence of NASH is associated with a significant risk of progression to fibrosis and cirrhosis, which are clinically the most critical aspects of NASH as a chronic liver disease. Few drugs, if any, have been shown to reduce or cure liver damage directly, independently of weight loss. In this article, we shall first examine the epidemiology and mechanisms of NAFLD. Then, we shall describe its clinical, biological and pathological features. Finally, we shall discuss treatment options according to the patho genesis of this disease.

## EPIDEMIOLOGY OF NAFLD

NAFLD is thought to be the most common liver disease in Western countries. In the US, the Third National Health and Nutritional Examination Survey estimated the prevalence of NAFLD on the basis of levels of the liver enzymes alanine aminotransferase, aspartate aminotransferase and ã -glutamyltranspeptidase.4 With the threshold value for these levels set at 30 U/l, the prevalence of NAFLD was estimated at about 23%, which increased to 74% among obese individuals. General population studies have used ultra-sound scans and liver function tests to diagnose NAFLD. For example, one population-based study enrolled 6,917 of a total of 10,150 citizens from two towns in northern Italy, from March 1991 through to March 1993.

The prevalence of steatosis in this population was estimated to be 55% in individuals who consumed less than 20 g of alcohol per day: within this group, the prevalence was 24.5% in those with a BMI of 25 kg/m 2 or less, 67% in those with a BMI between 25 kg/m 2gf and 30 kg/m 2 , and 91% in those with a BMI of 30 kg/m 2 or over. In white Europid individuals, NAFLD affects both sexes equally. Most patients with cryptogenetic cirrhosis have a history of diabetes (47%)7 or obesity (55–73%),(7,8) which can be hidden because of weight loss associated with aging or cirrhosis. This observation suggests that cryptogenetic cirrhosis is, in fact, the end stage of NAFLD. The metabolic syndrome, which is also known as insulin resistance syndrome, is an association of hypertension, central obesity, high fasting blood glucose concentration, high triglyceride concentration, and low HDL cholesterol concentration( 9) The prevalence of steatosis is exponentially increased in patients who have type 2 diabetes, hyper lipidemia, obesity, and hypertension: systemic hypertension is an independent predictor for NASH (odds ratio 5.2,95% CI 2.0–13.5).10 Low HDL-cholesterol levels and/or hyper triglyceridemia are found in more than 75% of patients with fatty liver.(11) The adjusted odds ratio for the development of NAFLD in persons with the metabolic syndrome is 4.00 (95% CI 2.63–6.08) for men and 11.20 (4.85–25.87) for women.(12) NAFLD should, there-fore, be seen as one e element of a multifactorial metabolic disease. Estimation of the exact prevalence of NASH is difficult, as only liver biopsy results can distinguish NASH from other stages of NAFLD, in particular steatosis without inflammation. If we consider that between one-third and one-half (13) of patients with NAFLD actually have NASH (assuming an overall prevalence of NAFLD of between 23%4 and 55%5 ), the estimated prevalence of NASH is between 8% (one-third of 23%) and 27% (half of 55%).

## PATHOGENESIS OF NAFLD—FROM INSULIN RESISTANCE TO STEATOHEPATITIS

NAFLD develops in two steps. A healthy liver first becomes steatotic and vulnerable to further damage. Oxidative stress then provides a 'second hit' that triggers NASH. The mechanisms that underlie NAFLD have generally been elucidated only in animal models of NAFLD or in *in vitro* experiments. Caution must, therefore, be used when extrapolating findings from animal models to humans.

### From insulin resistance to steatosis

Liver, fat and muscle have key roles in the patho-genesis of insulin resistance. Defective insulin signaling leads to ineffective suppression of glucose production via increased gluco neogenesis in the liver and the impairment of glucose uptake by muscle and adipose tissue. In adipose tissue, this insulin signal impairment leads to increased lipolysis. Lipid metabolism is also decreased by a fall in levels of sterol regulatory element binding protein 1, which regulates the transcription of several genes involved in lipid synthesis. In this context, storage of free fatty acids in the liver occurs as a result of elevated levels of circulating fatty acids that originate from adipose tissue, an increase in *de novo* liver lipogenesis and, to a lesser extent, an increase in the hydrolysis of chylomicrons from the intestine. Depending on nutritional and hormonal status, liver free fatty acids are oxidized in mitochondria or re-esterified to form tri glycerides, which then accumulate in the cytosol or are secreted as VLDL (Figure 1) (reviewed by Begriche *et a*l.14 ).

**Crosstalk between liver and adipose tissue**
Adipose tissue takes an active role in the establishment of insulin resistance via secretion of adipokines and cytokines (see Box 2).(15,16).

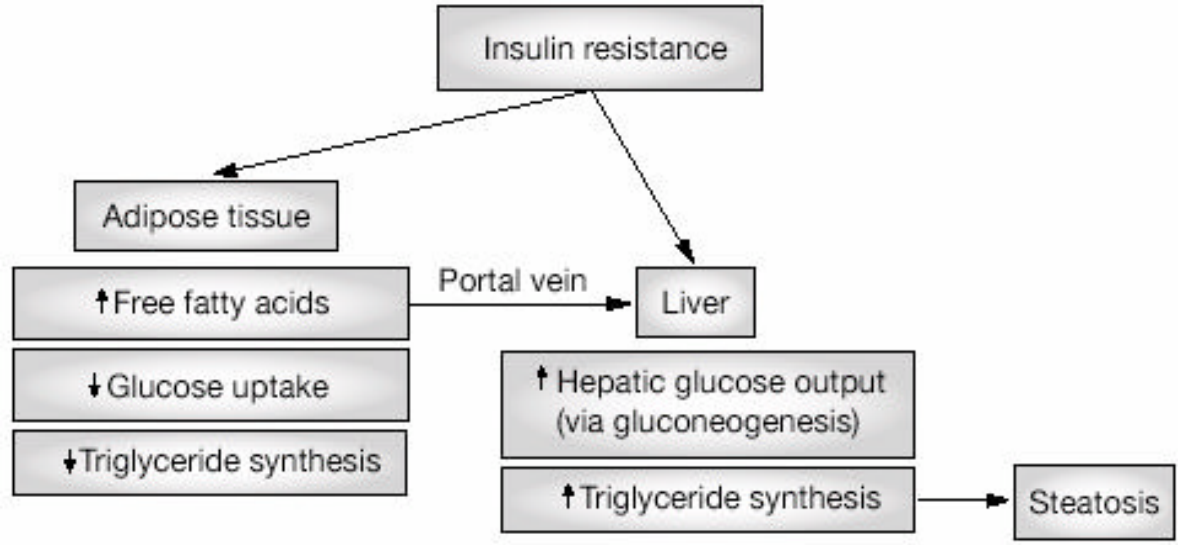


*Figure 1 Metabolic alterations that result in steatosis in nonalcoholic fatty liver disease. In insulin-resistant patients, the insulin-mediated regulation of lipolysis is impaired in adipose tissue, which leads to decreased glucose uptake, decreased triglyceride synthesis, and delivery of large amounts of free fatty acids to the liver. Insulin resistance in the liver increases glucose production via gluconeogenesis and the accumulation of fatty acids.*

Box 1 International Diabetes Federation (IDF) criteria for the metabolic syndrome.
Diagnosis of the metabolic syndrome requires central obesity (waist circumference =94 cm for Europid men and =80 cm for Europid women; there are specific values for other ethnic groups) plus any two of the following features: raised triglyceride levels ( =150 mg/dl [1.7 mmol/l]) or specific treatment for this lipid abnormality; reduced HDL-cholesterol level (<40 mg/dl [1.0 mmol/l] in males and <50 mg/dl [1.3 mmol/l] in females) or treatment for this lipid abnormality; raised blood pressure (systolic blood pressure =130 mmHg or diastolic blood pressure =85 mmHg) or treatment for previously diagnosed hypertension; raised fasting plasma glucose level ( =100 mg/dl [5.6 mmol/l) or previously diagnosed type 2 diabetes. If glucose levels are >100 mg/dl, an oral glucose tolerance test is strongly recommended but is not necessary to define presence of the metabolic syndrome.

Adiponectin, produced by adipocytes, decreases hepatic glucose production and increases muscle glucose and fatty acid utilization. Adiponectin also affects cytokine secretion and, therefore, has anti-inflammatory properties. Circulating adiponectin levels are low in obese patients, and these levels inversely correlate with hepatic fat content in patients with NAFLD. (17) In obese *ob/ob* mice, the delivery of adiponectin decreases steatosis and liver inflammation. (18) Although adipokines seem to have very promising therapeutic effects, caution is required as the bioactive form of adiponectin has not yet been identified (reviewed by Kadowaki *et a*l. (19 )).

Leptin, produced by adipocytes, is involved in regulation of food intake. Leptin also regulates T-cell and inflammatory responses, (20,21) and obese patients have high leptin levels. Leptin might also be involved in hepatic fibrogenesis, through activation of stellate cells.(22) Leptin treatment seems to be ineffective, however, because of the leptin resistance observed in obese patients. (23) Resistin, a potent proinflammatory agent, is produced in large amounts in patients with type 2 diabetes and can cause severe hepatic insulin resistance. Resistin levels are positively correlated with tumor necrosis factor (TNF) levels in patients with type 2 diabetes and with the histological inflammatory score in patients with NAFLD. (24,25) Two other adipokines, visfatin and vaspin, were described in 2005. (26,27) Both are specifically secreted by visceral adipose tissue. They lower glucose levels, but their involvement in the establishment of insulin resistance remains unclear (reviewed by Ailhaud *et a*l.(28) and Fantuzzi *et a*l.,(29) respectively). These adipokines are of considerable interest, because visceral fat is known to be involved in the development of complications that result from insulin resistance.(15)

Adipose tissue also produces other cytokines and chemokines, particularly TNF, interleukin (IL)-6 and CC chemokine ligand 2 (CCL2, also known as MCP1 or monocyte chemo-attractant protein 1). (28,29) TNF and IL-6 are both pro inflammatory cytokines that are involved in liver injury. TNF contributes to insulin resistance by inhibition of insulin-receptor signalling; although TNF levels are high in the adipose tissue of obese patients, (30) no currently available anti-TNF antibody can reduce insulin resistance in diabetic patients. (31) IL-6 levels are also high in the plasma and adipose tissue of obese patients, particularly at visceral sites. IL-6 induces hepatic production of acute-phase reactants, such as C-reactive protein, which is associated with the development of cardiovascular complications. CCL2, a chemokine involved in monocyte recruitment, is also produced in large amounts in obese patients; this chemokine upregulates inflammatory cell recruitment. (32)

These conditions lead to the development of steatosis and also actively contribute to a low-grade, chronic inflammatory state (see below). The precise mechanisms that underlie the shift from steatosis to steatohepatitis are, however, unknown. Hypothetical mechanisms have been deduced from animal models and from alcohol-induced liver diseases, both of which are histologically similar to NAFLD (Figure 2).

Box 2 Cytokines and chemokines in nonalcoholic fatty liver disease.

A balance between T-helper types 1 and 2 (T H 1 and T H 2) cytokines regulates the inflammatory process in nonalcoholic fatty liver disease.

T H 1 cells mediate proinflammatory activity such as attraction and activation of macrophages. Proinflammatory T H 1 cytokines include tumor necrosis factor, interferon-? , interleukin (IL)-1ß , IL-6, IL-12, and IL-8 (also known as the chemokine CXCL8, or CXC chemokine ligand 8).

T H 2 cells specialize in B-cell activation. They mediate inflammation, allergy and immunoglobulin synthesis. T H 2 cytokines include B-cell growth factors (for example IL-4, IL-5, IL-15) as well as the anti-inflammatory cytokine IL-10 and the profibrogenic cytokine transforming growth factor ß .

Natural killer T (NKT) cells express the cell-surface marker NK1.1, which is also found on natural killer cells. NKT cells, however, also express the aß T-cell receptor, which is found on T cells. Many NKT cells express CD4. They regulate T H 1 versus T H 2 responses.

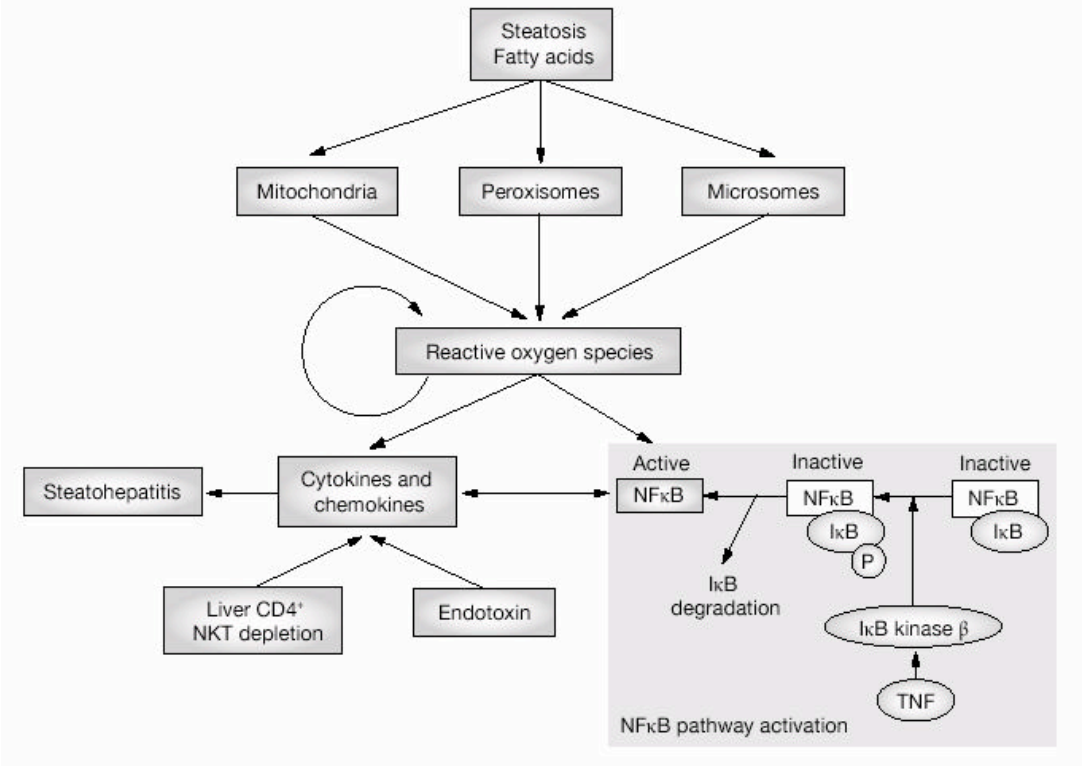


*Figure 2 From steatosis to steatohepatitis. Oxidation of free fatty acids can occur in mitochondria, peroxisomes or microsomes. This oxidation causes oxidative stress, owing to formation of reactive oxygen species, which can directly damage mitochondria. Reactive oxygen species can also increase their own production via positive feedback. They cause inflammation by inducing production of proinflammatory cytokines and chemokines. Reactive oxygen species and proinflammatory cytokines activate the NF?B pathway, which exacerbates inflammation and decreases insulin signaling. In the NF?B pathway, I?B binds to the transcription factor NF?B, and inhibits its actions by retaining it in the cytosol. I?B kinase ß is activated by TNF and phosphorylates I?B; once phosphorylated, I?B dissociates from NF?B, which can then enter the nucleus and activate genes involved in adaptive immunity and proinflammatory cytokine secretion. According to the intestine–liver interaction hypothesis, absorption of lipopolysaccharide (bacterial endotoxin) can trigger the release of TNF. Immune dysfunction of the liver, such as the depletion of CD4 + NKT cells, is accompanied by polarization of cytokine-producing liver cells to a T-helper type 1 phenotype and excessive production of proinflammatory cytokines. Abbreviations: I?B, inhibitor of NF?B; NF?B, nuclear factor ?B; NKT, natural killer T; TNF, tumor necrosis factor; P, phosphate group.*

**Oxidative stress**

Patients with NASH display decreased mitochondrial DNA levels and, therefore, have low levels of several proteins that are encoded by mitochondrial DNA. This decrease in mitochondrial proteins can lead to the over-reduction of respiratory chain complexes and the generation of reactive oxygen species (ROS), which damage mitochondrial DNA and impair mitochondrial function. (33) Fatty acids can also undergo ß oxidation in peroxisomes, which produces large amounts of hydrogen peroxide. Microsomal ? -oxidation of fatty acids, which is catalyzed by the cytochrome P450 enzymatic complex (isoforms 2E1, 4A10, and 4A14), also leads to the formation of ROS. (34) ROS can attack polyunsaturated fatty acids, which initiates lipid peroxidation within the cell (35) and leads to the formation of aldehyde products, such as 4-hydroxynonenal and malondial dehyde. They increase the production of proinflammatory cytokines (e.g. TNF, interferon-ã and IL-8, which is also known as CXC chemokine ligand 8 or CXCL8), the migration of inflammatory cells to the liver, and the activation of stellate cells. These effects lead to inflammation, hepatocyte death, necrosis and fibrosis.

**Production of tumor necrosis factor and other cytokines**

TNF production by Kupffer cells activates the nuclear factor ?B (NF?B) pathway through phosphorylation of the inhibitor of ?B by the inhibitor of ?B kinase ß (Figure 2). The NF?B pathway regulates the expression of pro inflammatory cytokines and their receptors, chemokines (e.g. IL-8), inflammatory enzymes (lipo-oxygenase, cyclo-oxygenase, nitric oxide synthetase), and adhesion molecules. NF?B also increases the production of TNF, which impairs insulin signaling by inhibiting the function of insulin receptor substrate 1. Kupffer cells are also a major source of transforming growth factor ß 1, which has a key role in the patho genesis of liver fibrosis. This cytokine upregulates smooth-muscle a-actin and collagen production and seems to be important for the activation of hepatic stellate cells, a key step in the process of liver fibrosis. (36) The livers of genetically obese *ob/ob* mice develop steatosis and, after exposure to lipo-polysaccharide, steatohepatitis. They are selectively depleted of CD4 + natural killer T (NKT) cells because of high rates of NKT cell apoptosis. This depletion is accompanied by polarization of the other cytokine-producing liver mono-nuclear cells towards a T H 1 (T helper type 1) phenotype (see Box 2). These *ob/ob* mice are also deficient in norepinephrine, and treatment with this neurotransmitter replenishes their hepatic NKT cell population and downregulates pro inflammatory cytokine production. (37)

**The intestine–liver interaction hypothesis**

Intestinal permeability is increased in genetically obese mice. (38) Bacterial products such as lipo-polysaccharide (endotoxin), which is derived from bacterial cell walls broken down in the digestive tract, are present in large amounts in the portal vein and can stimulate TNF production by Kupffer cells, increase hepatic oxidative stress and upregulate the endogenous production of ethanol in obese mice. (39) The administration of oral antibiotics to obese *ob/ob* mice decreases liver inflammation (Bigorgne A *et al*., unpublished data). In summary, liver lesions, and steatosis in particular, are manifestations of a systemic disease induced by obesity and, in particular, by insulin resistance. The inflammatory state induced by insulin resistance is essential for the development of acute liver diseases such as NASH. The precise factors that lead to the development of NASH from steatosis have not yet been clearly identified, but oxidative stress and bacterial endotoxin from the digestive tract seem to have an important role.

## CLINICAL MANIFESTATIONS AND DIAGNOSIS OF NAFLD

**Symptoms of NAFLD**

A diagnosis of NAFLD is clinically suspected in asymptomatic patients who have elevated aminotransferase levels (alanine aminotransferase and aspartate aminotransferase) or radio logical evidence of a fatty liver or hepatomegaly. Diagnosis of NAFLD might result from follow-up of patients newly diagnosed with type 2 diabetes mellitus, hypertension or dyslipidemia. When symptoms do occur, they are non-specific : vague right-upper-quadrant abdominal pain, fatigue, and malaise. Rarely, pruritus, anorexia and nausea can develop. The occurrence of ascites with abdominal distension, variceal hemor-rhage or hepatic encephalopathy is indicative of progression to decompensated cirrhosis.

**Physical examination**

Excess weight (BMI >25 kg/m 2 ) or obesity (BMI >30 kg/m 2 ) are the most common signs. Patients often have a large waist circumference and increased supine abdominal height, which reflect central fat accumulation. Hepatomegaly is observed in up to 50% of patients.1,40 Signs of chronic liver disease, such as jaundice, edema and asterixis, are usually absent at the time of diagnosis, although splenomegaly was reported in 25% of patients in one study. (1) Acanthosis nigricans (gray, brown or black pigmentation, chiefly in axillae and other body folds)—a clinical marker of insulin resistance—has been described in children with NAFLD.

**Laboratory data**

Elevated serum aminotransferase levels are often the only abnormality. The increase is moderate, to 1–4 times the upper limit of normal values. Patients with NAFLD, unlike those with alcohol-induced steatohepatitis, usually have a ratio of aspartate aminotransferase to alanine aminotransferase below 1; however, this ratio tends to increase with the development of fibrosis and, therefore, loses its diagnostic value. (1,41) Aminotransferase values do not reflect the extent of liver injury, and a low to normal alanine aminotransferase level does not guarantee the absence of underlying steatohepatitis or advanced fibrosis.(42) Levels of ?-glutamyltranspeptidase and alkaline phosphatase are also above the normal range. Prothrombin time and serum levels of albumin and bilirubin are normal, unless the patient presents with cirrhosis and liver failure.(2) A high ferritin concentration has been reported in approximately 50% of patients and an increase in transferrin saturation has been reported in 6–11% of patients; (41) however, the hepatic iron concentration is usually normal. (41,43) Heterozygosity for the hemochromatosis gene *(HF*E) has been suggested to be associated with iron overload and advanced stages of NAFLD; (43)however, this hypothesis was not confirmed in the most recent study, published in 2002. (44)

**Imaging studies**

Steatosis is diffuse in most patients, but can occasionally be focal. On ultrasound scans, the fatty liver shows increased echogenicity, described as 'bright liver', and appears brighter on the scan than the kidney. The sensitivity of ultra-sonography to detect steatosis is between 67% and 100% if fatty liver infiltration exceeds 33% on liver biopsy. (45) On CT scans, the fatty liver displays low-density hepatic parenchyma. Nuclear magnetic resonance can be used for the quantitative assessment of fatty infiltration of the liver, and this technique is better than ultrasonography for detecting and quantifying mild hepatic steatosis. (46) Magnetic resonance spectroscopy is a noninvasive method for measurement of tissue fat content, including intra hepatocellular lipids; this technique provides a sensitive method to quantify steatosis when applied to a large population.(47)

**Liver biopsy**

*Findings*

Only histological examination of the liver can confirm the diagnosis of NAFLD and evaluate its severity (i.e. steatosis alone or an advanced stage of NAFLD), in order to predict prognosis and guide management of these patients. The histological features of NAFLD are of three types: steatosis alone; NASH; and cirrhosis. Fatty changes are predominantly macro vesicular, and their severity can be estimated by determining the proportion of hepatocytes that contain fat droplets: less than 5%; 5–33%, 33–66%, and higher than 66%. In 2005, a scoring system for NAFLD was proposed. (48) This score provides a tool to assess improvement or worsening of NAFLD in longitudinal and interventional studies, but does not include fibrosis. The precise definition of a frontier that allows NASH to be distinguished from steatosis alone is very difficult in practice. (49) The characteristic features of steatohepatitis include various levels of hepatocyte ballooning and spotty necrosis, a scattered mixed inflammatory infiltrate that contains both polymorphonuclear and lymphocyte cells, and Mallory's bodies (hyaline cytoplasmic inclusions of cyto keratin within hepatocytes). Patients with NASH necessarily display all the features of steatohepatitis. A NAFLD activity score that includes only the features of active injury that are potentially reversible in the short term, has been proposed. This activity score comprises the unweighted sum of scores for steatosis (0–3), lobular inflammation (0–3), and hepatocyte ballooning (0–2) and, therefore, can range from 0 to 8. Fibrosis, which is less reversible and thought to result from disease activity, is not included in the activity score for NAFLD staging. (48) Fibrosis can progress to cirrhosis, which initially occurs in a micronodular pattern, but large nodules may develop. Typical features of NASH might disappear once cirrhosis has developed, (8) which explains why many cases of cirrhosis previously classified as cryptogenetic are now thought to correspond to late-stage NAFLD. (7) Hepatocellular carcinoma (HCC) can also be detected on liver biopsy. (50)

*Indications for liver biopsy*

A liver biopsy should be performed in patients at a high risk of fibrosis. A decision-making algorithm for liver biopsy is proposed in Figure 3. There are several independent clinical and biological predictors of hepatic fibrosis: two-thirds of patients who are over the age of 45 years and diabetic or obese have advanced fibrosis. (41) Patients with diabetes mellitus and NAFLD are more likely to develop cirrhosis and to have higher mortality rates than nondiabetic patients with NAFLD. (51)

The BAAT score (namely, the number [from 0 to 4] of the following factors that are present: BMI =28 kg/m 2 , age =50 years, alanine aminotransferase levels at least twofold the upper limit of normal, triglyceride levels =1.7 mmol/l) has been proposed to improve identification of patients at a high risk of fibrosis. (52) A BAAT score of 0 or 1 has 100% negative predictive value for septal fibrosis. Conversely, almost two-thirds of patients with a BAAT score of3 or 4 have significant fibrosis. Other similar scores that combine age, the ratio of aspartate aminotransferase to alanine aminotransferase, BMI, diabetes, triglycerides, hypertension, and insulin resistance index, are known by several acronyms (BARD, BARG, and HAIR). These scores, as well as the BAAT score, have been validated for the prediction of advanced fibrosis in patients with NAFLD. (10,13)

*Alternatives to liver biopsy*

A combination of serum biochemical markers has been validated for the diagnosis of liver fibrosis (i.e. the FibroTest–FibroSURE™ algorithm from Biopredictive, Paris, France, which is based on a2-macroglobulin, haptoglobin, gammaglobulin, apolipoprotein-AI, ?-glutamyl-transpeptidase, and total bilirubin levels) and liver inflammation (i.e. the ActiTest™ algorithm, which is also from Biopredictive and is based on the same markers plus alanine aminotransferase), respectively. Use of these algorithms can avoid the risks of liver biopsy for patients with hepatitis C virus (HCV) infection. The use of the FibroTest–FibroSURE™ algorithm for the prediction of liver fibrosis in patients with NAFLD was validated in 2006. (53) Results are given as a score within the range 0–1. With a cut-off of 0.30, the negative predictive value of this algorithm for a diagnosis of significant fibrosis (i.e. at least F2 by the Metavir classification) is 94%, and the positive predictive value (with a cut-off of 0.70) is 78%. (53)

Transient elastography (FibroScan ® , EchoSens, Paris, France) is a new, noninvasive, rapid and reproducible method for the evaluation of liver fibrosis, which measures liver stiffness. This technique readily detects advanced stages of liver fibrosis but is less powerful for diagnosing early stages of fibrosis. (54) The large amount of subcutaneous fat in obese patients can make elastography difficult, (55) but new ultra-sound probes should improve the reliability of this technique.

The place of these noninvasive procedures is still not well defined. Currently, the only way to definitively prove a diagnosis of NAFLD and to anticipate prognosis remains liver biopsy. It is likely, however, that liver biopsies will be performed less and less frequently with the improvement of these procedures.

## CLINICAL COURSE OF NAFLD

Long-term follow-up of patients with NAFLD is required to determine the natural history of the disease. The largest such study to date was part of the Rochester Epidemiology Project. (56) This study followed 420 patients between 1980 and 2000; the patients were from Olmsted County, MN, and were diagnosed with NAFLD. Patients with NAFLD had a higher than expected standardized mortality ratio (1.34, 95% CI 1.003–1.760; $P$ = 0.03). Liver disease was the third most important cause of death (13% of all deaths)—after cancer, the most common cause, and ischemic heart disease. By contrast, liver disease was only the 13$^{th}$ most common cause of death in the general population. (56)

Progression of NAFLD seems to reflect primarily the severity of tissue damage.2 Pure steatosis was found to progress to NASH, fibrosis and cirrhosis in only 5% of patients over a 5–17-year follow-up period, with no effect on overall mortality. (57,58) Up to 25% of patients with NASH, with or without fibrosis, progress to cirrhosis over 3–8 years of follow-up. (59,60)

Once a patient with NAFLD develops cirrhosis, the prognosis seems to be poor; one-third of these patients die or develop morbid conditions during short periods of follow-up. (61,62) In a retrospective study, the survival of overweight patients with cryptogenetic cirrhosis was found to be lower than that of untreated patients with HCV-related cirrhosis, who were matched for age and sex. The risk of HCC in patients with NAFLD seems to be similar to that in patients with HCV-related cirrhosis. (62) Diabetes increases the incidence of HCC.

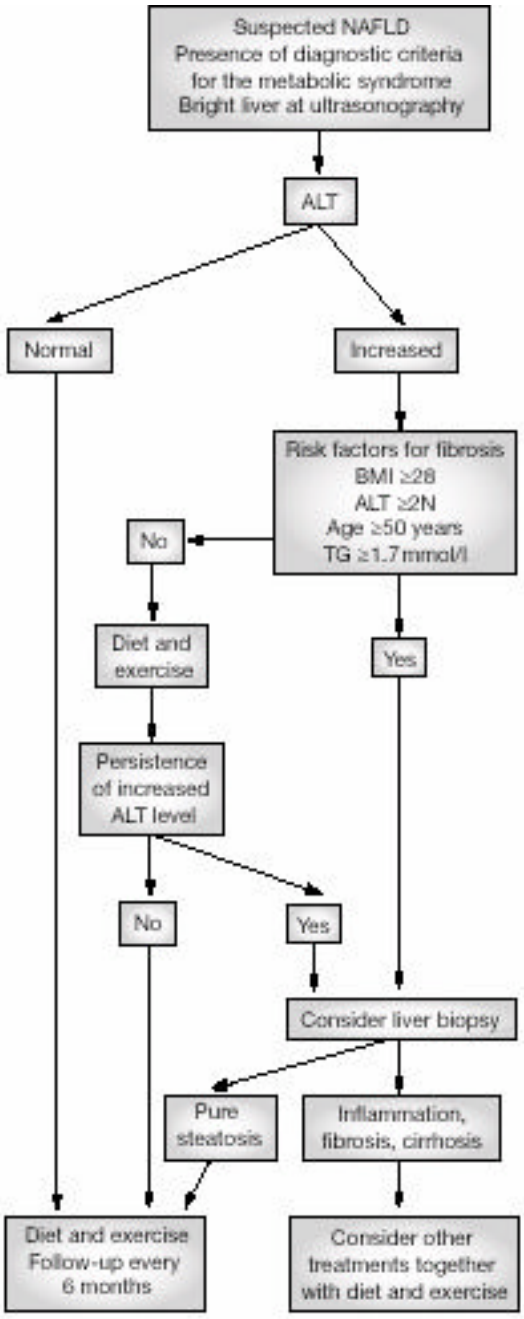


*Figure 3 Proposed algorithm for the diagnosis, confirmation, and treatment of NAFLD. Abbreviations: 2N, twice the upper limit of the normal range; ALT, alanine aminotransferase; NAFLD, nonalcoholic fatty liver disease; TG, triglyceride.*

**INTERACTION OF NAFDL WITH OTHER LIVER DISEASES**

**NAFLD and chronic hepatitis C**

Steatosis occurs in more than 50% of patients with chronic HCV infection, and results from both viral and host factors. (63,64) In patients infected with a genotype 1 virus, steatosis is related to the coexistence of NAFLD and HCV. Steatosis accelerates the development of fibrosis in chronic HCV infection. Weight reduction can improve serum alanine aminotransferase and insulin levels, as well as liver lesions. (65) Together with antiviral

treatment, weight loss can provide an important adjuvant treatment strategy for patients with steatosis and HCV infection.

**NAFLD and alcoholic liver disease**

Carrying excess weight can worsen alcoholic liver disease. Patients who have been overweight for at least 10 years are 2.15 times more likely to have cirrhosis than nonoverweight patients, after adjustment for age, sex, daily alcohol consumption and total duration of alcohol abuse; (66) moreover, BMI and blood glucose are also independent risk factors for fibrosis in alcoholic liver disease. (67) These studies suggest that excess body weight in alcoholic patients can exacerbate the metabolic
effects of ethanol ingestion.

**NAFLD and hepatic iron storage**

Abnormal serum iron concentrations have been described in patients with NAFLD, with hyperferritinemia most commonly observed. The role of iron in NAFLD is unclear. It might contribute to disease progression, as high iron levels are a source of oxidative stress. Conversely, obesity-related steatosis is independently associated with fibrosis in patients with genetic hemochromatosis.

**MANAGEMENT OF NAFLD**

**Exclusion of other causes of steatosis and steatohepatitis**

The metabolic syndrome and its components are the primary cause of NAFLD. It is, therefore, important to differentiate metabolic causes of NAFLD from other causes (Box 3), as their pathogenesis and outcomes are different. The management of cirrhosis is nonspecific. Screening for portal hypertension and HCC is essential, as for any other cause of cirrhosis. Patients with NAFLD who develop end-stage liver disease should be evaluated for liver transplantation.

The treatment of NAFLD is similar to that of the metabolic syndrome—interventions that target weight loss, insulin resistance or diabetes and hyperlipidemia—as no specific liver treatment has proven effective against NAFLD in the long term. Only a few randomized, controlled studies have been carried out to evaluate the efficacy of drug treatments for NASH. The choice of drug is, therefore, often based on pathophysiological hypotheses and the results of small, nonrandomized studies.

**Weight management**

Weight loss increases insulin sensitivity and can resolve NAFLD. A retrospective study on overweight patients showed that decreasing body weight by about 10% corrected abnormal hepatic function test results, and that every 1% decrease in body weight decreased alanine aminotransferase activity by 8.1%.68 The initial target for weight loss should be 10% of baseline weight within a period of 6 months. Exercise is important to achieve and maintain weight loss, and to increase insulin sensitivity.

Two drugs are approved for weight management : sibutramine and orlistat. (69) Rimonabant—an endocannabinoid CB1 receptor antagonist—reduces food intake, hunger and body weight, but its efficacy has not been demonstrated against this disease. Bariatric surgery is reserved for patients with a BMI over 35 kg/m$^2$. A recent report, published in 2006, suggested that bariatric surgery can decrease liver enzyme levels and improve liver lesions. (70)

**Box 3 Main causes of fatty liver.**

*Causes associated with insulin resistance*

- Diabetes, obesity, hyperlipidemia, polycystic ovary syndrome

Nutritional causes

- Alcohol, protein malnutrition, starvation and cachexia, rapid weight loss, gastrointestinal surgery (jejunoileal bypass, gastroplasty for morbid obesity, biliopancreatic diversion, extensive small-bowel resection, Roux-en-Y gastric bypass), total parenteral nutrition

*Metabolic or genetic causes*

- Acute fatty liver of pregnancy, lipodystrophy, dysbetalipoproteinemia, hypobetalipoproteinemia, cholesteryl ester storage disease, type 1 glycogen storage disease

*Drugs that can cause fatty liver*

- Corticosteroids, amiodarone, methotrexate, tamoxifen, aspirin, calcium-channel blockers,

tetracycline, cocaine, valproic acid, perhexilline maleate, nucleoside analogs

*Other causes*

- Small-bowel diverticulosis with bacterial overgrowth, HIV infection, *Bacillus cereus* toxin, environmental (hepatotoxins, phosphorus, petrochemicals, organic solvents), toxic

**Drug treatments that increase insulin sensitivity**

*Metformin*

Metformin, a biguanide, acts primarily to decrease hepatic glucose output (largely by inhibition of gluconeogenesis) and to increase insulin sensitivity. In one controlled trial, 36 patients with NASH were randomly allocated to two groups : metformin plus a diet, versus the diet alone. Levels of alanine aminotransferase improved in the two groups. Metformin, but not diet alone, significantly lowered insulin resistance whereas no significant effect on liver necrotic inflammatory activity or fibrosis was observed. (71) In an open-label, randomized trial published in 2005, (72) 55 nondiabetic patients who had NAFLD were given 2 g metformin daily for 12 months. The control individuals were given either vitamin E (800 IU daily, *n* = 28) or were treated with a prescriptive, weight-reducing diet *(n* = 27). After 1 year of treatment, aminotransferase levels had normalized in 50% of patients in the metformin group, 30% in the diet group, and 15% in the vitamin E group. In multivariate analysis, metformin treatment was associated with higher rates of aminotransferase normalization, after correction for age, sex, basal aminotransferase levels, and change in BMI. Features of the metabolic syndrome improved only in patients who received metformin. Post-treatment biopsies in 17 metformin-treated patients showed significant decreases in liver fat, necrotic inflammation and fibrosis. (72)

*Thiazolidinediones*

Thiazolidinediones improve insulin sensitivity by binding to the nuclear transcription factor peroxisome proliferator-activated receptor ?. Thiazolidinediones reduce plasma fatty acid concentrations and redistribute intracellular lipid from insulin-responsive organs to peripheral adipocytes. They also reduce extracellular-matrix deposition and hepatic stellate cell proliferation and activation. (73) Troglitazone decreased amino-transferase levels in obese patients with NASH, (74) but was withdrawn from the market because of the risk of severe liver toxicity. Rosiglitazone and pioglitazone have been evaluated in pilot studies. (75,76) A very recent, placebo-controlled trial reported in 2006 that 6 months of treatment with pioglitazone improved patients' aminotransferase levels, steatosis, necrosis and inflammation, but not fibrosis. (77) These promising results need confirmation in large, long-term clinical trials.

**Treatment with lipid-lowering agents**

Fibrates and statins have only been used in small pilot studies of patients with NAFLD, and their effectiveness needs to be confirmed.

**Drug treatments that protect hepatocytes**

*Ursodeoxycholic acid*

A large, randomized, placebo-controlled study in adults with NASH showed that 2 years of treatment with ursodeoxycholic acid at a daily dose of 13–15 mg/kg did not affect the degree of steatosis, necrotic inflammation or fibrosis. (78)

*Vitamin E and vitamin C*

Vitamin E (a-tocopherol) and vitamin C have antioxidant properties. Vitamin E (400–1,200 IU daily) can decrease levels of alanine aminotransferase and aspartate aminotransferase in children. (79) In a prospective, double-blind study that compared 6 months of vitamin treatment (1,000 IU vitamin C daily plus 1,000 mg vitamin E daily) with placebo, vitamins improved fibrosis but had no effect on necrotic inflammation or levels of alanine aminotransferase. (80) In another study, vitamin E combined with pioglitazone decreased steatosis and cytologic ballooning, whereas vitamin E alone did not. (81) As mentioned above, vitamin E was less effective than met formin or nutritional counseling for patients with NASH. (72)

**Other treatments**

Angiotensin-II-converting enzyme inhibitors, iron depletion and probiotics have shown interesting results in small, noncontrolled pilot studies. Large, randomized studies are needed to assess their efficacy in NAFLD.

**Conclusion**

In the context of the current obesity epidemic, NAFLD has become the leading cause of liver disease in Western countries. NAFLD can be considered as a liver manifestation of the metabolic syndrome, and is critically linked to insulin resistance. Oxidative stress and cytokine production have major roles in the pathogenesis of NAFLD. Clinical symptoms are nonspecific and are frequently absent in patients without cirrhosis. Several scoring methods have been proposed to stage this disease, but liver biopsy remains the gold stan-dard for evaluation of prognosis. Pure steatosis has a favorable prognosis, whereas steato-hepatitis may progress to fibrosis, cirrhosis, and even HCC.

As for other features of the metabolic syndrome, weight reduction can improve liver disease. Metformin and peroxisome proliferator-activated receptor ã agonists might be useful for the treatment of inflammation or fibrosis in these patients. Liver transplantation can be considered for patients with decompensated liver disease. Large, long-term, prospective studies that include liver biopsies are required, to provide clear information about the natural history, prognosis and specific therapeutic options of this disease.

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