Hepatic steatosis in obese patients: clinical aspects and prognostic significance

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Summary

Non-alcoholic fatty liver disease is a new clinicopathological condition of emerging importance, now recognized as the most common cause of abnormal liver tests. It is characterized by a wide spectrum of liver damage: simple steatosis may progress to advanced fibrosis and to cryptogenic cirrhosis through steatohepatitis, and ultimately to hepatocellular carcinoma. Obesity is the most significant single risk factor for the development of fatty liver, both in children and in adults; obesity is also predictive of the presence of fibrosis, potentially progressing to advanced liver disease. From a pathogenic point of view, insulin resistance plays a central role in the accumulation of triglycerides within the hepatocytes and in the initiation of the inflammatory cascade. Chronic hepatocellular injury, necroinflammation, stellate cell activation, progressive fibrosis and ultimately, cirrhosis may be initiated by peroxidation of hepatic lipids and injury-related cytokine release. In the last few years, several pilot studies have shown that treatment with insulin-sensitizing agents, anti-oxidants or cytoprotective drugs may be useful, but there is no evidence-based support from randomized clinical trials. Modifications in lifestyle (e.g. diet and exercise) to reduce obesity remain the mainstay of prevention and treatment of a disease, which puts a large number of individuals at risk of advanced liver disease in the near future.

Keywords: Insulin resistance, metabolic syndrome, non-alcoholic steatohepatitis, type 2 diabetes.

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Introduction

Obesity is now recognized as a major public health problem worldwide (1); body weight excess increases mortality rates (2) and several epidemiologic and clinical studies have confirmed the pathogenic contribution of obesity to several diseases, namely arterial hypertension and diabetes mellitus (3), but also to cardiovascular mortality. In developed countries, the total burden of obesity and related conditions (high blood pressure, high cholesterol, physical inactivity) has been estimated to be of the order of 30% of disability-adjusted life years (4).

Non-alcoholic fatty liver disease (NAFLD) is a new, emerging clinical problem among obese patients. NAFLD includes a broad spectrum of liver tissue alterations, ranging from pure steatosis to cirrhosis (5,6) through non-alcoholic steatohepatitis (NASH). The prevalence of NAFLD is high in the general population; although NAFLD is a syndrome with a multifactorial aetiology (7), obesity is the most common associated factor (8). The large majority of obese subjects have ultrasonographic (US) evidence of fatty liver (9–11), and 30% have histologically documented NASH (12,13). This link is particularly noteworthy not only because of the constantly increasing epidemics of obesity worldwide (1), but also because of the finding of an increased prevalence of liver damage in obese subjects (9).

The aim of the present review is to discuss the available evidence regarding the relationship between NAFLD and obesity, the clinical meaning of fatty liver disease, its prognostic significance, and treatment options.

Definition of NAFLD

NAFLD includes, in a single definition, a broad spectrum of liver function impairments and tissue damage similar to those observed in alcoholic liver disease, but that appear in subjects who do not drink or drink only a moderate amount of alcohol. This entity has been defined with a variety of terms: fatty-liver hepatitis, non-alcoholic Laennec's disease, diabetes hepatitis, etc. According to a recent Conference at the American Association for the Study of Liver Disease (14), NAFLD may be correctly defined only on a histopathological basis, as an accumulation of fat in the liver exceeding 5–10% of its weight, or as the percentage of fat-laden hepatocytes observed at light microscopy. In clinical practice, however, the diagnosis of NAFLD is based on US evidence of 'bright liver' and reduced posterior attenuation (15) in subjects with no or moderate alcohol consumption. The accepted cut-off level is currently set at 14 units per week ($20\,g\,d^{-1}$ or the equivalent of approximately two glasses of wine per day) (14).

Several factors have been associated with NAFLD. Recently, the terms 'primary' and 'secondary' NAFLD were introduced to indicate, respectively, NAFLD not attributable to any single factor and NAFLD in which drugs, surgical procedures and miscellaneous conditions play a primary role (Table 1). A new category is emerging for specific conditions, for example, hepatitis C infection, iron overload, bacterial overgrowth, where external factors seem to interact very closely with predisposing metabolic conditions.

The progression of NALFD from steatosis to advanced fibrosis and cirrhosis has been convincingly demonstrated. The turning point is NASH, a term first proposed by Ludwig *et al.* (13) to define a condition in which histologically documented steatosis is associated with fibrosis and necroinflammation.

Epidemiology of NAFLD

Despite the lack of specific, sensitive, reproducible and non-invasive tests, thus limiting the feasibility of large-scale epidemiological studies (16), recent data indicate that NAFLD represents the most common of all liver disorders and the most frequent cause of chronic liver disease (17–19). NAFLD affects 2.8–20% of the general population in various countries (11,20) and its prevalence may be as high as 76% in obese subjects (11,21). The prevalence of NAFLD is increasing not only in Western countries, as originally hypothesized (22). Recent community studies performed in Eastern Countries (23–25) reported US evidence of steatosis ranging from 16 to 30% of the population, a frequency comparable to that observed in Western studies (8,11,22).

NALFD affects all ages. It occurs also in children and, in recent years, has been recognized as an important, relatively common paediatric disease. A prevalence of 2.6% has been reported (26), which rises to 52.8% in obese children (27). Progression of NAFLD to fibrosis or cirrhosis has been documented in a few cases of childhood obesity (28).

There is evidence that NAFLD may also be concomitant with liver diseases of varying aetiology. A liver presenting with NAFLD runs a higher risk of being damaged by other factors, from viruses to endotoxins, from alcohol to industrial toxic compounds (29); in turn, a few data suggest that other liver diseases, such as hepatitis C virus infection, run a higher risk of progression to cirrhosis when associated with NAFLD (30).

Diagnosis of NAFLD

Most NAFLD patients are incidentally found, because of abnormal liver function tests or fatty liver at US, in the absence of clinical symptoms. Diagnosis of NAFLD is tentatively based on clinical, biochemical and radiological criteria. When present, clinical signs are non-specific (fatigue, weakness, malaise) in subjects without advanced liver disease. Hepatomegaly and related abdominal discomfort may be the only sign or symptom. Serum aspartate aminotrans-

Table 1 Proposed classification of non-alcoholic fatty liver disease (NAFLD)

Primary NAFLD	Conditions associated with insulin resistance syndrome: type 2 diabetes, obesity, hyperlipaemia
Secondary NAFLD	
Drugs	Corticosteroids, synthetic oestrogens, amiodarone, perhexiline, nifedipine, tamoxifen, calcium channel blockers
Surgical procedures	Jejunoileal bypass, extensive small bowel resection
Other conditions	Total parenteral nutrition, environmental toxins, Weber-Christian disease
Uncertain NAFLD	Intestinal bacterial overgrowth
	Iron overload
	HCV-related chronic hepatitis

HCV, Hepatitis C virus.

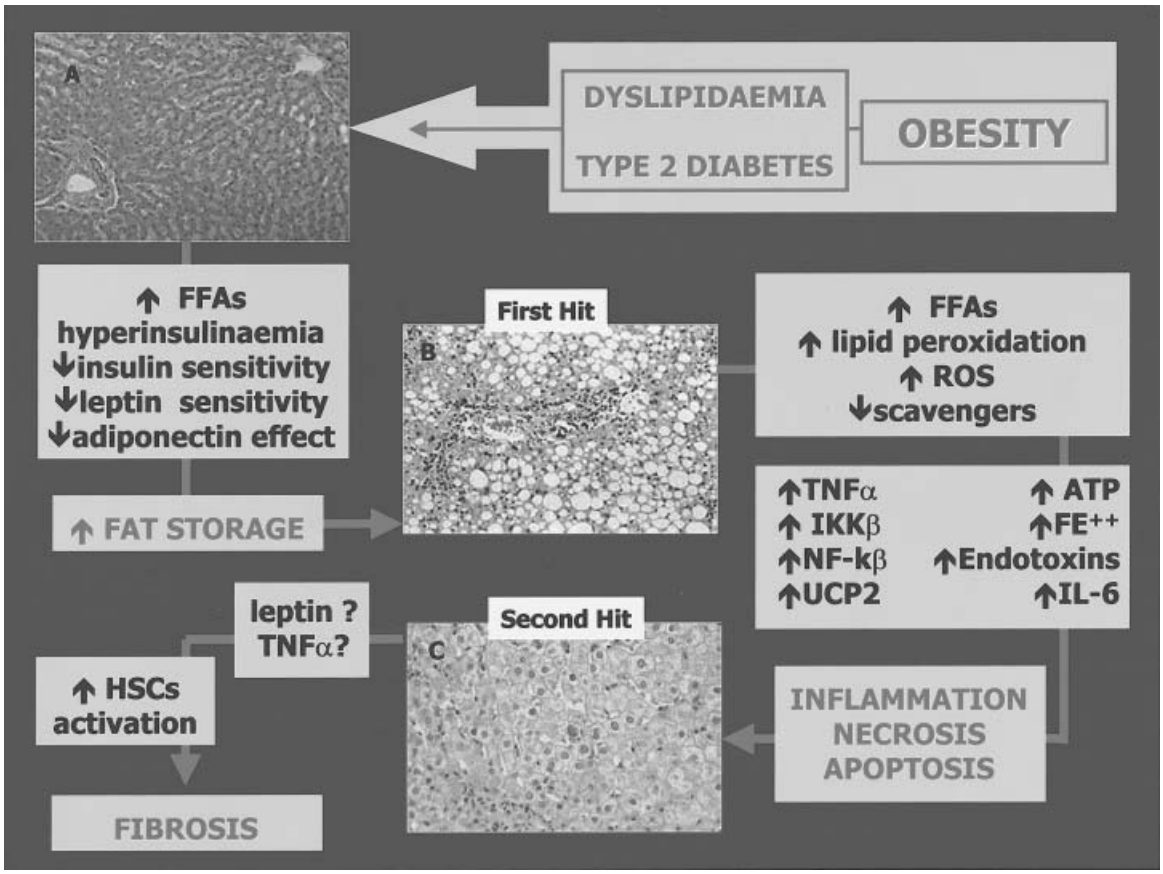

Figure 1 Pathogenesis of non-alcoholic fatty liver disease. Mechanisms of damage from normal liver (A), to steatosis (B: first hit), to necroinflammatory lesions (C: second hit). FFAs, free fatty acids; HSC, hepatic stellate cells; IL-6, interleukin-6; IKKβ: inhibitor kappa kinase beta; NF-kβ, nuclear factor kappa beta; ROS, reactive oxygen species; TNFα, tumour necrosis factor alpha; ATP, adenosinetriphosphate; UCP2, uncoupling protein-2; FE^{++}, iron.

ferase (AST) and, more commonly, alanine aminotransferase (ALT) may be mildly to moderately elevated; the AST/ALT ratio is usually <1, a sign distinguishing these cases from alcoholic liver disease (31). Levels of aminotransferase activity are not strictly correlated with staging or grading of NAFLD. In a retrospective study, NAFLD patients with normal ALT levels have been identified, also in the presence of advanced fibrosis (32).

Hepatic steatosis can be diagnosed by means of radiological techniques, for example, US, computed tomography (CT) and magnetic resonance imaging (MRI), but a recent, prospective study showed that these techniques have a low sensitivity (33). The minimum fatty liver infiltration detectable by imaging techniques is the presence of fatty droplets in 33% of hepatocytes. Liver biopsy remains the only accurate and specific means by which to grade and stage NAFLD and to diagnose NASH, as opposed to pure fatty liver (Figs 1 and 2). At present, there are no specific laboratory tests or imaging techniques by which to positively diagnose NASH. Imaging techniques (US, CT, MRI) completely fail to characterize NASH or distinguish it from pure steatosis (33).

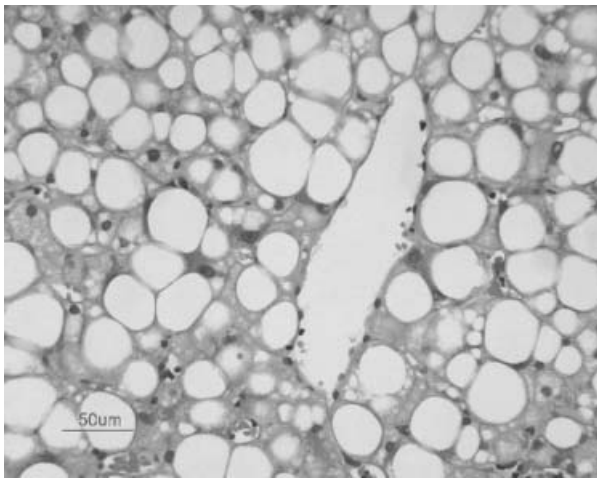

Figure 2 Liver biopsy: fatty liver. Macrovescicular steatosis is more evident in the perisinusoidal zone; ballooning is not present, as well as pericellular fibrosis (haematoxylin and eosin). Courtesy of Dr Silvia Casanova, Policlinico S. Orsola Malpighi, Bologna, Italy.

Table 2 Proposed grading and staging of histological lesions of non-alcoholic fatty liver disease

Grading for steatosis	
Grade 1	<33% of hepatocytes affected
Grade 2	33–66% of hepatocytes affected
Grade 3	>66% of hepatocytes affected
Grading for fibrosis	
Grade 1, mild	*Steatosis*: predominantly macrovescicular, involves up to 66% of lobules
	Ballooning: occasionally observed, zone 3 hepatocytes
	Lobular inflammation: scattered and mild acute (polymorphs) and chronic inflammation (mononuclear cells)
	Portal inflammation: none or mild
Grade 2, moderate	*Steatosis*: any degree, usually mixed macro- and microvescicular
	Ballooning: obvious and present in zone 3
	Lobular inflammation: polymorphs may be noted associated with ballooned hepatocytes, and/or pericellular fibrosis; mild chronic inflammation may be seen
	Portal inflammation: mild to severe
Grade 3, severe (florid steatohepatitis)	*Steatosis*: typically > 66% of lobules (panacinar); commonly mixed steatosis
	Ballooning: predominantly zone 3; marked
	Lobular inflammation: scattered acute and chronic inflammation; polymorphonuclear cells may be concentrated in zone 3 areas of ballooning and perisinusoidal fibrosis
	Portal inflammation: mild to moderate
Staging for fibrosis	
Stage 1	Zone 3 perivenular, perisinusoidal, or pericellular fibrosis; focal or extensive
Stage 2	As for Stage 1, plus focal or extensive portal fibrosis
Stage 3	Bridging fibrosis, focal or extensive
Stage 4	Cirrhosis with or without residual perisinusoidal fibrosis

Modified from Brunt *et al.* (34).

Table 3 Types of non-alcoholic fatty liver disease

Type 1, fatty liver alone
Type 2, fat accumulation and lobular inflammation
Type 3, fat accumulation and ballooning degeneration
Type 4, fat accumulation, ballooning degeneration and either Mallory hyaline or fibrosis

According to Matteoni *et al.* (35).

Table 4 Association and risk factors for non-alcoholic fatty liver disease

- Obesity (BMI > 27 kg m^{-2} in Asians, >30 in Caucasians)
- Central obesity: waist : hip > 0.90 (males) or >0.85 (females); waist > 102 cm or > 88 cm
- Diabetes mellitus, particularly type 2 (NIDDM), family history of NIDDM
- Glucose intolerance: insulin resistance
- Hypertriglyceridaemia (type 2b, type 4); low high-density lipoprotein cholesterol
- >45 years of age
- Rapid weight loss: after surgery for obesity, fasting and starvation, cachexia, associated medical disorders
- Small intestinal bacterial overgrowth
- Iron storage disorders

Recently, Brunt *et al.* (34) proposed a system in which the histological lesions of steatosis and necroinflammation are introduced into a 'grading' system and those of fibrosis into a 'staging' system for NAFLD (Table 2). Matteoni *et al.* (35) suggested a classification in which histological features are correlated with disease outcome: according to this classification, type 3 and 4 represent NASH that may be further described by using stages of fibrosis (Table 3). Patients with the most aggressive form of NAFLD (presence of steatonecrosis with Mallory hyaline and fibrosis) have a higher risk of a progressive clinical course, leading to cirrhosis and liver-related death.

By definition, NAFLD may only be diagnosed in the absence of significant alcohol consumption. An accurate history of alcohol consumption and/or drug use, also including validated questionnaires (36) and interviews with relatives for alcohol, is mandatory in all cases. Biochemical signs of other common liver diseases and of associated metabolic conditions may be usefully employed to define whether NAFLD may be classified as primary, secondary or uncertain (Table 1).

Risk factors for NAFLD

Several risk factors for NAFLD have been identified (Table 4). Focusing on primary NAFLD, overweight or frank obesity and type 2 diabetes mellitus are the most common associated factors. Any disorder of glucose regulation, from impaired fasting glucose to impaired glucose tolerance and diabetes, is significantly associated with NAFLD, and also a family history of type 2 diabetes increases the risk. This suggests that hyperglycaemia *per se*

is not the culprit, but that the metabolic impairment or the genetic milieu underlying diabetes is involved. Several studies point to insulin resistance as the link between NAFLD and metabolic diseases (37,38). This hypothesis is strongly supported by the association of NAFLD with obesity, hypertriglyceridaemia (type 2b, type 4), and reduced high-density lipoprotein cholesterol, all conditions which have hyperinsulinaemia and insulin resistance as common determinants.

Obesity, namely central obesity, is the metabolic condition most closely associated with steatosis and NASH. NAFLD is frequent in overweight or obese patients (11,21) and the likelihood of developing NASH increases with the severity of obesity; about 15–20% of morbidly obese patients have NASH (9). It has been shown that body mass index (BMI) represents the only independent predictor of the degree of fat infiltration of the liver (39) and histological examinations in potential living liver donors documented that BMI is a strong predictor of hepatic steatosis (40). However, obesity is not necessarily present in patients with NAFLD and a significant proportion of NAFLD cases have a normal body weight (31).

The pathogenesis of liver damage is strictly related to metabolic alterations related to insulin resistance. Several studies, carried out by various techniques, have convincingly associated NAFLD with both surrogate markers (Homeostasis Model Assessment method) (41,42) and dynamic measures of insulin sensitivity (Clamp technique) (38,43). In particular, insulin, in a range comparable to post-prandial levels, suppresses less efficiently both the endogenous glucose production and the high-levels of lipolysis-derived free fatty acids (38,43). Interestingly, these metabolic defects are not exclusively present in obese patients, but may be found also in non-obese, non-diabetic subjects with NAFLD (38). This suggests a genetic defect, accounting for insulin resistance, as the common factor responsible for fatty infiltration in the liver. Overweight and obesity, when present, simply magnify the defect.

The association of excess body fat with diabetes, hypertension and/or altered lipid metabolism has been identified as a distinct entity, referred to as 'metabolic syndrome' (44), carrying a high risk of cardiovascular disease. The metabolic disorder is probably much wider and most subjects have evidence also of other metabolic disorders (elevated urate concentrations, impaired fibrinolysis, endothelial dysfunction), including NAFLD. Metabolic disorders may progressively develop in the course of life, obesity usually coming first, followed by hyperlipidaemia and diabetes. According to the criteria set out in the Third Report of the National Cholesterol Education Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (45), which can be easily applied to the general population, the metabolic syndrome is present in over 60% of female and 30% of male NAFLD patients (46). Visceral obesity, expressed by a waist circumference exceeding the cut-off value of 102 cm for males and 88 cm for females, is present in 63% of females and 40% of males, in spite of a much lower prevalence of obesity (BMI = 30 kg m^{-2}). The prevalence of the metabolic syndrome increases with increasing BMI, from 18% in normal-weight subjects to 67% in obese subjects. The presence of the metabolic syndrome carries a threefold increased risk of NASH among NAFLD subjects, after correction for sex, age and body mass. In particular, the metabolic syndrome is associated with a high risk of bridging fibrosis or cirrhosis, without differences in the degree of steatosis and necro-inflammatory activity (46).

In summary, the presence of multiple metabolic disorders in subjects with hepatic steatosis is associated with a potentially progressive, more severe liver disease.

Pathogenesis of NAFLD

The pathogenesis of NAFLD is still unclear and a matter of debate, although the most prevailing theory is the 'two hit' hypothesis proposed by Day and James in 1998 (47). The 'first hit' consists of an accumulation of fat, specifically fatty acids and triglycerides, within the liver (Fig. 3). Once steatosis has developed, cellular adaptations occur through signalling pathways that are altered by chronic exposure to increased levels of oxidative stress. These allow the cell to survive in the new, stressful environment, but also enhance vulnerability to a 'second hit', possibly environmental or genetic in origin, leading to either apoptosis or necrosis, accompanied by inflammation (48). Various and differing

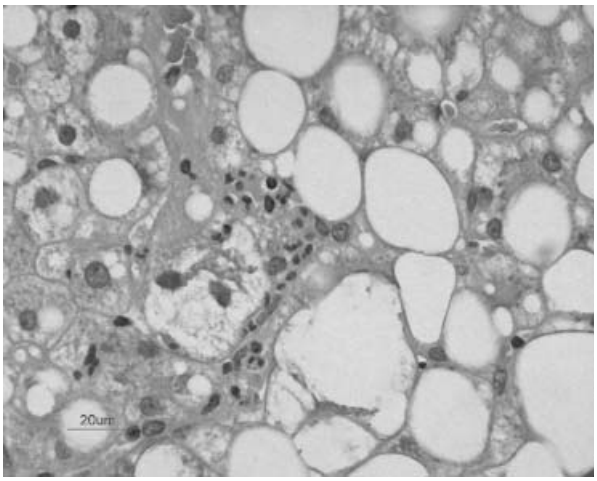

Figure 3 Liver biopsy: non-alcoholic steatohepatitis. Inflammation with polymorphonuclear leucocytes surrounding hepatocellular ballooning and Mallory's hyaline; presence of macrovescicular steatosis (haematoxylin and eosin).

mediators are involved in the pathogenesis of first and second hit. They play a role both in the development of the metabolic alterations (i.e. insulin resistance) and in the liver cell damage (Fig. 3).

A brief outline of the role of the liver in the metabolism of lipids and carbohydrates is useful to understand how steatosis and NAFLD in general, develop and eventually progress.

Hepatic glucose and lipid metabolism

The liver plays a central role in glucose and lipid metabolism. Dietary triglycerides and excess carbohydrates transformed into free fatty acids (FFAs) reach the liver through the portal vein and are partly released in the blood stream. FFAs are used as energy sources, mainly by muscle, or re-esterified to triglycerides and stored in the adipose tissue. During fasting or starvation, the triglycerides stored in adipose tissue are hydrolysed to FFAs and transported to the liver, where they are used to form phospholipids and cholesterol esters or converted into ketone bodies to be used as fuel by extrahepatic tissues (49). Mitochondria play a key role in fatty acid oxidation and are responsible for the majority of disturbances in lipid metabolism (49).

Glucose entry into hepatocytes is mediated by the GLUT 2 (insulin-responsive-glucose-transporter-2) isoform of glucose transporters (50) and liver glucose uptake cannot be saturated within the physiological range. Thus, when the portal vein glucose concentration is high, a proportional increase occurs in the flow of glucose into the hepatocyte. In the post-absorptive state glucose is stored as glycogen (51), or enters the glycolysis pathways providing pyruvate for FFA synthesis. A third metabolic fate is the pentose-phosphate pathway, which is also related to lipogenesis, increasing the intracellular NADPH (nicotinamide-adenine-dinucleotide-phosphate) concentration, necessary for fatty-acid synthase activity (52). Hepatic glycogen stores provide adequate glucose output during fasting or when the dietary carbohydrate content is low.

It is widely accepted that the abundancy of circulating FFAs, a common biochemical feature in obese and diabetic patients, plays a central role in the expression of the metabolic syndrome. Much is known about the FFA-mediated impairment of insulin action on skeletal muscle, whereas the effects on hepatic glucose output are a source of debate. Hepatic fat accumulation is associated with reduced insulin-mediated suppression of endogenous glucose production, both in obese and normal subjects (53), thus representing a major source of variability in the insulin requirements of diabetic patients (54), thus supporting the concept of 'hepatic insulin resistance'. One of the first mechanisms potentially involved in FFA-mediated disturbances of hepatic glucose metabolism is the classic Randle's hypothesis of substrate competition (55), but the increased oxidation of FFAs promotes several mechanisms leading to a decrease in glucose oxidation. FFA breakdown in mitochondria increases the production of reactive oxygen species, which may be responsible for FFA-induced insulin resistance, via mechanisms likely involving a protein kinase C-mediated pathway (56). FFAs are well-recognized modulators of the expression of genes involved in FFA oxidation metabolism, via the increase in peroxisome proliferator-activated receptor-α (PPAR-α) expression, and in hepatic lipogenesis. In the presence of abundant FFAs, PPAR stimulation self-limits hepatic lipid accumulation, but hepatic glucose production is also increased. The abundance of FFAs exerts diabetogenic effects, not only in skeletal muscles, but also in the liver.

In summary, hepatic lipid accumulation creates a vicious circle, which further increases hepatic steatosis, FFA and glucose levels by amplifying insulin resistance.

Development of hepatic steatosis (metabolic causes of first hit)

The primary metabolic abnormalities leading to hepatic lipid accumulation consist of alterations in the uptake, synthesis, degradation or secretion of lipid molecules, resulting from insulin resistance (6). Both obesity and primary genetic factors may be involved. Increased delivery of FFAs, increased hepatic synthesis of FFAs, decreased FFAs β-oxidation in the liver and/or decreased synthesis or secretion of very-low-density lipoproteins (VLDLs) represent key aspects of the steatosis-associated fat homeostasis. The increased FFA flow to the liver may be induced by one or more of the following mechanisms (49): increased release of FFAs from peripheral or abdominal adipocytes, relative excess of lipid content in the diet and increased endogenous synthesis of FFAs in the liver. Animal as well as human studies have suggested that the visceral adipose tissue may be a major source of massive FFA flow to the liver (9). Abdominal fat may more easily release lipids, thus promoting a direct flow to the liver through the portal vein. In the rat, large amounts of visceral fat are strictly related to the onset of insulin resistance in the liver and muscle tissue and visceral fat removal decreases hepatic insulin resistance (57). Both the release of FFAs from adipose tissue and their uptake and metabolism by the liver are influenced by various mediators, the secretion and activity of which are modified in patients with excess weight, type 2 diabetes or both.

Development of NAFLD is strictly related to reduced tissue sensitivity to insulin (37,42). Elevated levels of insulin exert a different influence on adipocytes and hepatocytes (58). In the adipocyte, insulin promotes lipolysis, resulting in increased FFA delivery to the liver; in the hepatocyte, insulin stimulates synthesis and inhibits oxidation of FFAs. Furthermore, because of the reduced hepatic

release secondary to hyperinsulinaemia, degradation of apolipoprotein B100 is enhanced. This mechanism reduces the release of triglycerides from the liver, as confirmed by Bjorkegren *et al.* in animal models (59). In conclusion, large amounts of triglycerides are stored in the liver because of increased synthesis induced by the greater flow of FFAs to the liver, as well as decreased production or secretion of VLDL.

Several peptide mediators are involved in the regulation of insulin sensitivity, such as tumour necrosis factor alpha (TNF-α), angiotensinogen, plasminogen activator inhibitor-1, complement components and leptin. Only a few of these are adipose-derived and probably they play a role both in the 'first hit' (development of liver steatosis) and in the 'second hit' (progression of fatty liver to NASH, fibrosis) (48) (Fig. 3).

TNF-α is involved in all phases of liver damage in NAFLD. It promotes liver steatosis stimulating lipogenesis in hepatocytes and increasing FFA release from adipocytes and their delivery to the liver. TNF-α modifies the activity of several mitochondrial enzymes reducing the availability of cytoprotective and anti-inflammatory cytokine and of anti-oxidant compounds. TNF-α might also directly induce apoptosis of hepatocytes and it influences the activity of cells involved in inflammatory processes promoting, in particular, the activation of hepatic stellate cells (HSCs), potentially contributing to the development of liver fibrosis.

Leptin, a satiety hormone synthesized by adipose tissue, inhibits feeding behaviour and increases energy expenditure through its effects on anorexigenic neurones in the ventral median nucleus of the hypothalamus (60). Elevated levels of leptin are found in the majority of patients with NASH (61), irrespective of BMI, and may be related to hyperlipidaemia. This hormone may have an important role in regulating the partitioning of fat between mitochondrial β-oxidation and triglyceride synthesis (62). Its contribution to hepatic steatosis might stem from its ability in promoting insulin resistance, modifying insulin signalling in hepatocytes and increasing hepatocellular fatty acid production. Leptin was recently shown to be necessary for the development of liver fibrosis in patients with NASH (63,64).

According to the results obtained in animal studies, leptin may also exert an anti-steatotic effect (65). In models in which obesity is induced by excessive calorie intake, serum leptin levels are increased. They first stimulate the storage of fat in the adipose tissue, but not in other tissues. Thereafter, for unknown reasons, leptin resistance develops, leading to fat deposits in organs and tissues other than the adipose tissue, such as the liver, pancreatic β-cells and the heart.

In a cohort of biopsy-proven NASH patients, a single missense mutation of the gene encoding PPAR-α has been observed (66). This could support the hypothesis of a genetic predisposition to NAFLD, because this receptor, expressed in tissues with high rates of oxidative metabolism, modulates lipid oxidation in mitochondria, peroxisomes and microsomes (67) and stimulates the synthesis of uncoupling protein-2 (UCP-2) (68). All these mechanisms could contribute to the development and progression of liver damage.

Taking into account the concerns about other mediators that are negatively affected by the hepatic metabolic damage present in NAFLD, adiponectin is the most interesting on account of its potential use for therapeutic purposes. This hormone, the receptors of which have recently been cloned (69), is a protein exclusively secreted by adipose tissue. Adiponectin is suppressed in states of insulin resistance and obesity (70) and an inverse relationship is present between plasma levels of adiponectin and adipose tissue-derived cytokines, particularly interleukin-6 (IL-6) (71), thus suggesting a cytokine inhibitory effect. The relationship between adiponectin and IL-6 is also confirmed by the *in vitro* observation that adiponectin gene expression is reversibly down-regulated by IL-6 (72). All these observations support the role of adiponectin as a modulator of insulin sensitivity, and suggest its possible protective role against fatty liver (73).

From steatosis to steatohepatitis (metabolic causes of second hit)

The main issue in NAFLD remains the study of biochemical events changing the natural history of the disease from a non-progressive condition (pure fatty liver) to fibrosis and necroinflammation (NASH).

Data from experimental and human studies (74) suggest that they may be either an amplification of first-hit events (exaggerated fat deposition, overproduction of cytokines) or new factors adding to lipid accumulation (oxidative stress/lipid peroxidation, bacterial toxins, alteration of hepatocyte adenosine triphosphate stores and cytochrome P450 Cyp2E1/Cyp4A enzyme activity).

FFAs *per se* are potentially hepatotoxic (75), but they may also increase cytochrome P450 Cyp2E1/Cyp4A enzyme activity, as observed in an animal model of NAFLD using a methionine-choline-deficient diet (76). These results suggest a role of oxidative stress/lipid peroxidation in NAFLD progression. Lipid peroxidation is caused by increased β- and ω-oxidation of various FFAs by the cytochrome P450 Cyp2E1 and Cyp4A system (67,77). Chronic oxidative stress leads to depletion of the natural antioxidant compounds (i.e. glutathione, Vitamin E), and results in an excess of reactive oxygen species (ROS) within the hepatocyte.

The presence of excessive intrahepatic iron may also represent a cofactor for oxidative stress (78). FFAs are shunted through peroxisomal β-oxidation, generating

hydrogen peroxide that is subsequently converted to reactive hydroxyl radicals in the presence of iron.

Large amounts of ROS not only trigger lipid peroxidation of cell membranes, but also stimulate insulin resistance (79) and the synthesis of cytokines (80), in particular of TNF-α, by the hepatocytes, Kupfer cells and adipose tissue. This increased availability of TNF-α activates specific redox-sensitive kinases, in particular inhibitor kappa kinase beta (IKK-β); this enzyme activates nuclear factor kappa beta (NF-kβ), a transcriptional factor that induces the synthesis of TNF-α (48,81). This mechanism initiates a self-reinforcing positive feedback that perpetuates insulin resistance and the production of TNF-α (48). In an animal model, intestinal bacterial products, specifically endotoxins, showed an additional effect on TNF-α release and, as a consequence, on increased FFA synthesis and insulin resistance (29). TNF-α promotes liver injury in different ways, and a direct correlation has been reported between serum TNF-α levels and the severity of NASH (82).

Chronic oxidative stress is accompanied by various mitochondrial adaptations (83), possibly associated with NAFLD progression. An increased expression of UCP-2, an inner mitochondrial membrane protein involved in mitochondrial oxidative phosphorylation [i.e. adenosinetriphosphate (ATP) synthesis], has been documented in fatty hepatocyte of ob/ob mice (84). The consequent reduction in ATP production could increase hepatocellular vulnerability to necrosis (85). Paracristalline mitochondrial abnormalities are present in patients with NASH, but not in patients with simple steatosis (43), although the ultrastructural defect is neither specific nor obligate of NASH.

From second hit to liver cirrhosis and hepatocellular carcinoma

Long-term prospective studies evaluating the natural history of NAFLD are not yet available, but there is growing evidence that most cases of cryptogenic cirrhosis may represent a late stage of NAFLD. Cirrhosis may be a presenting feature of NAFLD (86) or may develop in a far from negligible percentage of patients (19% of NASH patients during a 1–9-year follow-up), the natural history varying according to the histological type (35,86,87); these percentages are almost identical to those reported in much larger, long-term studies in patients with alcohol-induced steatohepatitis (88).

Emerging evidence indicates that NAFLD represents the most common cause of cryptogenic cirrhosis (89) and data from the United Network of Organ Sharing demonstrate that cryptogenic cirrhosis represents the third or fourth leading indication for liver transplantation in the USA (90). Adverse liver outcomes in NAFLD might even be underestimated, as common NAFLD-related comorbidities (e.g. obesity and type 2 diabetes mellitus) are often associated with cardiovascular diseases, lung failure, and extrahepatic malignancies that preclude liver transplantation candidacy (89,90). A few patients transplanted for cryptogenic cirrhosis develop NAFLD in the graft (91), thus supporting a role for genes in the pathogenesis and progression of the disease.

Patients with pure steatosis at liver biopsy have the most favourable prognosis within the spectrum of NAFLD, whereas features of NASH, with/without advanced fibrosis, are associated with progression (35). The outcome of patients with advanced fibrosis or cirrhosis is particularly poor. Matteoni *et al.* (35) found an age-adjusted death rate of 9.5/100 000 for chronic liver disease/cirrhosis in this category, with a liver-related death of 11% over 8 years; liver-related diseases were the second most common cause of death, only exceeded by cancer.

The majority of obese patients with NASH-related cirrhosis die from their liver disease, despite the corresponding high prevalence of cardiovascular disease (92,93). The liver-related mortality of NASH cirrhosis is similar to that of hepatitis C-related cirrhosis in those patients not treated with anti-virals or in whom the disease is refractory to treatment (92).

Predictive factors for the development of fibrosis/cirrhosis in patients with NAFLD have been consistently demonstrated (Table 5). In particular, age, obesity, diabetes, the

Table 5 Risk factor for progression of non-alcoholic steatohepatitis to cirrhosis and liver cancer

Risk factor	Author (year)	Reference no.
Age > 50 years	Garcia-Monzon *et al.* (2000)	137
	Ratziu *et al.* (2000)	138
	Angulo *et al.* (1999)	39
Obesity	Wanless *et al.* (1990)	139
	Ratziu *et al.* (2000)	138
	Angulo *et al.* (1999)	39
Type 2 diabetes	Wanless *et al.* (1990)	139
	Angulo *et al.* (1999)	39
↑ Grades of inflammation	Wanless *et al.* (1990)	139
	Garcia-Monzon *et al.* (2000)	137
	Ratziu *et al.* (2000)	138
Hypertension	Willner *et al.* (2001)	140
	Dixon *et al.* (2001)	142
AST/ALT ratio > 1	Angulo *et al.* (1999)	39
	Sorbi *et al.* (1999)	144
Iron	George *et al.* (1998)	95
Degree of steatosis, ↑ free fatty acids	Wanless *et al.* (1990)	139
	Garcia-Monzon *et al.* (2000)	137
	Willner *et al.* (2001)	140
Triglycerides > 151 mg dL^{-1}	Ratziu *et al.* (2000)	138
ALT > 2 × normal	Ratziu *et al.* (2000)	138
	Dixon *et al.* (2001)	142

AST, serum aspartate aminotransferase; ALT, serum alanine aminotransferase; ↑, increased.

severity of steatosis and necroinflammation have been identified in several studies. Iron overload is also considered to account for disease progression. Biochemical evidence of iron overload is present in up to 60% of patients with NASH (94), although the hepatic iron index does not exceed the diagnostic cut-off value of 1.9 for familial haemochromatosis. However, no relationship has been found between the severity of iron burden or gene mutations of haemochromatosis and NAFLD severity, thus the role of iron remains controversial (94–96).

Compared with patients with cirrhosis of a different aetiology, those cases with NAFLD-related cirrhosis have much more advanced liver disease at the time of presentation. This leads to the question of the possible role of late diagnosis in subjects primarily cared for metabolic disorders, where liver disease may be relatively underestimated.

Finally, an important aspect in the natural history of NAFLD is the risk of primary liver cancer. Obesity *per se* is a risk factor for several neoplasias, including hepatocellular carcinoma (HCC) (97). In a Danish population-based study, NAFLD patients showed a 4.4-fold increased risk of dying from liver cancer, compared with the general population (98). The relative role of obesity and fatty liver remains to be determined.

Retrospective data are also available on the progression of NASH to HCC, via fibrosis and cirrhosis (92,99,100). In the USA, at least 13% of cases with HCC have an underlying liver disease possibly having progressed from NASH (99), and similar results have been reported in Italy (100).

Features suggestive of the metabolic syndrome are more frequently observed in cases of HCC occurring in patients with cirrhosis of unknown aetiology than in carefully matched patients with HCC of viral or alcoholic origin, thus suggesting the existence of a metabolic disorder leading first to fatty liver and then to HCC via NASH, fibrosis and cirrhosis (100). HCC occurring in patients with cryptogenic cirrhosis is frequently associated with normal aminotransferase levels, possibly indicative of a slowly progressive hepatic disorder.

Treatment of NAFLD

No universally effective treatment has been identified for NAFLD and therapeutic strategies are still largely empirical. Only a few randomized controlled trials are available, usually vs. placebo. The majority of studies are purely observational, with short-term follow-up, and based on surrogate markers (ALT). Very few patients have a post-treatment liver biopsy to support biochemical data.

Patients with secondary NAFLD require specific interventions and the removal of putative toxin(s). In patients with primary NAFLD the presence and severity of excess weight and metabolic alterations (type 2 diabetes and dislipidaemia) should be evaluated. In the presence of overweight or obesity, weight loss strategies remain the primary therapeutic approach (101); achievement of this objective is expected to improve lipid and carbohydrate metabolism, and to improve or to halt NAFLD progression.

In normal weight subjects, specific pharmacological treatment of altered lipid and/or carbohydrate metabolism will reduce hepatic lipid overflow and liver damage. Finally, in patients whose NAFLD is not related to toxic compounds, excess weight and/or metabolic disturbances, drugs with known protective effect on liver cell may be tested.

Tables 6 and 7 summarize the results of the main therapeutic trials performed on NAFLD patients, subdivided according to the different therapeutic strategies, that is, aimed at the correction of risk factors for NAFLD (obesity, insulin resistance hyperlipidaemia) (Table 6) and protection of the hepatocyte (Table 7).

Treatment of risk factors

Obesity

The benefit of weight loss on NAFLD has been observed in studies in obese subjects undergoing weight management to improve the cardiovascular risk profile. In overweight individuals with elevated aminotransferase levels, weight reduction of 10% or more corrects aminotransferase levels and decreases hepatomegaly (102). There are no controlled studies on the usefulness of the diet in the management of NAFLD. Dietary supplementation with polyunsaturated fatty acids may improve insulin sensitivity as well as the cardiovascular risk profile; however, the effects of such a dietary modification on NAFLD are unknown. As recently suggested in a technical review on NAFLD by the American Gastroenterological Association (103), in the absence of clinical trials, it would be wise to commence a heart-healthy diet as recommended by the American Heart Association for those without diabetes (104) or an appropriate diet as recommended by the American Diabetes Association for those with diabetes (105).

Only progressive weight loss leads to benefits for liver damage; rapid and massive weight reductions, as in morbidly obese subjects undergoing bariatric surgery, may promote or worsen NAFLD, NASH, and even result in liver failure (9). The risk of liver damage is related to the rapidity of weight loss rather than to the type of surgery (21).

A very recent study (106) showed the efficacy of a lipase inhibitor, orlistat, on weight loss, liver enzymes, as well as on the grade of steatosis, inflammation and fibrosis. Withdrawal of treatment was not followed by the return of liver enzymes to pre-treatment levels, although no conclusions can be drawn from a single case report.

Table 6 Non-alcoholic fatty liver disease: results of clinical studies

Author (reference no.).	Therapy	Patients (*n*)	Study type	Duration	Liver enzyme levels	Histology
Diet						
Rozental *et al.* (141)	Diet	5	Open	1–4 weeks	=	Variable
Drenick *et al.* (143)	Diet	7	Open	2–7 months	n.p.	Variable
Drenick *et al.* (143)	Fasting	11	Open	1.5–3.5 months	n.p.	Variable
Eriksson *et al.* (145)	Diet	3	Case series	12 months	↑	↑
Andersen *et al.* (146)	Diet	41	Open	4–23 months	↑	Variable
Luyckx *et al.* (147)	Diet	8	Open	n.p.	n.p	↑
Diet, exercise						
Keeffe *et al.* (148)	Diet, exercise		Case series	4 months	↑	↑
Palmer *et al.* (102)	Diet, exercise	39	Case series	2–111 months	↑	n.p.
Vajro *et al.* (149)	Diet, exercise	9 (paed)	Open	6 months	↑	n.p.
Ueno *et al.* (150)	Diet, exercise	25	Open	3 months	↑	↑
Franzese *et al.* (27)	Dietexercise	58 (paed)	Open	6 months	↑	n.p.
Weight loss agents						
Harrison *et al.* (106)	Orlistat	10	Open	6 months	↑	↑
Surgical						
Silverman *et al.* (151)	Gastric by-pass	91	Case series	2.61 months	↑	↑

=, unchanged; n.p., not performed; ↑, improved; paed, paediatric study.

Table 7 Proposed treatments of non-alcoholic fatty liver disease

Author (reference no.).	Therapy	Patients (*n*)	Study type	Duration	Liver enzyme levels	Histology
Anti-diabetics						
Franzese *et al.* (27)	Metformin	14 (paed)	Open	4 months	↑	n.p.
Coyle *et al.* (107)	Metformin	2	Open	4–11 months	↑	↑
Marchesini *et al.* (108)	Metformin	14	Open	4 months	↑	n.p.
Caldwell *et al.* (110)	Troglitazone	10	Open	4–6 months	↑	↑
Acosta *et al.* (111)	Pioglitazone	8	Case series	2–12 months	↑	↑
Azuma *et al.* (112)	Pioglitazone	7	Open	3 months	↑	n.p.
Neuschwander-Tetri *et al.* (113)	Rosiglitazone	30	Open	48 weeks	↑	↑
Lipid lowering agents						
Laurin *et al.* (114)	Clofibrate	16	Open	12 months	=	=
Basaranogiu *et al.* (115)	Gemfibrozil	46	Randomized, open	1 months	↑	n.p.
Saibara *et al.* (116)	Bezafibrate	2	Open	not rep.	not rep.	↑
Horlander *et al.* (117)	Atorvastatin	7	Open	21 months	↑	↑
Nair *et al.* (118)	HMG-CoA RI	13	Case–control	> 6 months	not rep.	=
Merat *et al.* (152)	Probucol	27	Randomized	6 months	↑	n.p.
Cytoprotective agents						
Obinata *et al.* (153)	Taurine (diet)	10 (paed)	Open	6–17 months	↑	n.p.
Laurin *et al.* (114)	UDCA	24	Open	12 months	↑	↑
Guma *et al.* (125)	UDCA (diet)	24	Randomized, open	6 months	↑	n.p.
Ceriani *et al.* (126)	UDCA (diet)	31	Open	6 months	↑	n.p.
Anti-oxidants						
Fu *et al.* (154)	LAB	4	Open	12 weeks	↑	Variable
Levine (130)	Vitamin E	11 (paed)	Open	4–10 months	↑	Variable
Gulbahar *et al.* (155)	NAC	11	Open	3 months	↑	n.p.
Abdelmalek *et al.* (128)	Betaine	8	Open	12 months	↑	↑
Hasewaga *et al.* (133)	α-Tocopherol	22	Open	12 months	↑	↑
Miglio *et al.* (129)	Betaine nicotinamide	191	Randomized	12 months	not rep.	↑
Phlebotomy						
Macdonald *et al.* (134)	Phlebotomy	20	Open	As necessary	↑	n.p.
Facchini *et al.* (135)	Phlebotomy	17	Open	As necessary	↑	n.p.

↑, improved; =, unchanged; n.p., not performed; HMG-CoA RI, 3-hydroxy-3 methylglutary/Coenzyme A reductase; UDCA, ursodeoxycholic acid; LAB, lecithin, anti-oxidants, Vitamin B; NAC, N-acetylcysteine; not rep., not reported; paed, paediatric study.

Insulin resistance

Various compounds have been used to treat insulin resistance in NAFLD patients. Metformin, which down-regulates hepatic gluconeogenesis and diverts fatty acids from triglyceride production to mitochondrial β-oxidation, seems promising (27,107,108). Thiazolidinediones represent a new class of insulin-sensitizing agents, with various effects, the primary one on lipid metabolism (109). Troglitazone, the first compound to be studied, led to an improvement in liver tests as well as histological findings (110) before its withdrawal from the market because of potential hepatotoxicity. New agents, pioglitazone and rosiglitazone, have recently been released to treat diabetes, and post-marketing experience with these agents has not revealed any evidence of liver toxicity. Reports published in abstract form suggest a potential short-term usefulness for pioglitazone (111,112). Rosiglitazone improved liver biochemistry and histology in a 48-week open trial, but treatment stop was followed by a return of liver enzymes to pre-treatment levels (113). Further studies, however, are needed before these agents can be routinely used in the treatment of NAFLD.

Hyperlipidaemia

It is not known whether treatment of this condition improves NAFLD. Only a few studies are available, using various anti-hyperlipidaemic agents (clofibrate, gemfibrozil, bezafibrate, atorvastatin, simvastatin) with conflicting results (114–118). Moreover, as recently suggested (5,6), the potential role of lipid-lowering agents is questioned by observations of inherent defects of apoprotein metabolism in NASH and NAFLD patients (119).

Pharmacological treatment of liver damage

Several attempts have been made to reverse liver damage, independently of risk modification, with no definite result (5,6,58,120,121) (Table 7). Different categories of drugs have been studied: cytoprotective agents [e.g. ursodeoxycholic acid (UDCA), alone or in combination with dietary advice], anti-oxidants (e.g. Vitamin E), lecithin, betaine, N-acetylcysteine, etc.

Cytoprotective agents combine an attractive safety profile, few drug interactions and a plausible mechanism of action at the cellular or subcellular levels. UDCA, a hydrophilic bile acid that stabilizes cell membranes, has been shown to raise the apoptotic threshold to oxidative stress in hepatocytes by preventing mitochondrial membrane damage (122). Anti-oxidant properties of UDCA have been documented (123,124); in patients with NAFLD, it was either administered alone (114) or in combination with an appropriate diet (125–127); preliminary results are promising, showing an improvement in liver enzyme levels, but further studies are needed.

Betaine, a methyl donor in an alternative pathway of homocysteine to methionine remethylation, improved both the biochemical and the histological parameters in adult NAFLD patients (128,129), while Vitamin E was effective in normalizing liver tests in a paediatric series (130). Alpha-tocopherol was shown to decrease the production of superoxide and cytokines in patients with elevated triglycerides as well as in those with normal cholesterol levels (131) and to reduce fibrogenesis by stellate cells from patients with chronic hepatitis C (132). In a pilot study, alpha-tocopherol administration in patients with NASH improved inflammation and fibrosis in 55% of patients (133).

Although the role of iron overload in NAFLD is still controversial (82–84), excessive liver iron stores could accelerate NAFLD progression. Phlebotomy is not recommended in the routine treatment of NASH, but may be proposed for NASH patients with documented hepatic iron overload (134,135), with or without mutations in hereditary familial haemochromatosis gene (48).

Conclusions

Obesity is the most significant single risk factor for the development of NAFLD, both in children and in adults; obesity is also predictive of the presence of fibrosis, potentially progressing via NASH to advanced liver disease. Other predictive factors for liver disease progression have been identified, but at present the treatment of obesity remains the most effective preventive strategy. There is no evidence that NAFLD may influence the underlying metabolic disease, in particular obesity and type 2 diabetes, and its common cardiovascular complications. However, liver disease may become a major problem in a few patients, significantly contributing to morbidity and mortality.

Modifications in lifestyle, and in particular weight reduction and regular exercise, represent the mainstay of treatment and prevention. Epidemiological studies, with long-term follow-up and randomized clinical trials measuring histological progression, are needed to define the natural history of NAFLD and its link with metabolic diseases, as well as to validate effective therapeutic strategies.

Physicians caring for patients with obesity and related metabolic disorders must consider NAFLD as an additional complication, requiring correct classification and adequate control. A very recent re-analysis of the National Health and Nutrition Examination Survey revealed that obesity is associated with a 1.7 increased risk of cirrhosis-related death among persons who consume little or no alcohol (136). A systematic search for NAFLD in all obese subjects can no longer be overlooked. This is an additional task to which physicians have to comply in order to prevent the possible epidemics of advanced liver disease associated with the expected epidemics of obesity.

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