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Institut d'Estudis Catalans, Barcelona

Nonalcoholic steatohepatitis

J. Caballería and J. Rodés*

Unitat d'Hepatologia, Institut de Malalties Digestives i Metabòliques. Hospital Clínic i Provincial. Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS). Universitat de Barcelona

Abstract

Nonalcoholic fatty liver disease (NAFLD) is an increasingly recognized condition whose symptoms and pathology resemble those of alcohol-induced liver injury. NAFLD includes fatty liver, steatohepatitis (NASH) with inflammatory changes and fibrosis, and cirrhosis. NAFLD is usually seen in association with obesity, diabetes, hypertension, and hypertriglyceridemia, as the hepatic component of a metabolic syndrome. Most NAFLD patients are asymptomatic and usually present with mild elevations in aminotransferases. The diagnosis should be established by liver biopsy. The natural history of this disease is not well defined, but progression to cirrhosis and hepatocellular carcinoma is well recognized in some patients. The pathogenesis is not well known but the accumulation of fat in the liver is probably related to insulin resistance, which leads to altered free fatty acid metabolism. The progression from NAFLD to NASH and cirrhosis is less clear but increasing evidence suggests that oxidative stress may enhance proinflammatory and profibrogenic cytokines and that this leads to mitochondrial dysfunction followed by development of inflammation, necrosis, and fibrosis. There is no established treatment for NASH, and current therapies are focused on correcting the insulin resistance or reducing oxidative stress. Although the results of some recent pilot studies are promising, prospective, randomized studies with clearly defined histological endpoints are needed.

Keywords: fatty liver, steatohepatitis, insulin resistance, metabolic syndrome, free fatty acids.

Resum

El fetge gras no alcohòlic es caracteritza per unes lesions hepàtiques semblants a les de la malaltia hepàtica alcohòlica, que comprèn des de l'esteatosi simple i l'esteatohepatitis amb canvis inflamatoris i fibrosi fins a la cirrosi. És una malaltia cada vegada més freqüent que s'associa amb l'obesitat, la diabetis i la dislipèmia, i és probablement el component hepàtic d'una síndrome metabòlica. La seva patogènesi no és ben coneguda, però la resistència a la insulina i l'augment d'àcids grassos lliures al fetge hi tenen un paper fonamental, en provocar un estrès oxidatiu i activar els mecanismes responsables de la inflamació, la necrosi i la fibrosi. El diagnòstic s'ha de fer mitjançant una biòpsia del fetge, la qual també permet establir la gravetat de les lesions. A part del tractament dels factors associats, l'esteatohepatitis no alcohòlica no té un tractament establert, tot i que s'estan assajant fàrmacs que milloren la resistència a la insulina i d'altres amb acció antioxidant. Els resultats d'alguns estudis pilot són esperançadors, encara que falten estudis controlats, amb grups mes grans de malalts i que valorin els canvis histològics.

Nonalcoholic steatohepatitis (NASH) is a disease which is characterized by hepatic lesions similar to those produced by alcohol but which appear in subjects who do not consume excessive alcohol. The term NASH was proposed by Ludwig and cols. in 1980 [1] and covers a wide spectrum of liver damage including simple steatosis, steatohepatitis with fatty vacuoles, necroinflammatory changes and/or a variable degree of fibrosis and, lastly, well-established cirrhosis. Thus, a more correct term would be nonalcoholic fatty liver, although most authors continue to use the term NASH to denominate this disease. Interest in NASH has increased in recent years and the number of publications related to this disease has increased exponentially [2-11]. However, many aspects of the physiopathology, the natural history, and the treatment of NASH remain to be elucidated.

The prevalence of NASH is not well known and may be underestimated since a large proportion of the patients are asymptomatic and present discrete biological alterations,

*Author for correspondence: Juan Rodés, Direcció General, Hospital Clínic i Provincial. Villarroel 170. 08036 Barcelona, Catalonia (Spain). Tel. 34 932275775. Email: rodes@medicina.ub.es

and liver biopsy is not performed. The diagnosis of this disease is, however, very frequent and it is considered that from 1% to 8% of liver biopsies correspond to NASH [7]. One of the factors thought to influence the rise in the prevalence of NASH is the increasing incidence of obesity among the Western population and which is already manifested during adolescence. While in the past NASH was considered to be a disease which fundamentally affected middle-aged, obese women, many of whom were diabetic, NASH has currently been found to affect both sexes equally and may be observed in younger individuals and even adolescents.

Etiology

NASH has been associated with multiple etiological factors such as congenital or acquired metabolic alterations, nutritional disturbances, surgical procedures, drugs and other toxic products. Obesity is the most frequently associated factor and the prevalence of NASH appears to be associated with the degree of obesity. Thus, in some series from 9% to 26% of obese patients present NASH lesions [12], while the prevalence may be greater than 60% among patients with morbid obesity who undergo surgery [13]. On the other hand, steatosis has been detected in 75% of the obese population [14]. Type 2 diabetes mellitus is often associated with NASH. A clear relationship has also been observed between this process and increased resistance to insulin, even in patients with normal glucose tolerance [15]. A high percentage of patients with NASH present dyslipemia, hypercholesterolemia, hypertriglyceridemia or both. In clinical practice, most patients with NASH present obesity, type 2 diabetes mellitus or dyslipemia as the etiologic factor, with the association of several of these factors being frequent. Therefore, nonalcoholic fatty liver is beginning to be considered as the hepatic component in a metabolic syndrome characterized by obesity, hyperinsulinemia, increase in resistance to insulin, diabetes, hypertriglyceridemia and hypertension [16,17].

To a lesser extent, NASH may be due to other causes. Total parenteral nutrition and a rapid loss of weight may cause NASH. Some hereditary metabolic diseases such as abetalipoproteinemia, the Weber Christian syndrome, galactosemia, and thyrosinemia are also causes of NASH [6-8,10]. Jejunoileal bypass for the treatment of morbid obesity was associated with NASH lesions in more than 40 % of the patients, with some developing signs of acute hepatic failure, which led to the abandonment of this procedure [18]. Other surgical procedures such as jejunocolic bypass, biliopancreatic bypass and gastroplasty may also cause NASH, although with a lower frequency. Many drugs have also been associated with NASH. Among these, amiodarone, perhexillin maleate, tamoxifen, estrogens, nifedipine and corticosteroids are of note. The appearance of NASH has recently been described in workers who are chronically exposed to petrochemical vapors [19]. In Spain, NASH has been associated with the toxic oil syndrome with 9% of the patients in the late phase of this syndrome presenting hepatic lesions compatible with NASH [20].

Pathogenesis

In recent years great advances have been made in the knowledge of the pathogenesis of nonalcoholic fatty liver and its progression to steatohepatitis, although many points remain to be elucidated [21-23]. The most prevailing theory is the "two hit" hypothesis proposed by Day and James in 1998 [24]. The first "hit" is the accumulation of fat in the liver in the form of fatty acids and triglycerides. This increase in fat produces oxidative stress, this being the so-called "second hit", which makes the hepatocyte vulnerable to apoptosis and necrosis (Figure1).

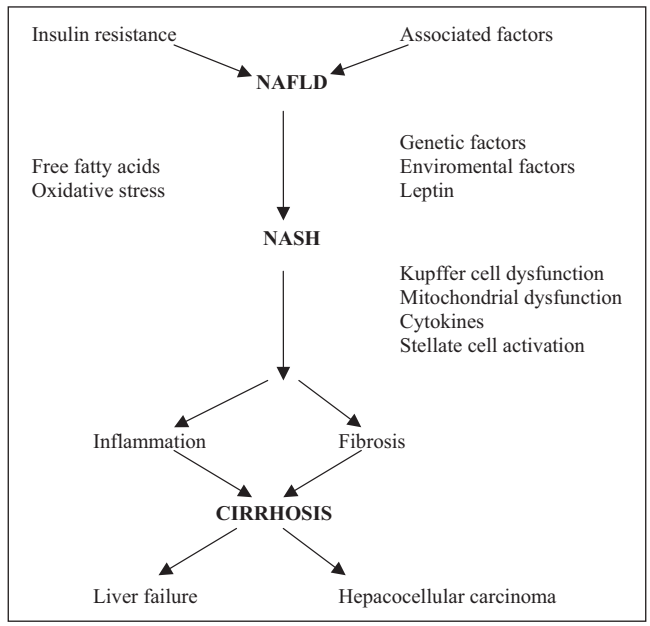

Figure 1. Schematic representation of the pathogenesis of nonalcoholic steatohepatitis.

The deposit of fatty acids and triglycerides in the liver is produced when there is a greater contribution and/or difficulty in the use of these nutrients. Free fatty acids are synthesized in the liver or transported to the liver bound to albumin proceeding from the intestinal absorption or lipolysis in the adipose tissue. Likewise, free fatty acids may accumulate in the liver when their oxidation is reduced or when there is an alteration in triglyceride transport out of the liver in the form of very low density lipoproteins (VLDL).

The increase in resistance to insulin is very frequent in patients with nonalcoholic fatty liver and it is believed to be responsible for most of the changes in lipid metabolism [25-27]. Insulin is fundamental in energetic regulation at the level of the skeletal muscle, the adipocytes and hepatocytes. In muscle, insulin facilitates the enhancement of glucose by the translocation of the glucose transporter-4 (GLUT-4) from the cytoplasm to the cell surface [28]. In adipocytes, insulin inhibits lipoprotein-lipase, impeding the rupture of the triglyc-

erides and the release of the free fatty acids [29]. In hepatocytes, insulin favors glycogenesis and inhibits glycogenolysis and gluconeogenesis [30]. In contrast, insulin resistance is characterized by an increase in the circulating levels of free fatty acids due to an increase in lipolysis and a lower oxidation both in muscle and in the liver [27]. In addition, the hyperinsulinemia associated with insulin resistance decreases the synthesis of apolipoprotein B-100, which is an essential component of the VLDL, thus diminishing the transport of the triglycerides outside the hepatocytes [31,32].

The development of fatty liver may also be influenced by other mechanisms such as diet [33], genetic factors such as polymorphisms of the microsomal protein transporter of the triglycerides [35,36], an increase in the circulating levels of leptin [36], which has been related to the increase in the resistance to insulin, and some cytokines such as tumoral necrosis factor alfa (TNFα) and adiponectin [37]. TNFα levels are increased in patients with fatty liver and enhance insulin resistance [38]. By contrast, the expression of mRNA of adiponectin is reduced while the administration of adiponectin to experimental models normalizes insulin resistance and reduces the deposit of triglycerides in the liver [30]. Likewise, TNFα may inhibit the expression of adiponectin, thereby contributing to insulin resistance [29].

Some patients only develop steatosis while in others the lesions progress to steatohepatitis. Several factors may influence the different evolution of the patients, although the mechanisms remain unclear. In the second stage of the disease, the increase in the deposit of fatty acids also plays an important role in disease progression. Mitochondrial ß-oxidation is the normal metabolic route of free fatty acids. This route is less effective in NASH due to mitochondrial alterations [39,40] and, moreover, it is saturated by the excess of fatty acids activating other routes such as microsomal oxidation, producing overexpression of cytochromes P450 2E1 and 3A4 [41,42] and an increase in peroxisomal oxidation [43]. All of this induces the formation of reactive oxygen species, oxidative stress and lipid peroxidation [39]. The final products of lipid peroxidation, malondialdehyde and hydroxinonenal stimulate the synthesis of cytokines and adhesion molecules [44].

The generation of reactive oxygen species saturates the physiological antioxidant systems. It also induces the synthesis of the nuclear kβ transcription factor, and this in turn stimulates the production of proinflammatory cytokines such as TNFα and IL-8 and the synthesis of adhesion molecules which increase neutrophilic chemotaxis [22]. The greater production of TNFα and other proinflammatory cytokines is also related to a rise in endotoxin [45] and Kupffer cell dysfunction [46].

The activation of the hepatic stellate cells to initiate the process of fibrogenesis in NASH depends on several factors such as free radicals, cytokines, and products derived from damaged hepatocytes [47-49]. The transforming growth factor beta (TGFβ) is probably the cytokine with strongest profibrogenetic properties [50]. In experimental models, leptin is very important in the development of fibrosis [51]. In these models leptin is produced by activated stellate cells and interacts in a paracrine fashion with Kupffer cell receptors, thus favoring the production of TGFβ and increasing fibrogenesis [52,53]. Similarly, patients with NASH and fibrosis present overexpression of the leptin receptor in hepatic tissue (REF).

The role of the rise in hepatic iron deposits as a coadjuvant in the pathogenesis of NASH is a matter of debate. Some studies have shown that a high percentage of patients with NASH present an increase in ferritin or transferrin saturation together with a moderate increase in hepatic iron deposits, although it remains unclear whether these alterations are a cause or a consequence of liver damage [54]. Indeed, iron may contribute to the pathogenesis of NASH by several mechanisms. Experimental studies have demonstrated that iron stimulates lipid peroxidation and cell damage [55] and also activates stellate cells, thereby favoring fibrogenesis [56]. After the demonstration of the rise in ferritin and iron deposits in some patients with NASH, a possible association between this disease and the gene of hemochromatosis was sought. In a recent study, George *et al.* [57] found that patients with NASH showed a prevalence of the Cys 282Tyr mutation of 31 %, and this situation was accompanied by an increase in iron deposits as well as a higher degree of fibrosis. In another study, Bonkovsky *et al.* [58] observed that patients with NASH and the Cys282Tyr or H6D3 mutations of the hemochromatosis gene had higher levels of ferritin and transferrin saturation as well as increased iron deposits but with no relationship between hepatic iron concentrations and the degree of fibrosis. No have more recent studies shown that hepatic iron concentrations are a predictive factor for the degree of fibrosis in patients with NASH [59]. These discrepancies may be explained by geographical and methodological differences in the evaluation of the hepatic iron deposits. However, the most recent results suggest that the role of iron in the pathogenesis of NASH is small.

In summary, NASH develops in two stages. In the first stage, steatosis develops as a consequence of the alterations in lipid metabolism related to an increase in resistance to insulin. In the second stage, inflammatory changes and fibrosis appear. Oxidative stress plays an essential role in this second stage and is triggered by the accumulation of fatty acids. Moreover, in the second stage both lipid peroxidation and liver damage and fibrosis may be influenced by different factors such as the induction of CYP2E1, endotoxin, iron, Kupffer cell dysfunction and changes in mitochondria [60] and ATP homeostasis [61,62]. Neither the interactions among all these factors nor the importance of each factor has been well established.

Clinical manifestations

Most of the patients with NASH are asymptomatic and in these patients the diagnosis is usually the result of the casual discovery of hepatomegaly and/or alterations in hepatic biology during the course of a health examination or because of consultation for an extrahepatic disease. In symptomatic cases, the symptomatology is unspecific, with asthenia, general malaise, and discomfort on the right side of the upper abdomen. On

physical exploration, hepatomegaly is the most frequent and, in general, the only finding. Only patients with cirrhosis may present cutaneous stigmata and splenomegaly [6-8,10].

Laboratory tests are also unspecific. In most patients there is a moderate elevation in transaminases and, contrary to what occurs in alcoholic liver disease, the AST/ALT ratio is usually less than one. A moderate elevation in alkaline phosphatase is also frequent, as is that of gammaglutamyl transpeptidase. On the other hand, bilirubin, albumin, and the prothrombin rate are usually normal and are only altered in patients with cirrhosis. Some patients present low titers of antitissular antibodies, especially antinuclear antibodies. Depending on the cause of NASH, there is a frequent elevation of serum lipid (hypercholesterolemia and/or hypertriglyceridemia) and hyperglycemia. Patients with NASH may show an increase of iron in the liver which is accompanied by an elevated saturation of transferrin and serum ferritin. Some authors have also reported a high prevalence of the Cys282Tyr mutation of the hemochromatosis gene [6-8,10,63].

Ultrasonography is the least expensive and most effective imaging technique to detect steatosis and also allows a semiquantitative estimation of the intensity of the disease. However, the sensitivity of ultrasonography or the remaining imaging techniques to detect the fibrosis associated with steatosis is much lower [64]. The liver can be observed as irregular or nodular and extrahepatic signs of portal hypertension can be seen only in the cirrhotic phase. Axial tomography and magnetic resonance have the same limitations as ultrasonagraphy. Only in the infrequent cases of focal steatosis can magnetic resonance imaging distinguish space-occupying lesions from focal fatty infiltration [65].

Histologic findings

The spectrum of lesions in NASH is the same as that observed in alcoholic liver disease, from simple steatosis to cirrhosis. The minimum lesions necessary for the diagnosis of NASH is not yet well established, although most authors consider that steatosis, lobulillar inflammation, hepatocyte degeneration or ballooning and/or fibrosis would then be present. Steatosis is usually macrovacuolar with varying intensity, from slight, only affecting the perivenular region, to practically massive. In the cirrhotic phase, steatosis may be minimal. Mallory hyaline bodies are not a requisite for the diagnosis but are often present. Glycogenic degeneration of the nuclei may also be observed. Neutrophils predominate in inflammatory infiltrates, although lymphocytes and macrophages may also be present. Positive staining for iron varies according to the series. Pericellular, perisinudoidal or periportal fibrosis is found in 40% to 80% of the patients with NASH. Fibrosis is marked in the pericentral region and ranges from fine septa around the centrolobulillar vein or around groups of hepatocytes to thick septa and bridges of fibrosis which distort the hepatic architecture. In some series from 7% to 16% of the patients with NASH have cirrhosis in the initial biopsy. In the cirrhosis phase both steatosis and the inflammatory changes may have disappeared, making the etiological diagnosis difficult to establish.

A classification has been proposed for nonalcoholic fatty liver according to the histologic lesions [66]. Steatosis is classified into 3 grades according to hepatocyte involvement: 33 %, from 33 % to 66 % or more than 66 % of the hepatocytes, respectively. In regard to steatohepatitis, a scoring system has been proposed which establishes the activity according to the intensity of liver damage and inflammation as the stage according to the degree of fibrosis. Grade 1 or mild steatohepatitis presents steatosis, occasional hepatocyte ballooning, and mild lobulillar or portal inflammation. Grade 2 or moderate steatohepatitis is characterized by more marked steatosis, evident ballooning, and more intense inflammatory infiltrates than those observed in grade 1. Grade 3 or intense steatohepatitis presents important steatosis, and both lobulillar and portal ballooning and inflammation. Stage 1 fibrosis includes perisinusoidal, especially centrolobulillar, fibrosis. Stage 2 fibrosis is perisinosoidal and portal and stage 3 is similar to stage 2 plus fibrotic bridges and stage 4, cirrhosis (table 1).

Table 1. Histological classification of NASH

Fatty liver
Grade 1 (mild): <33% of hepatocytes
Grade 2 (moderate): 33%-66% of hepatocytes
Grade 3 (severe): >66% of hepatocytes

Steatohepatitis

Necroinflammatory grade
Grade 1 (mild): steatosis <66% of hepatocytes, occasional ballooned zone 3 hepatocytes, mild intra-acinar inflammation and polymorphonuclear infiltration, and mild or absent portal inflammation.
Grade 2 (moderate): Steatosis of any *degree*, ballooning of hepatocytes patent, mild or moderate intra-acinar and portal inflammation.
Grade 3 (severe): steatosis, diffuse ballooning of hepatocytes, inflammation and polimorphonuclear infiltration in zone 3, and mild or moderate portal inflammation.

Fibrosis
Stage 1: Zone 3 perisinusoidal/pericellular fibrosis.
Stage 2: Zone 3 perisinusoidal/pericellular fibrosis with focal or extensive periportal fibrosis.
Stage 3: Zone 3 perisinusoidal/pericellular fibrosis and portal fibrosis with bridging fibrosis.
Stage 4: Cirrhosis.

Diagnosis

Although NASH may be suspected on the basis of the clinical manifestations, laboratory data, and complementary explorations, the diagnosis of this disease should be based on liver biopsy. In all cases, liver disease of another etiology should be excluded. Thus, apart from liver function tests, viral markers, antitissular antibodies, ferritin, cupremia, cupruria and ceruloplasmin, and alpha-1-antitrypsin should be studied. Ultrasonography is the most indicated imaging test

due to its relatively low cost and easy applicability. As previously mentioned, it is sensitive enough to detect steatosis but much less sensitive for the detection of associated fibrosis. Steatosis may also be observed by axial tomography or magnetic resonance imaging techniques [10,67].

Excessive alcohol intake should also be carefully ruled out. To do this the patients and their relatives should be interviewed, and laboratory tests should be evaluated since an elevated mean corpuscular erythrocyte volume or an AST/ALT ratio greater than one may be orientative [68-70]. When in doubt, more specific tests such as serial alcohol determinations in urine or the determination of markers such as carbohydrate deficient transferrin may be useful [71-73]. Although the limits of alcohol intake are not well established, alcohol ingestion of up to 20 g/day may be considered as not being responsible for the disease in these patients.

The need for performing a liver biopsy to confirm the suspicion of nonalcoholic steatohepatitis is still under debate [9,74]. This is because the patients are, generally, asymptomatic, the prognosis is good in many cases, the treatment of these patients is not well established, and biopsy is an expensive technique which carries a risk which should be taken into account. For all these reasons, many authors consider that from a practical point of view the establishment of the diagnosis of suspicion and periodic monitoring of the patient are sufficient.

The liver biopsy is not limited to confirming the diagnosis, it also allows pure steatosis to be distinguished from steatohepatitis and classification of the patients according to the intensity of the hepatic lesions [66]. This is important since a high percentage of patients already have intense fibrosis on the initial biopsy, and a recent study has shown that in patients with normal transaminases the whole spectrum of lesions may be observed, including advanced fibrosis [75]. The possibility of determining the intensity of the lesions allows the prognosis of the patients to be established. Likewise, due to the heterogeneity of the patients, a liver biopsy is necessary for the inclusion of a patient in a therapeutic study to allow correct interpretation of the results. Patients with the diagnosis of suspicion of NASH should be informed of the need for a liver biopsy to confirm the diagnosis. They should be told of the advantages and inconveniences of carrying out a biopsy in this disease and they should actively participate in the decision to undertake this exploration.

Prognosis

Knowledge of the natural history of NASH is not precise since few studies with a long-term follow-up and histologic control have been performed in these patients. Simple steatosis has a good prognosis. A series of 40 patients was followed over a mean of 11 years with no observed progression of the lesions in any of the patients [76]. In another series of 49 patients with simple steatosis, only one died due to liver-related causes [77]. Despite the similarity of the lesions and their pathogenesis, NASH has better prognosis than alcoholic hepatitis, although the lesions may also progress to cirrhosis and even hepatocarcinoma. In a review of 3 series with a total of 28 patients with a long term histologic follow-up, the lesions remained unmodified in 54 % of the patients, the fibrosis progressed in 43 %, and only one patient showed improvement [55]. All the studies coincide in concluding that age, obesity and diabetes are factors of poor prognosis [13, 78-80]. In a recent series including 22 patients with NASH with a control biopsy, progression of fibrosis was observed in one third of the patients, and in one third of these patients the progression from mild to advanced fibrosis was rapid [81].

There is increasing evidence that NASH is one of the major precursors of cryptogenetic cirrhosis. Recent studies have shown that 70% of the patients with cryptogenetic cirrhosis over the age of 60 years have a history of obesity, type 2 diabetes mellitus or dyslipemia suggesting NASH as the cause of cirrhosis [82,83]. In this phase the steatosis and other lesions characteristic of NASH are mild or even absent in the liver biopsy. Recent evidence also suggests that some patients with NASH that progresses to cirrhosis will develop hepatocellular carcinoma [84-86].

Treatment

At present there is no specific treatment for NASH. The current treatment consists of a series of general measures such as the abstinence from alcohol, a balanced diet, moderate physical exercise, and the avoidance of drugs and exposure to environmental toxins which may potentially cause the disease. Likewise, the etiological factor should be treated. Thanks to a better knowledge of the pathogenesis of NASH, a series of pharmacological treatments has been proposed. However, to date the experience with these drugs has been limited. In patients with advanced cirrhosis, as in those with cirrhosis of another etiology, liver transplantation is also indicated [10] (table 2).

Table 2. Treatment of non alcoholic steatohepatitis

1. Treatment of associated disorders
- – Gradual weight loss
- – Control of diabetes
- – Control of dyslipidemia

2. Potential pharmacological approaches

Improved insulin resistance
- – Metformin
- – Thiazolidinediones: rosiglitazone, pioglitazone

Improved dyslipidemia
- – Clofibrate
- – Gemfibrozil
- – Atorvastatin
- – Probucol

Antioxidants
- – Tocopherol
- – Tocopherol/ascorbic acid
- – Betaine
- – Ursodeoxycholic acid
- – S-adenosylmethionine

3. Liver transplantation

Treatment of the etiological factor

The treatment of obesity has been shown to be effective in both adolescents and adults. In most cases, progressive weight loss together with the treatment of diabetes and/or dyslipemia, when associated with obesity, is accompanied by an improvement in analytical alterations and ultrasonographic changes [87]. The method of achieving weight loss is important and should be progressive, with physical exercise and a balanced diet. Rapid weight loss with a very strict diet is counterproductive since mobilization of fatty acids in the adipose tissue of the liver may be produced which may deprive the patient of the correct metabolism of proteins and other essential nutrients, all of which produce steatosis and worsening of the lesions [89]. On many occasions it is difficult to ensure that the patients maintain the weight loss, and they recover the weight within a few months with the consequent reappearance of the lesions. Orlistat is a reversible inhibitor of gastric and pancreatic lipases which is beginning to be used with good results in the treatment of obesity. Recent pilot studies have shown that treatment with orlistat in patients with NASH can improve liver function tests, steatosis, inflammation and fibrosis [90]. Nonetheless, it is doubtful whether only weight loss is effective in cases with marked fibrosis.

In patients with intestinal bypass for the treatment of obesity, the restoration of normal transit and the administration of metronidazole to reduce endotoxin levels have been shown to improve the lesions. When the disease appears during the course of parenteral nutrition, the composition of this treatment should be modified by increasing lipid contribution in the place of glucose, and choline supplementation should be provided [91]. Glucose stimulates insulin secretion, which in turn inhibits mitochondrial oxidation of fatty acids and increases their hepatic synthesis. Choline is necessary for the synthesis of lecitin which, in turn, is necessary for the formation of VLDL lipoproteins.

Pharmacological treatment

The slight clinical expressiveness of most of the patients and the belief that the course of the disease is, in general, benign, explain why few therapeutic studies on treatment efficacy with an evaluation according to the histologic changes have been carried out. Moreover, the studies performed to date have been pilot studies, most of them uncontrolled and with a small number of patients. Therefore, there is no established treatment for NASH. The potential therapeutic strategies may be divided into three groups according to their mechanism of action: drugs which improve the resistance to insulin, hypolipemic drugs and drugs with an antioxidant and/or hepatoprotective effect [10,11,21,22].

Among the drugs which improve resistance to insulin, biguanides such as metformin and thiazolidindiones have been studied. In experimental models, metformin has been shown to improve steatosis and inflammation [92]. In patients with NASH, metformin reduces AST values, although possible histologic changes have not been evaluated [93]. Troglitazone was the first thiazolidindione tested and also showed an improvement in transaminases in a small group of patients [94]. Cases of severe hepatitis have recently been reported in patients treated with troglitazone and this drug has therefore been withdrawn [95]. Recently, the first results of a study on treatment with pioglitazone and rosiglitazone have been published. In 26 patients treated for 48 weeks with 4 mg of rosiglitazone every 12 hours, improvement was observed in the resistance to insulin, ALT values, and histologic lesions, especially inflammation and necrosis, although the ALT values returned to the basal levels 6 months after discontinuation of treatment [96]. One pilot study including 18 patients treated for 48 weeks with 30 mg/day of pioglitazone demonstrated similar results, that is, an improvement in resistance to insulin, transaminases and histologic lesions [97]. Both rosiglitazone and pioglitazone were, in general, well tolerated, with the most frequent adverse event being a significant increase in weight. These promising results should be confirmed in controlled studies.

In relation to hypolipemic drugs, one controlled study did not show any beneficial effect with treatment with clofibrate [98]. Two controlled studies with a small number of patients demonstrated a significant improvement in transaminases with gemfibrozyl [99] and probucol [100], respectively. Histologic changes were not evaluated in any of these studies. Atorvastatin was also evaluated in a pilot study in seven patients and showed a decrease in plasmatic lipid values as well as an improvement in steatosis, inflammation and fibrosis in the biopsy carried out after one year of treatment [101].

The administration of vitamin E at a dose of 400 to 1200 mg/day achieved an improvement in transaminases in a group of adolescents with steatohepatitis [102]. The administration of tocoferol alone or combined with ascorbic acid showed relatively small changes in the histologic lesions in adults [103,104]. Likewise, in another pilot study, the administration of vitamin E did not produce modifications in the levels of the cytokines involved in the pathogenesis of NASH (105).

In still another pilot study, one year of treatment with ursodeoxycholic acid at a dose of 13 to 15 mg/day achieved an improvement in both the biochemical parameters and the degree of steatosis in the liver biopsy [98]. However, the same authors have recently published the results of a controlled, double-blind study including 166 patients treated with the same dose of ursodeoxycholic acid for two years. Pre– and post-treatment biopsies were evaluated in 107 patients and showed the same changes in the group treated with the drugs and in the placebo group [106]. Thus, although well tolerated, from this study it may be deduced that ursodeoxycholic acid is not effective in the treatment of NASH.

Treatment with betaine, a precursor of s-adenosylmethionine, a drug with hepatoprotective effects, has been evaluated in a pilot study including 10 patients. After one year of treatment all the patients showed normalization or improve-

ment in transaminase values as well as improvement in the steatosis, the necroinflammatory lesions and the degree of fibrosis [107]. These results should be confirmed with controlled studies. Other drugs with antioxidant effects such as s-adenosylmethionine or metadoxin have not been evaluated in NASH.

In conclusion, controlled studies including a sufficient number of patients are required with the main objective being the histologic changes to evaluate correctly the different therapies proposed. Likewise, studies should perhaps also be designed to evaluate the effects of the combination of different therapeutic approaches [108].

Liver transplantation

Liver transplantation is a therapeutic option which should be considered in patients with NASH in an advanced phase, although in some cases morbid obesity or the complications of diabetes mellitus may contraindicate this procedure. The immediate results of transplantation in these patients are good [109], although the lesions of steatohepatitis may reappear in the mid-term [110,111]. The cause of the recurrence of lesions is unknown but may be due to the persistence of an etiological factor such as prolonged treatment with corticosteroids. Cyclosporine administration may also cause mitochondrial damage.

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About the authors

Juan Rodés is Professor of the Department of Medicine at the University of Barcelona. Since its foundation in 1969 to the present he has been Director of the Liver Unit of the Hospital Clínic of Barcelona. Under his direction the Liver Unit has become one of the most prestigious and an international reference for clinical and basic research in liver diseases. He has published 463 original articles, 28 books and 93 chapters of books and many reviews covering most aspects of liver diseases. He has been president of the Spanish, European and International Societies for the Study of the Liver and the International Liver Transplant Society. At present, he is General Director of the Hospital Clínic of Barcelona, Scientific Director of the Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), and Editor of Journal of Hepatology. *He is member of the Biological Sciences Section of the Institute for Catalan Studies.*

Juan Caballería is a Senior Consultant of the Liver Unit at the Hospital Clinic of Barcelona. Since his graduation in 1971 all his clinical and research work has been done in this Unit. He spent a year as a postdoctoral fellow in the Alcohol Research Center, Bronx V.A. Medical Center, Mount Sinai School of Medicine, New York University. His work in the Liver Unit has been devoted to alcohol-related liver disease, covering both clinical and basic aspects. The results of his work have been published in the most prestigious medical journals. In recent years he has also developed a new research line related to non alcoholic fatty liver disease.