Approach to the Pathogenesis and Treatment of Nonalcoholic Steatohepatitis

JESÚS MEDINA, PHD[1]
LUIS I. FERNÁNDEZ-SALAZAR, MD[2]
LUISA GARCÍA-BUEY, MD[1]
RICARDO MORENO-OTERO, MD[1]

Nonalcoholic steatohepatitis (NASH) represents an advanced stage of fatty liver disease developed in the absence of alcohol abuse. Its increasing prevalence in western countries, the diagnostic difficulties by noninvasive tests, and the possibility of progression to advanced fibrosis and even cirrhosis make NASH a challenge for hepatologists. NASH is frequently associated with type 2 diabetes and the metabolic syndrome, and several genetic and acquired factors are involved in its pathogenesis. Insulin resistance plays a central role in the development of a steatotic liver, which becomes vulnerable to additional injuries. Several cyclic mechanisms leading to self-enhancement of insulin resistance and hepatic accumulation of fat have been recently identified. Excess intracellular fatty acids, oxidant stress, tumor necrosis factor-α, and mitochondrial dysfunction are causes of hepatocellular injury, thereby leading to disease progression and to the establishment of NASH. Intestinal bacterial overgrowth also plays a role, by increasing production of endogenous ethanol and proinflammatory cytokines. Therapeutic strategies aimed at modulating insulin resistance, normalizing lipoprotein metabolism, and downregulating inflammatory mediators with probiotics have promising potential.

Diabetes Care 27:2057–2066, 2004

Nonalcoholic steatohepatitis (NASH) is a liver disease characterized by steatosis and periportal and lobular inflammation. In its initial phases, during which fat accumulates in the liver, no clinical symptoms are evident. In advanced stages, fibrosis (eventually progressing to established cirrhosis in some patients) is detectable histologically, along with a mixed inflammatory cell infiltrate, glycogen nuclei, and Mallory's hyaline (1). Because its adequate diagnosis requires histological evaluation of the liver, the prevalence of NASH is probably underestimated. Nonalcoholic fatty liver disease has been suggested to be the most common cause of chronic liver disease in the U.S., with a suggested incidence of 10–24% in the general population and probably similar figures in Europe and Japan (2,3).

Two types of NASH exist: primary NASH (which is associated with metabolic syndrome–related conditions, such as obesity, type 2 diabetes, and hyperlipemia) and secondary NASH (which occurs after obesity-related intestinal surgery, rapid weight loss in the obese, total parenteral nutrition, treatment with drugs such as amiodarone or perhexiline maleate, lipodystrophy, or Wilson's disease). Although many aspects of the disease are common to both presentations, this short review focuses mainly on the pathogenesis of primary NASH.

The actual prevalence of NASH in type 2 diabetes and obesity is unknown. It is estimated that 75% of type 2 diabetic patients present some form of nonalcoholic fatty liver of different degrees. An association of NASH with hyperinsulinemia, as well as with clinical features of insulin resistance, has frequently been reported (4–9). As far as obesity is regarded, steatosis has been reported in 70% of obese and 35% of lean patients and NASH in 18.5% of obese and 2.7% of lean patients in a consecutive study (10), although some authors have reported even higher figures (up to 95% in some studies [11]). The prevalence of simple steatosis in obese patients is ~60%, whereas 20–25% present NASH and 2–3% present cirrhosis (7,12,13).

Criteria for diagnosis of NASH were established at the American Association for the Study of Liver Diseases single topic conference on NASH (1). In most cases, asymptomatic patients are referred to the hepatologist after abnormal liver enzyme levels obtained during routine evaluation or during antihyperlipidemic drug therapy. Because clinical signs and liver test values have a poor predictive value for making a specific diagnosis, histological evaluation of morphological changes in a liver biopsy may be required, in particular, to differentiate between simple steatosis and steatohepatitis. The presence of obesity or type 2 diabetes, high (at least two times that of normal) levels of alanine aminotransferase (ALT) and triglycerides, hypertension, and an aspartate aminotransferase/ALT ratio greater than unity may justify performing a biopsy, since prognostic information is greater in these subgroups of patients (12,14–16). A standardized scoring system for nonalcoholic fatty liver disease has been published (17).

PATHOGENIC CONSIDERATIONS

— According to Day et al. (14,18), the pathogenesis of NASH comprises two steps. First, the

From the [1]Liver Unit, University Hospital La Princesa, Autonomous University, Madrid, Spain; and the [2]Digestive Diseases Service, University Hospital, Valladolid, Spain.

Address correspondence and reprint requests to Ricardo Moreno-Otero, Unidad de Hepatología (planta 3), Hospital Universitario de la Princesa, Diego de León 62, E-28006 Madrid, Spain. E-mail: rmoreno.hlpr@salud.madrid.org.

Received for publication 24 October 2003 and accepted in revised form 29 April 2004.

J.M. and L.I.F.-S. contributed equally to this work.

Abbreviations: ALT, alanine aminotransferase; FFA, free fatty acid; IKKβ, IκB kinase β; IRS, insulin receptor substrate; LPS, lipopolysaccharide; NASH, nonalcoholic steatohepatitis; NF, nuclear factor; PPAR, peroxisome proliferator–activated receptor; TGF, transforming growth factor; TNF, tumor necrosis factor.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

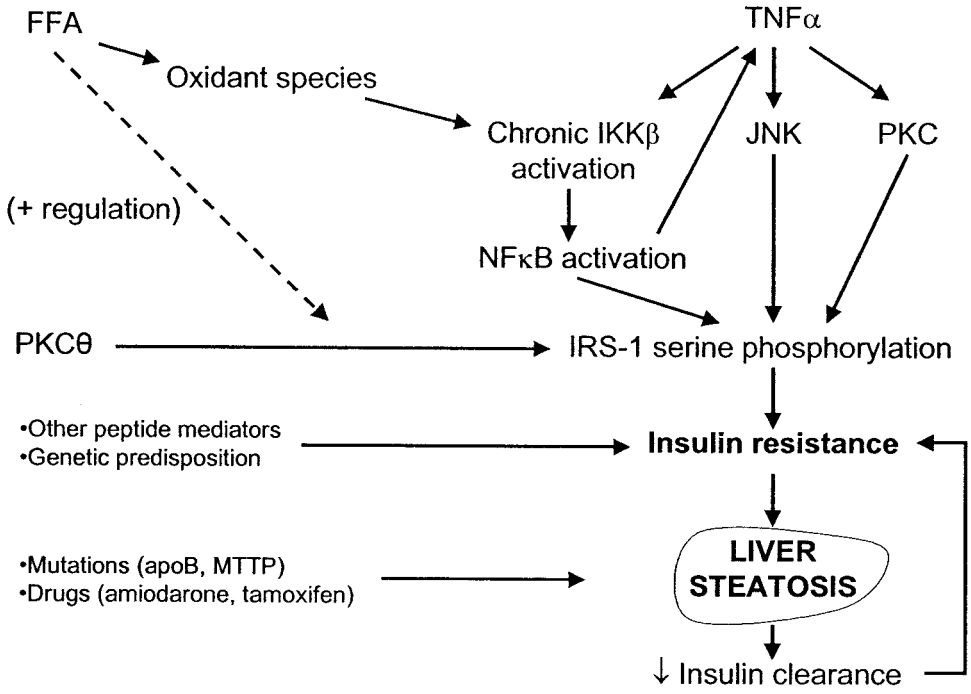

Figure 1—*Mechanisms leading to liver steatosis. Insulin resistance, occurring in response to a variety of genetic and acquired factors, is intimately associated with the development of a steatotic liver. At least two mechanisms contribute to exacerbation of insulin resistance by cyclic self-perpetuation:* 1*) chronic activation of IKKβ, which plays a key role not only in the production of and response to proinflammatory cytokines, such as TNF-α, but also in the development of insulin resistance; and* 2*) the decreased clearance of insulin that occurs in the presence of a steatotic liver, thus creating a hyperinsulinemic medium that in turn enhances insulin resistance. Serine phosphorylation (rather than tyrosine phosphorylation) of IRS-1 (which leads to disruption of insulin signaling) represents a central step in the stimulation of insulin resistance by a variety of factors. apoB, apolipoprotein B; JNK, c-Jun NH_2-terminal kinase; MTTP, microsomal triglyceride transfer protein.*

healthy liver becomes steatotic. This is mainly a consequence of peripheral resistance to insulin, whereby the transport of fatty acids from adipose tissue to the liver is increased. Although some protective mechanisms are developed to survive this stress, the fatty liver is in most cases particularly fragile and vulnerable to additional insults, such as ethanol or bacterial lipopolysaccharide. Then, a second step elicited by oxidative stress and cytokines (basically, tumor necrosis factor [TNF]-α) occurs. This leads to exacerbation of insulin resistance, further oxidative stress, and organelle dysfunction within liver cells, resulting in an inflammatory process, hepatocellular degeneration, and fibrosis.

Insulin resistance and development of steatosis

A combination of genetic and acquired factors contribute to the first hit–originating liver steatosis through increased lipolysis and delivery of free fatty acids (FFAs) to the liver (Fig. 1). Insulin resistance plays a primary role: it is the most specific finding in NASH, and it can be observed in most patients, whether they present overweight or with type 2 diabetes (4,9). This primary resistance leads to hyperinsulinemia and is associated with the metabolic syndrome (4,9,19), of which NASH is considered to represent the hepatic expression (20). The severity of insulin resistance has been shown to parallel the severity of fatty liver disease, with clinically overt type 2 diabetes being most common in NASH patients with cirrhosis (21). In this regard, the presence of diabetes has been suggested to be useful for the identification of those elderly NASH patients who might have severe liver fibrosis (16). These findings further support the notion that insulin resistance plays a pathogenetic role in NASH.

Several mechanisms for the development of peripheral resistance to insulin have been described, including acquired and inherited factors (22–24) (Fig. 1). A breakthrough in this regard was the discovery that chronic stimulation of IκB kinase β (IKKβ), which promotes activation of nuclear factor (NF)-κB (a transcription factor involved in inflammatory cytokine production) also causes insulin resistance (25). This is caused by IKKβ-mediated changes in phosphorylation (in serine rather than in tyrosine) of insulin receptor substrate (IRS)-1, thus disrupting the intracellular signaling triggered by binding of insulin to its receptor (25).

Interestingly, IKKβ is activated by two main types of stimuli (Fig. 1). The first is increased hepatic oxidative stress. This may be caused, among other factors, by an increased mitochondrial, peroxisomal, and/or microsomal oxidation of FFAs as a result of either environmental factors (fat diets, lipopolysaccharide, ethanol, and drugs) or genetic factors (β-oxidative defects). The second type of stimuli is proinflammatory cytokines, particularly TNF-α. This permits perpetuation of the cycle, whereby TNF-α activates IKKβ, which in turn induces TNF-α production. TNF-α stimulation of IRS-1 serine phosphorylation may also be mediated by c-Jun NH_2-terminal kinase and several protein kinase C isoforms (1). Data supporting the major role played by TNF-α in the development of insulin resistance come from studies with TNF-α

knockout mice, which do not develop insulin resistance in response to obesity induction (26). Notably, TNF-α expression in adipose tissue and liver is augmented in NASH patients, whose serum TNF-α levels correlate with insulin resistance (27).

As mentioned, the diet may be responsible for steatosis and oxidative stress in some liver diseases (28). Evidence exists demonstrating that the diet of NASH patients is rich in unsaturated fat and cholesterol but poor in polyunsaturated fat, fiber, and vitamins E and C compared with that of healthy subjects. These levels of unsaturated fat in the diet correlate with a lower sensitivity to insulin, with high postprandial triglyceride levels in these patients, and with other aspects of the metabolic syndrome (29).

As a consequence of insulin resistance, the lipogenic effects of this hormone on adipose tissue are modulated, resulting in the degradation of triglycerides into FFAs, which are then released into the blood flow (30). This situation is similar to that observed in overweight patients undergoing a fast and disproportioned weight loss (10). There are indications that visceral and central adipose tissues play a more critical role than peripheral adipose tissue in FFA release and steatotic liver formation (4,19). The reason might be a more direct access to the liver through the portal system for fat derived from those tissues, as well as a lower secretion of leptin (31).

This situation is extreme in lipodystrophies—genetic diseases characterized by absence of or insensitivity to leptin. In these patients, adipose tissue is not developed, fat accumulates in organs such as the liver (thereby originating a secondary steatosis [32]), and peripheral resistance to insulin occurs (22). Leptin is a hepatocyte-derived 16-kDa peptidic hormone mainly involved in lipid metabolism regulation. It precludes fat accumulation in nonadipose tissues by inhibiting lipogenesis and potentiating lipolysis after excessive caloric intakes (33,34). Controversial data have been reported on the involvement of leptin in the pathogenesis of NASH. Uygun et al. (35) found increased serum leptin levels in NASH patients and suggested that leptin might represent an independent predictive factor of the intensity of liver steatosis in NASH, along with C-peptide (which reflects pancreatic insulin secretion) and age (36). However, these results might have been influenced by the fact that subjects in the control group had a lower BMI than NASH patients. The apparent paradox of the correlation between leptin levels and liver steatosis (in principle, leptin reduces fat accumulation in the liver) could be explained by the development of resistance to leptin actions or by a close relation between leptin levels and insulin resistance (4). In this respect, leptin inhibits insulin-induced IRS-1 phosphorylation (37). However, in a separate clinical study that used appropriately matched control subjects (BMI, percent body fat, and abdominal fat distribution), no association between serum leptin levels and NASH could be observed (NASH: 21 ± 13 ng leptin/ml [$n = 26$]; control group: 18 ± 11 ng leptin/ml [$n = 20$]; $P = 0.5$) (38). Furthermore, there was no correlation between serum leptin and hepatic histology, serum transaminases, fasting insulin levels, or a measure of insulin resistance. Although these data suggest that leptin may not be relevant in NASH, additional studies with larger cohorts of patients and an improved control for factors such as interindividual leptin levels and the absence of liver pathology in control subjects could help to clarify the involvement of this hormone in the pathogenesis of NASH.

Exaggerated levels of FFAs may be deleterious for the liver through a variety of mechanisms (1), including *1*) de novo synthesis of ceramides, which may cause apoptosis; *2*) resistance to insulin, by interfering with intracellular phosphorylation processes; and *3*) lipid peroxidation. Under normal conditions, the liver is prepared to defend against FFA-induced toxicity through their sterification and triglyceride formation, their oxidation, or the synthesis and release of VLDLs. Nuclear peroxisome proliferator–activated receptor (PPAR)-α plays an important role in these processes, by sensing excess FFAs and upregulating the genetic program of their disposal (39). However, under hyperinsulinemic conditions, the following processes will take place: *1*) the expected increase in FFA sterification and triglyceride formation, but also *2*) an increase in glycolysis and fatty acid synthesis, *3*) an inhibition of oxidation (similarly to amiodarone and perhexyline maleate) (40), and *4*) a reduced release of triglycerides in the form of VLDL (41). Apolipoprotein B secretion, necessary to form VLDL, has been shown to be reduced in NASH (29,42). Besides, NASH patients show hypertriglyceridemia in both fasting and postprandial situations, resulting in a higher fat upload to the liver (29,42,43). These alterations in lipid metabolism also contribute to making a healthy liver become steatotic.

The steatotic liver is a particularly susceptible organ to become resistant to insulin (19). However, the mediating mechanisms are not fully understood: apart from some of those already mentioned (TNF-α, NF-κB–mediated FFA effects), PPAR-α–mediated inhibition by polyunsaturated fatty acids of insulin-induced lipogenic and glycolytic enzymes may play a role (44). Leptin might also regulate hepatic sensitivity to insulin (4,36,37).

Injury to the steatotic liver and progression of liver disease

Although in most patients the process does not progress, a minority of subjects undergo a second stage, characterized by inflammation and hepatocellular degeneration. Steatosis renders hepatocytes vulnerable against external aggressions, and apoptosis becomes a frequent mechanism of cell death (45,46). Recent results obtained in a cohort of 264 prospectively enrolled patients with nonalcoholic fatty liver disease indicate that insulin resistance is a major, independent risk factor for advanced fibrosis, thus acting both as the first and second hits (47).

The reasons for progression of the disease are not fully understood, although oxidative stress and cytokines are the main effectors of this second hit on the vulnerable liver (Fig. 2). Furthermore, a certain genetic predisposition has been suggested to play a role in the development of NASH.

Oxidative stress. Peripheral resistance to insulin and high levels of leptin allow entrance to the mitochondria of those FFAs reaching the liver as a consequence of the previously inhibited oxidation (14). Although oxidation of long-chain and very-long-chain fatty acids is partly extramitochondrial (in microsomes and peroxisomes), free oxygen radical production occurs mainly in mitochondria (48,49). Massive FFA hepatic upload, and particularly acyl-CoA, lead to PPAR-α–mediated activation of the synthesis of enzymes responsible for oxidation, thereby increasing peroxide levels (14,50). Formation of free oxygen radicals in a fat-rich

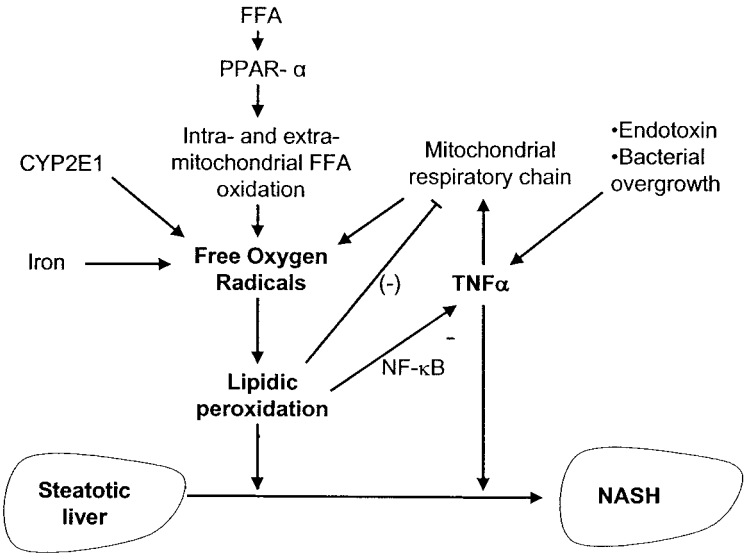

Figure 2—*Factors contributing to the development of NASH from a steatotic liver. Iron overload, activation of CYP2E1, and induction of enzymes involved in FFA oxidation result in an increase in free oxygen radical production, which in turn induce lipidic peroxidation, leading to inhibition of mitochondrial respiration and NF-κB–mediated TNF-α synthesis. Bacterial endotoxin-stimulated TNF-α synthesis also uncouples mitochondrial respiration. Both lipidic peroxidation and TNF-α are key triggering factors for NASH development.*

medium induces lipidic peroxidation. Increased oxidative stress and lipidic peroxidation induce damage in plasmatic membranes, intracellular organelles, mitochondrial DNA, and respiratory chain-related proteins (51). Additionally, the end products of oxidative stress activate NF-κB–mediated nitric oxide synthesis, leading to the formation of peroxynitrites (13). FFAs also increase the expression of microsome oxidases CYP4A and CYP2E1, responsible for the production of hydroxyethil radicals (52–54). Because CYP2E1 is inhibited by insulin, its expression levels are higher in case of peripheral resistance to this hormone (55).

Another consequence of the increased production of free oxygen radicals is the induction of Fas ligand expression in hepatocellular membranes (not expressed under normal conditions), since its promoter contains a binding site for NF-κB. Interaction of Fas ligand with Fas-expressing hepatocytes leads to their death through a process termed "fratricidal apoptosis" (49).

Mitochondrial dysfunction seems to be a critical event in the second hit of NASH. Whether induced by lipidic peroxidation products secondary to oxidative stress or directly by TNF-α, it leads to alterations in electron transfer along the respiratory chain, thus generating more free oxygen radicals (49). The expression of mitochondrial oxidative phosphorylation uncoupling protein 2 is increased. This protein reduces free oxygen radical synthesis, but it also decreases ATP levels, thus making the cell more sensitive to insults (46,56,57) and facilitating hepatocellular apoptosis and necrosis. Decreased ATP synthesis has been reported in NASH patients after perfusion with fructose (58). Nevertheless, other authors question the detrimental potential of uncoupling protein 2 (59). Electron microscopic evaluation of liver biopsies from NASH patients has shown paracrystaline inclusions in 10–30% of hepatocellular mitochondria (6). Although the significance of this finding is unknown, these inclusions are observed in patients with myopathies associated with mitochondrial DNA alterations that affect respiratory chain enzymes (60) and might therefore represent a genetic basis determining the evolution of fatty liver in NASH (6).

TNF-α. TNF-α also contributes to the second hit in NASH pathogenesis. An increased TNF-α synthesis by hepatocytes and Kupffer cells may be caused by *1*) NF-κB–mediated FFA oxidation-induced oxidative stress (61) or *2*) endotoxemia resulting from intestinal bacterial overgrowth (see below). TNF-α has several modes of action: *1*) it induces resistance to insulin, thus producing increased levels of FFAs; *2*) it uncouples mitochondrial respiration, thereby inducing oxygen radical formation, similarly to amiodarone and perhexyline maleate (40); and *3*) it induces hepatocyte apoptosis and necrosis (62).

Genetic predisposition. NASH has been suggested to have an inherited component, involving genes related to *1*) determination of sensitivity to insulin (22,63); *2*) hepatic lipid storage, oxidation, or release into the blood flow (64); *3*) obesity and its distribution (65,66); *4*) regulation of hepatic iron levels and oxidative stress generation (67–69); or *5*) cytokine synthesis (8,70,71).

Iron overload. Iron overload may play a role in NASH pathogenesis. An association between iron overload and the metabolic syndrome (68,72–75) or advanced liver disease (76) has been demonstrated. Iron catalyzes the transformation of hydrogen peroxide into hydroxyl groups through the Fenton reaction. Nevertheless, the relevance of mutations in the *HFE* gene and serum iron levels in the pathogenesis of NASH is a matter of discussion. Some authors have found higher prevalence of mutations in the *HFE* gene along with elevated hepatic iron and more advanced stages of fibrosis in NASH patients (67,69), whereas others have failed to identify an excess of iron and its supposed fibrogenic action (16,47,68,74,77) in these patients.

Fibrogenesis. Additional mechanisms have been described to potentially contribute to fibrogenesis in NASH. A marked expression of connective tissue growth factor, which correlates with fibrosis (78), has been shown. Interestingly, glucose and insulin potentiate its synthesis. Hepatic stellate cell–derived leptin might stimulate fibrogenesis through an autocrine action and through the induction of transforming growth factor (TGF)-β1 by Kupffer and endothelial cells (79,80). However, serum leptin levels are not associated to more advanced stages of fibrosis (36). Finally, the expression of the TGF-β1 receptor endoglin is increased in sinusoidal endothelial cells and probably in hepatic stellate cells of NASH patients (13).

Role of intestinal bacterial overgrowth in the pathogenesis of NASH

A clear link between intestinal bacterial overgrowth and liver damage during NASH has recently been established (81). Bacterial overgrowth has been detected in NASH patients with breath tests with lactulose and D-xilose (82), as well as in some forms of secondary NASH, such as that associated with obesity-related intestinal surgery (83).

Studies about the pathogenesis of alcoholic fatty liver disease demonstrated protection of the liver from ethanol when intestinal bacterial overgrowth was inhibited (84,85). The reason was the reduced hepatic exposure to bacterial lipopolysaccharide (LPS). Intestinal bacteria may increase hepatic oxidative stress by at least two mechanisms (81): *1*) increased endogenous ethanol production and *2*) release of LPS. Both ethanol and LPS stimulate inflammatory cytokine production through an IKKβ-mediated mechanism (86), with Kupffer cells (a cell type that plays a critical role in NASH) as the main source of TNF-α. Because TNF-α is a central mediator in the pathogenesis of NASH, inhibition of bacterial overgrowth was also shown to result in protective mechanisms in this disorder (49). Further support for these effects is provided by evidence that Kupffer cells are oversensitive in NASH, as well as in obesity, probably because of leptin actions (87–89).

Ethanol synthesized by intestinal bacteria might be involved in NASH pathogenesis, since obese female NASH patients present higher levels of breath ethanol (90). This is supported by data obtained from leptin-deficient obese mice (*ob/ob* mice), regarded as a good experimental model for human NASH (91). These mice show a reduced endogenous ethanol production after treatment with neomycin to eliminate bacterial overgrowth (92). Other authors, however, propose a protective role for low doses of alcohol, by reducing resistance to insulin and inhibiting TNF-α synthesis by monocytes (7,93).

THERAPEUTIC ALTERNATIVES FOR NASH

At present, there is no widely accepted approach to treat NASH. The various therapeutic alternatives, as follows, are aimed at interfering with the risk factors considered to be involved in the etiology of NASH.

1. Correction of obesity with hypocaloric diets and physical exercise (94–97). Rapid weight loss and long-lasting fasting periods should be avoided, since they lead to an increase in the flow of FFAs to the liver. A gradual weight reduction has been associated with an improvement of hepatic lesions, including fibrosis (98).
2. Control of hyperglycemia with diet, insulin, or oral antidiabetic agents. Simultaneous treatment of overweight in these patients is of paramount importance.
3. Withdrawal from treatment with amiodarone, perhexiline maleate, tamoxifen, or other drugs to which NASH development has been attributed. Likewise, exposure to hepatotoxic environmental agents, including alcohol, should be avoided, particularly where fibrosis is histologically detected in a biopsy.
4. Control of hyperlipemia with diet, or, when indicated, with hypolipemic drugs. Nevertheless, the efficacy of this measure is controversial, according to a study in hypertriglyceridemic patients who received clofibrate (2 g/day) for 1 year, showing no biochemical or histological improvement (99). Gemfibrozil (600 mg/day) or bezafibrate showed more favorable results in terms of biochemical parameters and development of steatosis (100,101). Orlistat, an inhibitor of lipoprotein lipase, has been recently proven to be beneficial for NASH patients, inducing normalization of transaminases and reduction in liver steatosis and inflammatory activity (102).
5. In parenteral nutrition-associated NASH, modifying the composition of the infusion, replacing glucose with lipids. Glucose stimulates insulin secretion, thus inhibiting FFA oxidation and leading to their accumulation and synthesis in the liver. Supplementation with choline is indicated to increase the synthesis of lecitin, necessary for VLDL formation (98).
6. In patients undergoing surgery to treat obesity, reconstructing intestinal transit to help improve hepatic lesions. Metronidazol may prevent the development of NASH by preventing the absorption of bacterial overgrowth-derived endotoxin in excluded loops (1,98).

Progression of NASH histological lesions is not always precluded by measures that target the etiological factors of NASH, as those mentioned above. Therefore, alternative treatments directed against specific pathogenetic mechanisms are currently under investigation, including clinical trials with anti–TNF-α antibodies (103).

Antibiotics

Because bacterial overgrowth–derived lipoproteins may be involved in the development of NASH, oral metronidazol (0.75–2 g/day for 3 months, followed by a similar period without treatment) may be efficacious in reverting steatosis and, in some cases, inflammation and fibrosis (104,105). Oral polymixin B may improve parenteral nutrition–associated NASH by reducing liver exposure to intestinal flora-derived endotoxin.

Probiotics

The knowledge of the role of bacterial overgrowth in the pathogenesis of NASH has led to the proposal of probiotics as a therapeutic strategy for this disorder. Probiotics may interfere with the development of NASH at various levels: *1*) decreases in proinflammatory cytokines, such as TNF-α; *2*) alteration of the inflammatory effects of pathogenic strains of intestinal bacteria, through changes in cytokine signaling; *3*) replacement of pathogenic strains of bacteria; and *4*) improved epithelial barrier function (thereby avoiding excessive exposure of the liver to LPS and bacterial ethanol). Evidence in experimental animal models of fatty liver disease (103), as well as clinical data on other gastrointestinal diseases, strongly suggest that probiotics might be beneficial in NASH (81). Data from an uncontrolled clinical trial with NASH patients show promising results, with improvement of liver enzymes in treated patients (106).

Cytoprotective agents and antioxidants

Several agents are included in this group, including ursodeoxycholic acid, vitamin E, lecitin, β-carotene, selenium, S-adenosyl-methionine, metadoxine, or silimarin.

Ursodeoxycholic acid has several mechanisms of action that justify its use in NASH: hydrophilic effect (resulting in the displacement of toxic hydrophobic biliary salts), and immunomodulatory and cytoprotective properties. An oral dose of 13–15 mg/day for 12 months was efficacious in improving liver biochemistry alterations and steatosis, although no favorable changes occurred in the rest of the histological lesions of NASH (99). However, recently reported results of a randomized multicenter study in which NASH patients received between 13 and 15 mg · kg^{-1} · day^{-1} of ursodeoxycholic acid for 2 years showed no improvement of liver disease with respect to placebo-treated patients (107).

The therapeutic indication of S-adenosyl-methionine in intrahepatic cholestasis and alcoholic liver disease is based on its anti-steatotic, anti-inflammatory, anti-oxidant, and anti-fibrotic properties. Oral treatment with 600 mg/day or intramuscular administration of 50–100 mg/day have shown efficacy in terms of biochemical, histological, and echographic parameters of liver steatosis, in the absence of adverse effects (108).

α-Tocoferol is effective in improving liver biochemistry and histological lesions of NASH because of its actions as an antioxidant agent and as an inhibitor of TGF-β, a cytokine involved in liver fibrogenesis (109).

Metadoxine has proved efficacious in the treatment of alcoholic liver steatosis, as shown by biochemical data and echographical signs (110). This drug restores hepatic glutathione concentrations and acts as an antifibrogenic agent. These therapeutic effects, along with the proven efficacy in steatosis, may justify its indication for the treatment of NASH.

Silymarin also possesses antioxidant and antifibrogenic properties, with beneficial effects in alcoholic liver disease (111), supporting its indication for the treatment of NASH. Betaine treatment has shown beneficial biochemical and histological effects in a pilot study of NASH patients (112). Additional drugs currently in assessment include ghrelin (113) and pentoxyphylline (114). Other promising, potentially useful antioxidant agents include vitamin E and *N*-acetylcysteine.

Reduction of peripheral resistance to insulin

The thiazolidinediones are compounds that improve insulin sensitivity by binding the PPAR-γ class of nuclear transcription factors. Troglitazone led to a decrease in the transaminase levels in obese NASH patients (115), although no weight reduction was observed. Nevertheless, troglitazone was later discarded because of its risk for severe liver toxicity. Second-generation agents such as rosiglitazone have also shown some efficacy in improving liver enzyme levels and histology (116). In a pilot study in which 30 NASH patients were treated with rosiglitazone, 4 mg twice daily for 48 weeks, reductions in liver fat content and in mean ALT (from 86 to 37 units/l) were already observed by 24 weeks of treatment, along with the expected improvement (although not complete normalization) in insulin sensitivity and an acceptable tolerance profile (117). Whether the observed liver effects were caused by improved insulin sensitivity or by the anti-inflammatory actions of this class of compounds remains to be elucidated. Similarly, pioglitazone has also been tested in a pilot study with 18 nondiabetic NASH patients (118). Administration of a daily dose of 30 mg for 48 weeks resulted in normalization of ALT levels in 72% of patients. Hepatic fat content and size, as well as glucose and FFA sensitivity to insulin, were consistently improved, as well as histological signs of steatosis. Treatment side effects were manageable. Metformin has also been tested as a therapy for NASH. In addition to improving hyperinsulinemia and insulin sensitivity in animals and humans (119), metformin inhibits hepatic TNF-α and several TNF-inducible responses, which, as stated above, are likely to promote hepatic steatosis and necrosis. When administered to insulin-resistant obese *ob/ob* leptin-deficient mice, metformin reduced both hepatic steatosis and hepatic TNF-α expression (120). In humans, treatment of 14 NASH patients with 500 mg t.i.d. metformin for 4 months resulted in normalization of transaminase levels, improvement of insulin sensitivity, and a decrease in liver volume in 50% of patients (121). In a recent clinical study, 17 NASH patients were given 850 mg b.i.d. metformin plus dietary treatment and were compared with a similar group with calorie-restricted dietary treatment alone. Metformin statistically significantly improved serum alanine/aspartate aminotransferase levels as well as insulin resistance, whereas it decreased insulin and C-peptide levels (122). However, no significant differences between groups were observed in terms of necro-inflammatory activity or fibrosis. Although larger studies are needed, these data suggest that metformin could be a promising agent for the treatment of NASH patients.

Reduction of liver iron content

Because iron deposits are associated with a higher intensity of liver damage in NASH patients, repeated phlebotomies might prevent the development of lesions, delay their evolution, or even reduce or eliminate those already established (69).

Liver transplantation

Liver transplantation is indicated for NASH patients with decompensated cirrhosis (123). However, some cases of disease recurrence have been reported in female NASH patients and in cases of jejunum-ileum derivation in which the intestinal transit was not restored by eliminating the dysfunctional loop, simultaneously to transplantation.

In summary, the pathogenesis of NASH is multifactorial: the development of steatosis seems to be mainly determined by insulin resistance, whereas the causes of hepatocellular injury include factors such as excess intracellular fatty acids, mitochondrial dysfunction, oxidant stress, cytokines, iron overload, bacterial overgrowth, and genetic predisposition. At present, therapeutic strategies targeting the various etiologic and pathogenetic mechanisms are being investigated, including behavioral and pharmacological approaches to insulin resistance, cytoprotective agents, antioxidant agents, iron reduction therapy, and antihyperlipidemic agents.

Acknowledgments— Financial support was obtained from a grant (C03/02) from the Instituto de Salud Carlos III, Spain, SAF 2001-1414 from Ministerio de Ciencia y Tecnología, Spain (to R.M.O.), and a grant (02/3015) from Fondo de Investigaciones Sanitarias, Spain (to J.M.).

The authors are grateful to Brenda Ashley for assistance with English.

References

1. Neuschwander-Tetri BA, Caldwell S: Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. *Hepatology* 37:1202–1219, 2003
2. Clark JM, Brancati F, Diehl A: The prevalence and etiology of elevated aminotransferase levels in the United States. *Am J Gastroenterol* 98:960–967, 2003
3. Clark JM, Brancati F, Diehl A: Nonalcoholic fatty liver disease. *Gastroenterology* 122:1649–1657, 2002
4. Chitturi S, Abeygunasekera S, Farrell G, Holmes-Walker J, Hui J, Fung C, Karim R, Lin R, Samarasinghe D, Liddle C, Weltman M, George J: NASH and insulin resistance: insulin hypersecretion and specific association with the insulin resistance syndrome. *Hepatology* 35: 373–379, 2002
5. Willner IR, Waters B, Patil S, Reuben A, Morelli J, Riely C: Ninety patients with nonalcoholic steatohepatitis: insulin resistance, familial tendency, and severity of disease. *Am J Gastroenterol* 96:2957–2961, 2001
6. Sanyal AJ, Campbell-Sargent C, Mirshahi F, Rizzo W, Contos M, Sterling R, Luketic V, Shiffman M, Clore J: Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology* 120:1183–1192, 2001
7. Dixon JB, Bhathal P, O'Brien P: Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. *Gastroenterology* 121:91–100, 2001
8. Valenti L, Fracanzani A, Dongiovanni P, Santorelli G, Branchi A, Taioli E, Fiorelli G, Fargion S: Tumor necrosis factor alpha promoter polymorphisms and insulin resistance in nonalcoholic fatty liver disease. *Gastroenterology* 122:274–280, 2002
9. Pagano G, Pacini G, Musso G, Gambino R, Mecca F, Depetris N, Cassader M, David E, Cavallo-Perin P, Rizzetto M: Nonalcoholic steatohepatitis, insulin resistance, and metabolic syndrome: further evidence for an etiologic association. *Hepatology* 35:367–372, 2002
10. Wanless IR, Lentz J: Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. *Hepatology* 12:1106–1110, 1990
11. Falck-Ytter Y, Younossi Z, Marchesini G, McCullough A: Clinical features and natural history of nonalcoholic steatosis syndromes. *Semin Liver Dis* 21:17–26, 2001
12. Ratziu V, Giral P, Charlotte F, Bruckert E, Thibault V, Theodorou I, Khalil L, Turpin G, Opolon P, Poynard T: Liver fibrosis in overweight patients. *Gastroenterology* 118:1117–1123, 2000
13. Garcia-Monzon C, Martin-Perez E, Iacono O, Fernandez-Bermejo M, Majano P, Apolinario A, Larranaga E, Moreno-Otero R: Characterization of pathogenic and prognostic factors of nonalcoholic steatohepatitis associated with obesity. *J Hepatol* 33:716–724, 2000
14. Day CP: Non-alcoholic steatohepatitis (NASH): where are we now and where are we going? *Gut* 50:585–588, 2002
15. Alba LM, Lindor K: Non-alcoholic fatty liver disease (Review Article). *Aliment Pharmacol Ther* 17:977–986, 2003
16. Angulo P, Keach J, Batts K, Lindor K: Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. *Hepatology* 30:1356–1362, 1999
17. Brunt EM, Janney C, Bisceglie AD, Neuschwander-Tetri B, Bacon B: Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 94:2467–2474, 1999
18. Day CP, James O: Steatohepatitis: a tale of two "hits"? *Gastroenterology* 114:842–845, 1998
19. Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, McCullough A, Natale S, Forlani G, Melchionda N: Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes* 50:1844–1850, 2001
20. Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, Natale S, Vanni E, Villanova N, Melchionda N, Rizzetto M: Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* 37:917–923, 2003
21. Matteoni CA, Younossi Z, Gramlich T, Boparai N, Liu Y, McCullough A: Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology* 116:1413–1419, 1999
22. Savage DB, Tan G, Acerini C, Jebb S, Agostini M, Gurnell M, Williams R, Umpleby A, Thomas E, Bell J, Dixon A, Dunne F, Boiani R, Cinti S, Vidal-Puig A, Karpe F, Chatterjee V, O'Rahilly S: Human metabolic syndrome resulting from dominant-negative mutations in the nuclear receptor peroxisome proliferator-activated receptor-γ. *Diabetes* 52:910–917, 2003
23. Reynet C, Kahn C: Rad: a member of the Ras family overexpressed in muscle of type II diabetic humans. *Science* 262: 1441–1444, 1993
24. Maddux BA, Sbraccia P, Kumakura S, Sasson S, Youngren J, Fisher A, Spencer S, Grupe A, Henzel W, Stewart T, et al.: Membrane glycoprotein PC-1 and insulin resistance in non-insulin-dependent diabetes mellitus. *Nature* 373:448–451, 1995
25. Yuan M, Konstantopoulos N, Lee J, Hansen L, Li Z, Karin M, Shoelson S: Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. *Science* 293: 1673–1677, 2001
26. Uysal KT, Wiesbrock S, Marino M, Hotamisligil G: Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. *Nature* 389: 610–614, 1997
27. Crespo J, Cayon A, Fernandez-Gil P, Hernandez-Guerra M, Mayorga M, Dominguez-Diez A, Fernandez-Escalante J, Pons-Romero F: Gene expression of tumor necrosis factor alpha and TNF-receptors, p55 and p75, in nonalcoholic steatohepatitis patients. *Hepatology* 34: 1158–1163, 2001
28. Mezey E: Dietary fat and alcoholic liver disease. *Hepatology* 28:901–905, 1998
29. Musso G, Gambino R, Michieli FD, Cassader M, Rizzetto M, Durazzo M, Faga E, Silli B, Pagano G: Dietary habits and their relations to insulin resistance and postprandial lipemia in nonalcoholic steatohepatitis. *Hepatology* 37:909–916, 2003
30. Jensen MD, Haymond M, Rizza R, Cryer P, Miles J: Influence of body fat distribution on free fatty acid metabolism in obesity. *J Clin Invest* 83:1168–1173, 1989
31. Arner P: Not all fat is alike. *Lancet* 351: 1301–1302, 1998
32. Powell EE, Searle J, Mortimer R: Steatohepatitis associated with limb lipodystrophy. *Gastroenterology* 97:1022–1024, 1989
33. Friedman JM, Halaas J: Leptin and the regulation of body weight in mammals. *Nature* 395:763–770, 1998
34. Lee Y, Wang M, Kakuma T, Wang Z, Babcock E, McCorkle K, Higa M, Zhou Y, Unger R: Liporegulation in diet-induced obesity: the antisteatotic role of hyperleptinemia. *J Biol Chem* 276:5629–5635, 2001
35. Uygun A, Kadayifci A, Yesilova Z, Erdil A, Yaman H, Saka M, Deveci M, Bagci S, Gulsen M, Karaeren N, Dagalp K: Serum leptin levels in patients with nonalcoholic steatohepatitis. *Am J Gastroenterol* 95:3584–3589, 2000
36. Chitturi S, Farrell G, Frost L, Kriketos A, Lin R, Fung C, Liddle C, Samarasinghe D, George J: Serum leptin in NASH correlates with hepatic steatosis but not fibrosis: a manifestation of lipotoxicity? *Hepatology* 36:403–409, 2002
37. Cohen B, Novick D, Rubinstein M: Modulation of insulin activities by leptin. *Science* 274:1185–1188, 1996
38. Chalasani N, Crabb D, Cummings O, Kwo P, Asghar A, Pandya P, Considine R: Does leptin play a role in the pathogenesis of human nonalcoholic steato-

hepatitis? *Am J Gastroenterol* 98:2771–2776, 2003
39. Galli A, Pinaire J, Fischer M, Dorris R, Crabb D: The transcriptional and DNA binding activity of peroxisome proliferator-activated receptor alpha is inhibited by ethanol metabolism: a novel mechanism for the development of ethanol-induced fatty liver. *J Biol Chem* 276:68–75, 2001
40. Berson A, Beco VD, Letteron P, Robin M, Moreau C, Kahwaji JE, Verthier N, Feldmann G, Fromenty B, Pessayre D: Steatohepatitis-inducing drugs cause mitochondrial dysfunction and lipid peroxidation in rat hepatocytes. *Gastroenterology* 114:764–774, 1998
41. Kaplan LM: Leptin, obesity, and liver disease. *Gastroenterology* 115:997–1001, 1998
42. Charlton M, Sreekumar R, Rasmussen D, Lindor K, Nair K: Apolipoprotein synthesis in nonalcoholic steatohepatitis. *Hepatology* 35:898–904, 2002
43. Cassader M, Gambino R, Musso G, Depetris N, Mecca F, Cavallo-Perin P, Pacini G, Rizzetto M, Pagano G: Postprandial triglyceride-rich lipoprotein metabolism and insulin sensitivity in nonalcoholic steatohepatitis patients. *Lipids* 36:1117–1124, 2001
44. Clarke SD: Nonalcoholic steatosis and steatohepatitis. I. Molecular mechanism for polyunsaturated fatty acid regulation of gene transcription. *Am J Physiol Gastrointest Liver Physiol* 281:G865–G869, 2001
45. Unger RH, Orci L: Lipoapoptosis: its mechanism and its diseases. *Biochim Biophys Acta* 1585:202–212, 2002
46. Rashid A, Wu T, Huang C, Chen C, Lin H, Yang S, Lee F, Diehl A: Mitochondrial proteins that regulate apoptosis and necrosis are induced in mouse fatty liver. *Hepatology* 29:1131–1138, 1999
47. Bugianesi E, Manzini P, D'Antico S, Vanni E, Longo F, Leone N, Massarenti P, Piga A, Marchesini G, Rizzetto M: Relative contribution of iron burden, HFE mutations, and insulin resistance to fibrosis in nonalcoholic fatty liver. *Hepatology* 39:179–187, 2004
48. Pessayre D, Berson A, Fromenty B, Mansouri A: Mitochondria in steatohepatitis. *Semin Liver Dis* 21:57–69, 2001
49. Pessayre D, Mansouri A, Fromenty B: Nonalcoholic steatosis and steatohepatitis. V. Mitochondrial dysfunction in steatohepatitis. *Am J Physiol Gastrointest Liver Physiol* 282:G193–G199, 2002
50. Reddy JK: Nonalcoholic steatosis and steatohepatitis. III. Peroxisomal beta-oxidation, PPAR alpha, and steatohepatitis. *Am J Physiol Gastrointest Liver Physiol* 281:G1333–G1339, 2001
51. Hruszkewycz AM: Evidence for mitochondrial DNA damage by lipid peroxidation. *Biochem Biophys Res Commun* 153:191–197, 1988
52. Leclercq IA, Farrell G, Field J, Bell D, Gonzalez F, Robertson G: CYP2E1 and CYP4A as microsomal catalysts of lipid peroxides in murine nonalcoholic steatohepatitis. *J Clin Invest* 105:1067–1075, 2000
53. Chalasani N, Gorski J, Asghar M, Asghar A, Foresman B, Hall S, Crabb D: Hepatic cytochrome P450 2E1 activity in nondiabetic patients with nonalcoholic steatohepatitis. *Hepatology* 37:544–550, 2003
54. Robertson G, Leclercq I, Farrell G: Nonalcoholic steatosis and steatohepatitis. II. Cytochrome P-450 enzymes and oxidative stress. *Am J Physiol Gastrointest Liver Physiol* 281:G1135–G1139, 2001
55. Weltman MD, Farrell G, Hall P, Ingelman-Sundberg M, Liddle C: Hepatic cytochrome P450 2E1 is increased in patients with nonalcoholic steatohepatitis. *Hepatology* 27:128–133, 1998
56. Chavin KD, Yang S, Lin H, Chatham J, Chacko V, Hoek J, Walajtys-Rode E, Rashid A, Chen C, Huang C, Wu T, Lane M, Diehl A: Obesity induces expression of uncoupling protein-2 in hepatocytes and promotes liver ATP depletion. *J Biol Chem* 274:5692–5700, 1999
57. Echtay KS, Roussel D, St-Pierre J, Jekabsons M, Cadenas S, Stuart J, Harper J, Roebuck S, Morrison A, Pickering S, Clapham J, Brand M: Superoxide activates mitochondrial uncoupling proteins. *Nature* 415:96–99, 2002
58. Cortez-Pinto H, Chatham J, Chacko V, Arnold C, Rashid A, Diehl A: Alterations in liver ATP homeostasis in human nonalcoholic steatohepatitis: a pilot study. *JAMA* 282:1659–1664, 1999
59. Baffy G, Zhang C, Glickman J, Lowell B: Obesity-related fatty liver is unchanged in mice deficient for mitochondrial uncoupling protein 2. *Hepatology* 35:753–761, 2002
60. Zeviani M, Tiranti V, Piantadosi C: Mitochondrial disorders. *Medicine (Baltimore)* 77:59–72, 1998
61. Kern PA, Saghizadeh M, Ong J, Bosch R, Deem R, Simsolo R: The expression of tumor necrosis factor in human adipose tissue: regulation by obesity, weight loss, and relationship to lipoprotein lipase. *J Clin Invest* 95:2111–2119, 1995
62. Tilg H, Diehl A: Cytokines in alcoholic and nonalcoholic steatohepatitis. *N Engl J Med* 343:1467–1476, 2000
63. Shepherd PR, Kahn B: Glucose transporters and insulin action: implications for insulin resistance and diabetes mellitus. *N Engl J Med* 341:248–257, 1999
64. Bernard S, Touzet S, Personne I, Lapras V, Bondon P, Berthezene F, Moulin P: Association between microsomal triglyceride transfer protein gene polymorphism and the biological features of liver steatosis in patients with type II diabetes. *Diabetologia* 43:995–999, 2000
65. Masuzaki H, Paterson J, Shinyama H, Morton N, Mullins J, Seckl J, Flier J: A transgenic model of visceral obesity and the metabolic syndrome. *Science* 294:2166–2170, 2001
66. Barsh GS, Farooqi I, O'Rahilly S: Genetics of body-weight regulation. *Nature* 404:644–651, 2000
67. Bonkovsky HL, Jawaid Q, Tortorelli K, LeClair P, Cobb J, Lambrecht R, Banner B: Non-alcoholic steatohepatitis and iron: increased prevalence of mutations of the HFE gene in non-alcoholic steatohepatitis. *J Hepatol* 31:421–429, 1999
68. Chitturi S, Weltman M, Farrell G, McDonald D, Kench J, Liddle C, Samarasinghe D, Lin R, Abeygunasekera S, George J: HFE mutations, hepatic iron, and fibrosis: ethnic-specific association of NASH with C282Y but not with fibrotic severity. *Hepatology* 36:142–149, 2002
69. George DK, Goldwurm S, MacDonald G, Cowley L, Walker N, Ward P, Jazwinska E, Powell L: Increased hepatic iron concentration in nonalcoholic steatohepatitis is associated with increased fibrosis. *Gastroenterology* 114:311–318, 1998
70. Grove J, Daly A, Bassendine M, Day C: Association of a tumor necrosis factor promoter polymorphism with susceptibility to alcoholic steatohepatitis. *Hepatology* 26:143–146, 1997
71. Grove J, Daly A, Bassendine M, Gilvarry E, Day C: Interleukin 10 promoter region polymorphisms and susceptibility to advanced alcoholic liver disease. *Gut* 46:540–545, 2000
72. Mendler MH, Turlin B, Moirand R, Jouanolle A, Sapey T, Guyader D, Gall JL, Brissot P, David V, Deugnier Y: Insulin resistance-associated hepatic iron overload. *Gastroenterology* 117:1155–1163, 1999
73. Moirand R, Mortaji A, Loreal O, Paillard F, Brissot P, Deugnier Y: A new syndrome of liver iron overload with normal transferrin saturation. *Lancet* 349:95–97, 1997
74. Chitturi S, George J: Interaction of iron, insulin resistance, and nonalcoholic steatohepatitis. *Curr Gastroenterol Rep* 5:18–25, 2003
75. Fargion S, Mattioli M, Fracanzani A, Sampietro M, Tavazzi D, Fociani P, Taioli E, Valenti L, Fiorelli G: Hyperferritinemia, iron overload, and multiple metabolic alterations identify patients at risk for nonalcoholic steatohepatitis. *Am J Gastroenterol* 96:2448–2455, 2001
76. Ludwig J, Hashimoto E, Porayko M, Moyer T, Baldus W: Hemosiderosis in

cirrhosis: a study of 447 native livers. *Gastroenterology* 112:882–888, 1997
77. Younossi ZM, Gramlich T, Bacon B, Matteoni C, Boparai N, O'Neill R, McCullough A: Hepatic iron and nonalcoholic fatty liver disease. *Hepatology* 30:847–850, 1999
78. Paradis V, Perlemuter G, Bonvoust F, Dargere D, Parfait B, Vidaud M, Conti M, Huet S, Ba N, Buffet C, Bedossa P: High glucose and hyperinsulinemia stimulate connective tissue growth factor expression: a potential mechanism involved in progression to fibrosis in nonalcoholic steatohepatitis. *Hepatology* 34:738–744, 2001
79. Saxena NK, Ikeda K, Rockey D, Friedman S, Anania F: Leptin in hepatic fibrosis: evidence for increased collagen production in stellate cells and lean littermates of ob/ob mice. *Hepatology* 35: 762–771, 2002
80. Honda H, Ikejima K, Hirose M, Yoshikawa M, Lang T, Enomoto N, Kitamura T, Takei Y, Sato N: Leptin is required for fibrogenic responses induced by thioacetamide in the murine liver. *Hepatology* 36:12–21, 2002
81. Solga SF, Diehl A: Non-alcoholic fatty liver disease: lumen-liver interactions and possible role for probiotics. *J Hepatol* 38:681–687, 2003
82. Wigg AJ, Roberts-Thomson I, Dymock R, McCarthy P, Grose R, Cummins A: The role of small intestinal bacterial overgrowth, intestinal permeability, endotoxaemia, and tumour necrosis factor alpha in the pathogenesis of non-alcoholic steatohepatitis. *Gut* 48:206–211, 2001
83. Drenick EJ, Fisler J, Johnson D: Hepatic steatosis after intestinal bypass: prevention and reversal by metronidazole, irrespective of protein-calorie malnutrition. *Gastroenterology* 82:535–548, 1982
84. Adachi Y, Moore L, Bradford B, Gao W, Thurman R: Antibiotics prevent liver injury in rats following long-term exposure to ethanol. *Gastroenterology* 108: 218–224, 1995
85. Yin M, Wheeler M, Kono H, Bradford B, Gallucci R, Luster M, Thurman R: Essential role of tumor necrosis factor alpha in alcohol-induced liver injury in mice. *Gastroenterology* 117:942–952, 1999
86. Chitturi S, Farrell G: Etiopathogenesis of nonalcoholic steatohepatitis. *Semin Liver Dis* 21:27–41, 2001
87. Yang SQ, Lin H, Lane M, Clemens M, Diehl A: Obesity increases sensitivity to endotoxin liver injury: implications for the pathogenesis of steatohepatitis. *Proc Natl Acad Sci U S A* 94:2557–2562, 1997
88. Loffreda S, Yang S, Lin H, Karp C, Brengman M, Wang D, Klein A, Bulkley G, Bao C, Noble P, Lane M, Diehl A: Leptin regulates proinflammatory immune responses. *FASEB J* 12:57–65, 1998
89. Li Z, Lin H, Yang S, Diehl A: Murine leptin deficiency alters Kupffer cell production of cytokines that regulate the innate immune system. *Gastroenterology* 123:1304–1310, 2002
90. Nair S, Cope K, Risby T, Diehl A, Terence R: Obesity and female gender increase breath ethanol concentration: potential implications for the pathogenesis of nonalcoholic steatohepatitis. *Am J Gastroenterol* 96:1200–1204, 2001
91. Koteish A, Diehl A: Animal models of steatosis. *Semin Liver Dis* 21:89–104, 2001
92. Cope K, Risby T, Diehl A: Increased gastrointestinal ethanol production in obese mice: implications for fatty liver disease pathogenesis. *Gastroenterology* 119:1340–1347, 2000
93. Poullis A, Mendall M: Alcohol, obesity, and TNF-alpha. *Gut* 49:313–314, 2001
94. Kugelmas M, Hill D, Vivian B, Marsano L, McClain C: Cytokines and NASH: a pilot study of the effects of lifestyle modification and vitamin E. *Hepatology* 38: 413–419, 2003
95. Ueno T, Sugawara H, Sujaku K, Hashimoto O, Tsuji R, Tamaki S, Torimura T, Inuzuka S, Sata M, Tanikawa K: Therapeutic effects of restricted diet and exercise in obese patients with fatty liver. *J Hepatol* 27:103–107, 1997
96. Palmer M, Schaffner F: Effect of weight reduction on hepatic abnormalities in overweight patients. *Gastroenterology* 99:1408–1413, 1990
97. Drenick EJ, Simmons F, Murphy J: Effect on hepatic morphology of treatment of obesity by fasting, reducing diets and small-bowel bypass. *N Engl J Med* 282: 829–834, 1970
98. Neuschwander-Tetri BA, Bacon E: Nonalcoholic steatohepatitis. *Med Clin North Am* 80:1147–1166, 1996
99. Laurin J, Lindor K, Crippin J, Gossard A, Gores G, Ludwig J, Rakela J, McGill D: Ursodeoxycholic acid or clofibrate in the treatment of non-alcohol-induced steatohepatitis: a pilot study. *Hepatology* 23:1464–1467, 1996
100 Basaranoglu M, Acbay O, Sonsuz A: A controlled trial of gemfibrozil in the treatment of patients with nonalcoholic steatohepatitis. *J Hepatol* 31:384, 1999
101. Saibara T, Onishi S, Ogawa Y, Yoshida S, Enzan H: Bezafibrate for tamoxifen-induced non-alcoholic steatohepatitis. *Lancet* 353:1802, 1999
102. Harrison SA, Ramrakhiari S, Brunt E, Anbari M, Cortese C, Bacon B: Orlistat in the treatment of NASH: a case series. *Am J Gastroenterol* 98:926–930, 2003
103. Li Z, Yang S, Lin H, Huang J, Watkins P, Moser A, Desimone C, Song X, Diehl A: Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease. *Hepatology* 37:343–350, 2003
104. Sheth SG, Gordon F, Chopra S: Nonalcoholic steatohepatitis. *Ann Intern Med* 126:137–145, 1997
105. Ludwig J, McGill D, Lindor K: Review: nonalcoholic steatohepatitis. *J Gastroenterol Hepatol* 12:398–403, 1997
106. Loguercio C, Simone TD, Federico A, Terracciano F, Tuccillo C, Chicco MD, Carteni M: Gut-liver axis: a new point of attack to treat chronic liver damage? *Am J Gastroenterol* 97:2144–2146, 2002
107. Lindor KD, Kowdley K, Heathcote E, Harrison M, Jorgensen R, Angulo P, Lymp J, Burgart L, Colin P: Ursodeoxycholic acid for treatment of nonalcoholic steatohepatitis: results of a randomized trial. *Hepatology* 39:770–778, 2004
108. Osman E, Owen J, Burroughs A: S-adenosyl-L-methionine: a new therapeutic agent in liver disease? (Review Article) *Aliment Pharmacol Ther* 7:21–28, 1993
109. Hasegawa T, Yoneda M, Nakamura K, Makino I, Terano A: Plasma transforming growth factor-beta1 level and efficacy of alpha-tocopherol in patients with non-alcoholic steatohepatitis: a pilot study. *Aliment Pharmacol Ther* 15: 1667–1672, 2001
110. Caballeria J, Pares A, Bru C, Mercader J, Garcia-Plaza A, Caballeria L, Clemente G, Rodrigo L, Rodes J: Metadoxine accelerates fatty liver recovery in alcoholic patients: results of a randomized double-blind, placebo-control trial: Spanish Group for the Study of Alcoholic Fatty Liver. *J Hepatol* 28:54–60, 1998
111. Flora K, Hahn M, Rosen H, Benner K: Milk thistle (Silybum marianum) for the therapy of liver disease. *Am J Gastroenterol* 93:139–143, 1998
112. Abdelmalek MF, Angulo P, Jorgensen R, Sylvetre P, Lindor K: Betaine, a promising new agent for patients with nonalcoholic steatohepatitis: results of a pilot study. *Am J Gastroenterol* 96:2711–2717, 2001
113. Bugranesi E, Marchesini G, Pagotto U: Ghrelin in non-alcoholic fatty liver disease (Abstract). *J Hepatol* 38:A-3620, 2003
114. Satapathy SK, Garg S, Sakhuja P, Malhatra V, Sorin S: Pentoxyphylline as a novel therapy for non-alcoholic steatohepatitis: a pilot study (Abstract). *Gastroenterology* 124:A-728, 2003
115. Caldwell SH, Hespenheide E, Redick J, Iezzoni J, Battle E, Sheppard B: A pilot study of a thiazolidinedione, troglitazone, in nonalcoholic steatohepatitis. *Am J Gastroenterol* 96:519–525, 2001
116. Neuschwander-Tetri BA, Brunt E, Wehmeier K, Oliver D, Bacon B: Improved nonalcoholic steatohepatitis after 48

weeks of treatment with the PPAR-gamma ligand rosiglitazone. *Hepatology* 38:1008–1017, 2003
117. Neuschwander-Tetri BA, Brunt E, Wehmeier K, Sponseller C, Hampton K, Bacon B: Interim results of a pilot study demonstrating the early effects of the PPAR-gamma ligand rosiglitazone on insulin sensitivity, aminotransferases, hepatic steatosis and body weight in patients with non-alcoholic steatohepatitis. *J Hepatol* 38:434–440, 2003
118. Promrat K, Lutchman G, Uwaifo G, Freedman R, Soza A, Heller T, Doo E, Ghany M, Premkumar A, Park Y, Liang T, Janovski J, Kleiner D, Hoofnagle J: A pilot study of pioglitazone treatment for nonalcoholic steatohepatitis. *Hepatology* 39:188–196, 2004
119. Kirpichnikov D, McFarlane S, Sowers J: Metformin: an update. *Ann Intern Med* 137:25–33, 2002
120. Lin HZ, Yang S, Chuckaree C, Kuhajda F, Ronnet G, Diehl A: Metformin reverses fatty liver disease in obese, leptin-deficient mice. *Nat Med* 6:998–1003, 2000
121. Marchesini G, Brizi M, Bianchi G, Tomasetti S, Zoli M, Melchionda N: Metformin in non-alcoholic steatohepatitis. *Lancet* 358:893–894, 2001
122. Uygun A, Kadayifci A, Isik A, Ozgurtas T, Deveci S, Tuzun A, Yesilova Z, Gulsen M, Dagalp K: Metformin in the treatment of patients with non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 19: 537–544, 2004
123. D'Souza-Gburek SM, Batts K, Nikias G, Wiesner R, Krom R: Liver transplantation for jejunoileal bypass-associated cirrhosis: allograft histology in the setting of an intact bypassed limb. *Liver Transpl Surg* 3:23–27, 1997