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REVIEW

Nonalcoholic steatohepatitis

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ABSTRACT

Nonalcoholic steatohepatitis (NASH) is an underdiagnosed liver disease characterised by steatosis, necroinflammation and fibrosis. This disease may eventually develop into cirrhosis and hepatocellular carcinoma. NASH is highly prevalent among obese individuals and among patients with diabetes mellitus type 2. Nonalcoholic fatty liver (NAFL), a precursor of NASH, is the main cause of elevated serum liver enzymes among the general population. Insulin resistance is a major aetiological factor in NASH. Gradual weight loss, physical exercise and drugs that improve insulin sensitivity are potential therapies.

A recent survey showed that 8% of an American population has elevated serum aminotransferase levels.[1] Most of these persons have nonalcoholic fatty liver (NAFL). NAFL is characterised by steatosis without inflammation or fibrosis. It is a benign and reversible condition. However, in 20% of cases, liver histology shows necroinflammation and some degree of pericellular fibrosis. This is called nonalcoholic steatohepatitis (NASH). NAFL can be considered as a precursor lesion of NASH. NASH progresses to cirrhosis in 20% of cases.[2] To complete the terminology, both NAFL and NASH are nonalcoholic fatty liver diseases (NAFLD).

NAFL is highly prevalent among individuals with mild (body mass index BMI 25-30 kg/m^2) or severe central obesity (BMI >30 kg/m^2), among patients with diabetes mellitus type 2 or dyslipidaemia.[3,4] In Western societies, including the Netherlands, obesity is one of the most common abnormal conditions.[5] Likewise NAFL/NASH is the most frequent liver disease and its incidence will increase. Asymptomatic individuals with some degree of hepatomegaly may have NAFL[6] but even without any signs or symptoms, the liver may contain too much fat. This became apparent in the screening of potential donors for living-related liver transplantation wherein histological examination of a liver biopsy is a standard procedure. Completely asymptomatic persons may have a steatotic liver and have to be excluded from liver donation. Steatotic livers show a high degree of initial poor function and have an impaired capacity to regenerate.[7,8] Steatosis is also more common than hitherto realised in the elderly.[9]

The transition of NAFL to NASH is not clearly demarcated. In both conditions liver enzymes may be elevated and the liver fat content, as diagnosed by ultrasonography, may be equal. Inflammation and fibrosis are features of NASH. Thus, for the differentiation of NAFL and NASH, a liver biopsy is needed.[10] NASH is a major cause of so-called cryptogenic liver cirrhosis. The diagnosis of NASH is missed in these patients because the steatosis often disappears once cirrhosis develops.[2,11] The prognosis of NASH-related cirrhosis was worse than that of hepatitis C-related cirrhosis in one study (three-year survival 50 *vs* 70%),[12,13] but showed no difference in another study.[13] Both conditions may give rise to hepatocellular carcinoma[2,14] and both may recur after liver transplantation.[15,16]

PATHOLOGY

The pathology of NASH was first described by Ludwig.[17] NASH is characterised by macrovesicular steatosis, hepato-

cellular ballooning and mild lobular inflammation with scattered polymorphonuclear leucocytes and monocytes (*table 1*).[18] Perisinusoidal or pericellular (chickenwire) fibrosis is present, in particular in acinar zone 3. Mallory bodies are occasionally seen but are not necessary for the diagnosis. Liver histopathology in alcoholic liver disease and NASH is very similar. Cholestasis is not a feature of NASH but often occurs in alcoholic liver disease. Likewise central-central and central-portal bridging necrosis frequently occurs in alcoholic liver disease but is uncommon in NASH.[19] Macrovesicular steatosis is a common feature of a variety of liver diseases including hepatitis C, Wilson's disease, alcoholic liver disease, primary biliary cirrhosis and toxic liver injury (*table 2*). All features of NASH may be present in hepatitis C but then the inflammation and fibrosis are mainly localised in the portal or periportal areas.[18] Ballooning degeneration, Mallory's hyaline and fibrosis are discriminating features suggestive of progressive NASH.[20]

Table 1
Histopathology of NASH

• Necessary components - Steatosis (macro > micro) - Hepatocellular ballooning* - Mixed mild lobular inflammation
• Usually present - Zone 3 perisinusoidal/pericellular fibrosis* - Zone 1 hepatocellular glycogenated nuclei - Small lipogranulomas, acidophil bodies, PAS-positive Kupffer cells
• Maybe present - Mallory's hyaline* - Hepatocellular iron granules in zone 1 - Megamitochondria
• Unusual for NASH - Predominantly microvesicular steatosis - Sclerosing hyaline necrosis - Veno-occlusive lesions, phlebosclerosis, perivenular fibrosis - Portal changes - Acute/chronic cholestasis

*Adapted from: EM Brunt, Pathologic spectrum of fatty liver disease. In: Liver disease in the 21st century. American Association for the Study of Liver Disease 2003. * Dicriminant features with prognostic significance.*[20]

ASSOCIATIONS

NASH is associated with central obesity, diabetes type 2, syndrome X (obesity, dyslipidaemia, insulin resistance and hypertension), polycystic ovary syndrome and hyper-triglyceridaemia.[21] Almost all patients with NASH are insulin resistant. It is clear that insulin resistance must play a key role in the pathogenesis of NASH.[22,23] However, insulin-resistance, hyperinsulinaemia and hyperglycaemia are not limited to NASH but also occur in other liver diseases such as hepatitis C.[24,25] In contrast to alcoholic liver disease, liver pathology in patients with NASH does not disappear upon complete abstinence. As for obesity, a person does not have to be morbidly obese to have NASH. In moderately overweight patients with elevated serum liver enzymes (BMI >25 kg/m^2), NASH with septal fibrosis was present in 30% and cirrhosis in 11%.[26] NASH is related to diabetes mellitus type 2 but its true incidence among these patients is unknown.[27] NASH is increasingly recognised among obese children.[28]

DIAGNOSIS

NASH is an underdiagnosed disease. For its diagnosis a liver biopsy is required. This undoubtedly constitutes a threshold for its detection among obese and diabetic popu-

Table 2
Liver diseases with steatosis as an important component

MACROVESICULAR AND MIXED MACRO/MICROVESICULAR STEATOSIS
• Alcoholic liver disease
• NASH
• Drugs and toxins - Tamoxifen, methotrexate, nifedipine, coralgil, tetracyline - Phospholipidosis: smiodarone, perhexiline - Petrochemicals (solvents), dimethylformamide - Cocaine
• Viral hepatitis, hepatitis C
• Inherited disorders - Abetalipoproteinaemia - Familial hypobetalipoproteinaemia
• Nutritional disorders - Obesity - Total parenteral nutrition - Kwashiorkor (protein-calorie malnutrition) - Celiac disease - Schwachman's syndrome (pancreatic insufficiency with bone marrow suppression) - Bariatric surgery for treatment of obesity (jejunoileal bypass)
• Systemic disorders - Inflammatory bowel disease - Weber-Christian disease - Cystic fibrosis
• Metabolic disorders - Galactosaemia - Tyrosinaemia - Hereditary fructose intolerance - Cystinuria
• Others - Wilson's disease - Hepatic ischaemia
• Bacterial overgrowth
MICROVESICULAR STEATOSIS
- Acute fatty liver of pregnancy
- Reye's syndrome
- Valproic acid
- Nucleoside analogues

Adapted from: EM Brunt, Pathologic spectrum of fatty liver disease. In: Liver disease in the 21st century. American Association for the Study of Liver Disease 2003.

lations. Indeed a liver biopsy in obese people carries an increased risk. Moreover, a liver biopsy is too invasive for screening purposes. Ultrasonography misses about one third of cases.[29,30] Most patients with NASH have elevated serum aminotransferases and many have some degree of hepatomegaly. However, normal liver enzymes and a normal liver size do not exclude the diagnosis. Elevated liver enzymes in target groups, such as obese persons, patients with diabetes mellitus type 2 and patients on total parenteral nutrition, should raise the level of suspicion and this may lead to a more frequent diagnosis of NASH. It is clear, however, that new diagnostic markers or noninvasive procedures to detect and quantify liver fat are needed. Magnetic resonance proton spectroscopy of the liver may be such a procedure.[31,32]

PATHOGENESIS

In the pathogenesis of NASH two steps or 'hits' can be recognised (*figure 1*). Fat accumulation in the liver is the *sine qua non*, the 'first hit'. Fat *per se* is not toxic and fat accumulation is to a certain degree a physiological response. In many species fat in the liver constitutes a rapidly mobilisable source of energy. For instance, migratory birds increase their liver (and body) weight considerably before migration. During the long migratory flights, triglycerides are hydrolysed and free fatty acids (FFA) are used as energy source for muscle action. However, fat in the liver is not innocuous. Fat makes the liver vulnerable to endotoxins and ischaemic reperfusion damage and fat impairs liver regeneration.[33-36] Fat in the liver causes hepatic insulin resistance.[37] Activation of a serine kinase cascade that leads to a defect of insulin signalling may be the underlying mechanism.[38] Furthermore, insulin-mediated activation of sterol regulatory element-binding protein 1 (SREBP-1) stimulates lipogenic enzymes in and outside the liver.[39] Meanwhile apolipoprotein synthesis in the liver is impaired and this leads to a reduced production of very-low-density lipoprotein (VLDL), a lipoprotein that constitutes a rate-determining step in hepatic lipid export.[40] Despite the active oxidation of FFA, influx and neosynthesis outweigh FFA degradation and secretion, the net effect being hepatic fat accumulation.

Reactive oxygen species (ROS) and inflammation represent the 'second hit'. FFAs can in fact deliver both hits. When in abundance as in over-nutrition, FFAs in the liver contribute to the synthesis of triglycerides and the hepatic accumulation of fat. In addition, the oxidation of FFAs in mitochondria and peroxisomes contributes to ROS generation.[41] FFAs are ligands for the peroxisomal proliferator-activated receptor α, PPARα. This stimulates mitochondrial and peroxisomal β-oxidation as well as the expression of several cytochrome P450s, in particular Cyp2E1 and Cyp4A.[42-45] Cyp2E1 and CYP4A are elevated in patients with NASH.[46,47] This shows that in these patients PPARα is indeed activated. PPARα is a master-regulator of FFA metabolism in the liver. PPARα-knockout mice on a high-fat diet develop severe hepatic steatosis. In contrast, activation of PPARα in mice on a high-fat diet prevents triglyceride accumulation.[48] Oxidation of FFAs helps to clear the fat and the massive amounts of ROS produced under these conditions does not seem to harm the mouse liver. Thus, although ROS production and oxidative stress may set the scene for development of NASH, inflammation is a major additional factor in the transition of NAFL to NASH. FFAs may also play a key role here. FFAs directly stimulate IκB/NFκB, factors involved in the inflammation cascade.[49,50] Moreover, adipose tissue expresses TNFα, interleukin 6 and inducable nitric oxide synthase (iNOS).[51] Therefore obesity is a pro-inflammatory condition and likewise in obese persons the liver is exposed to cytokines produced in their adipose tissues. Moreover, TNFα and interleukin 1 and 6 reduce the activity of Jun N-terminal kinase and this inactivates insulin receptor substrate with insulin resistance as a consequence.[52] Thus insulin resistance is both cause and consequence of NASH.

Whether intestinal dysmotility and bacterial overgrowth also contribute to NASH is controversial. Bacterial overgrowth of the small intestine has been reported in NASH and conditions with bacterial overgrowth such as jejunoileal bypass cause NASH.[53] It can be hypothesised that lipohilic endotoxins from the gut may accumulate in the fat of hepatocytes from where they are slowly but continuously released. This stimulates Kupffer cells to produce TNFα, interleukin 1 and 6 and the profibrotic cytokine TGFβ. TGFβ stimulates the transformation of hepatic stellate cells into collagen-producing myofibroblasts. In addition, chemokines are produced that attract monocytes and neutrophils which greatly contribute to the perpetuation of oxidative stress and cell injury.[54-56]

Adiponectin deficiency may be important in the development of NASH. Adiponectin (also known as 30-kDa adipocyte complement-related protein; Acrp30) is a hormone produced by peripheral adipose tissue. It circulates in the blood in a globular form and as a full-length molecule. Liver and muscle have adiponectin receptors. Stimulation of the adipoR2 receptor in the liver leads to the activation of AMP-activated protein kinase and PPARα.[57,58] Thus, adiponectin increases fatty acid β-oxidation thereby decreasing the hepatic triglyceride content and ameliorating insulin resistance. Adiponectin-deficient mice show an increased sensitivity to carbon tetrachloride-induced liver damage. The liver damage in these mice could be significantly attenuated by administration of recombinant adiponectin.[59] Also other studies show that adiponectin has a protective

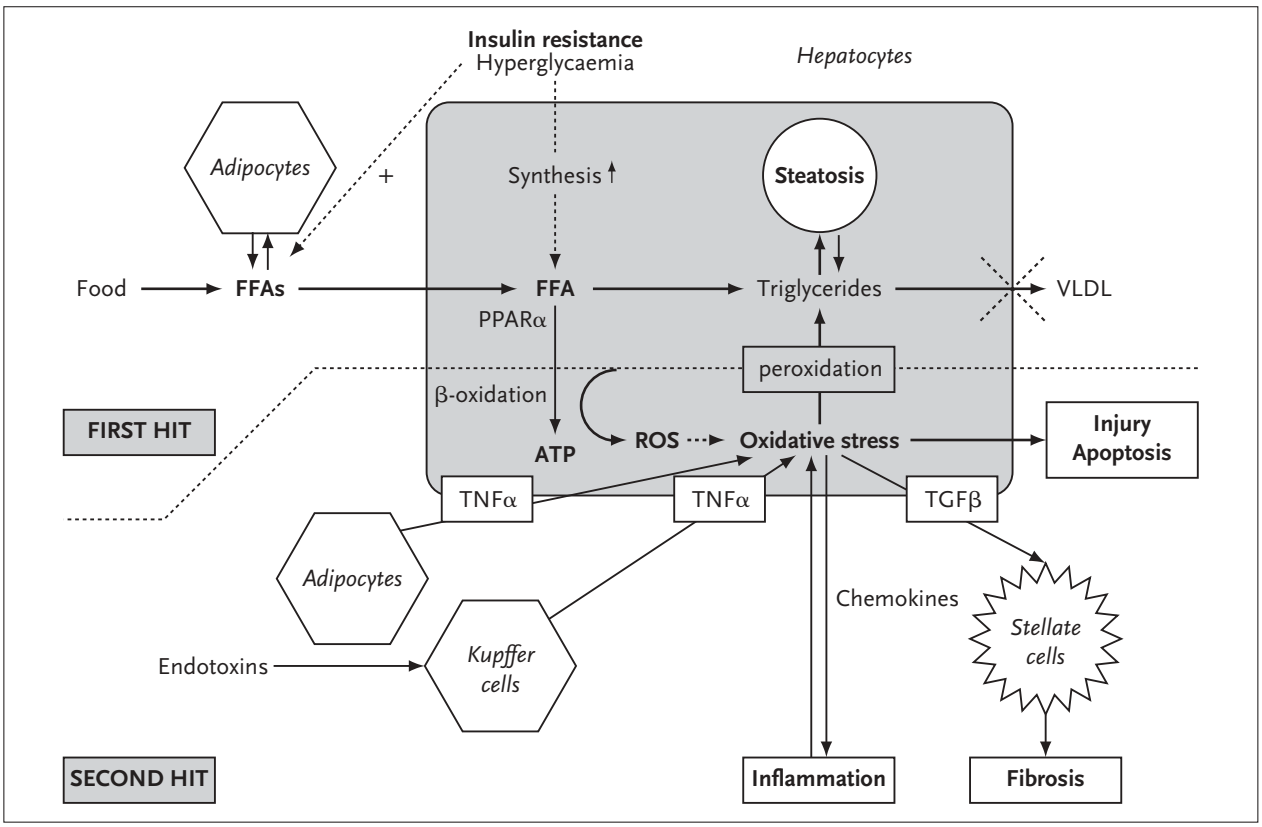

Figure 1

From fat to liver injury

Free fatty acids are taken up in the hepatocytes where they are either metabolised via peroxisomal or mitochondrial β-oxidation or stored as triglycerides. VLDL is the rate-determining step in triglyceride export from the liver. In NASH its synthesis is decreased. The insulin-resistant state favours lipolysis in the adipose tissues, FFA synthesis and lipogenesis in the liver. The net result is hepatic fat accumulation. Continuous and superfluous FFA oxidation causes the generation of ROS in excess with oxidant stress as a result. Cytokines are produced in hepatocytes as well as in Kupffer cells. TGFβ stimulates hepatic stellate cells to produce collagen and cause liver fibrosis, in addition these cells transform into myofibroblasts with more collagen formation and an increase of intrahepatic vascular resistance and portal hypertension. Chemokines attract monocytes and neutrophils with inflammation, more oxidant stress, hepatocellular apoptosis and injury as a result.

effect on the liver where it decreases necrosis and inflammation.[60] In NASH, obesity and type 2 diabetes mellitus, adiponectin production is decreased.[61] In the absence of this protective factor the liver is vulnerable to the action of cytokines and ROS.

THERAPY

Treatment of NASH has to be seen against this pathophysiological background (*table 3*). Lifestyle adjustments should be tried first: weight reduction in combination with exercise is the most rational remedy.[54] Unfortunately compliance in obese people is low and episodic rapid weight loss, followed by binge eating and weight gain, is counterproductive. Thus, diets may often make things worse, in particular when they lead to a yoyo effect on body weight. Programmed exercise would be a rational therapy. It diverts the fatty acids away from the liver to be metabolised in the muscles and it decreases insulin resistance.

Diets with polyunsaturated fatty acids are effective and have a sound physiological background. Polyunsaturated fatty acids stimulate PPARα and repress the sterol regulatory element-binding protein SREBP-1.[62] Indeed, disruption of the *SREBP-1* gene in leptin-deficient ob/ob mice causes a reduction of lipogenesis and clearance of liver fat but did reduce obesity and insulin resistance.[63] Polyunsaturated fatty acid-enriched diets in ob/ob mice reduced both hepatic steatosis and insulin resistance.[64]

Stimulation of PPARα by drugs could be an effective therapy. Gemfibrozil showed a positive effect in a small pilot study.[65] A recent study showed that arachidyl-amidocholanoic acid, a liver-specific agent with presumed PPARα

Table 3
Possible therapies of NASH

THERAPY	EFFICACY
Reduce insulin resistance	
10% weight reduction	Effective[54,77,78]
Orlistat	Case report only[79,79]
Exercise	Effective[54,78]
Low-fat diet	Effective[78]
Metformin	Positive data in obese mice,[68] effective in uncontrolled human study[69]
Thiazolidinediones	Rosiglitazone effective in uncontrolled study[72]
	Troglitazone is hepatotoxic[70,71,80]
	Pioglitazone effective in pilot study[73]
Reduce oxidative stress	
Vitamins	Vitamin E not effective in adults,[54] combination of vitamin E and C reduced
	fibrosis in small randomised study,[67] vitamin E was effective in children in
	an open-label study[81]
Hepatic iron reduction	Unproved[82]
Anti-inflammatory agents	
Antibiotics	Unproved[83]
Probiotics	Unproved[84]
Anti-TNF	Effective in ob/ob mice[85]
Cytoprotection	
Ursodeoxycholic acid	Beneficial in pilot study[86]
Lipotropic agents	
Choline	No reports
Betaine	Effective in pilot study[87]
Hypolipidaemic agents	
Statins	Pitavastatin effective in aromatase-deficient mice,[88] no human trials
Fibrates	Clofibrate not effective,[86] gemfibrozil may be effective (letter only)[65]
Probucol	Effective in small randomised study[89]

agonistic activity, prevents diet-induced fatty livers in rodents.[66] Drug therapy should aim at several levels and it is unlikely that a single drug could do the job. Anti-inflammatory, antioxidative, cytoprotective and lipolytic agents have been tried but given alone none of these have been shown to be very effective. For example, supplementation with vitamin E is ineffective[54] but vitamin E in combination with vitamin C has shown to reduce fibrosis in a small randomised study.[67] Current trials focus on drugs that increase insulin sensitivity. Metformin was successfully tried in an animal model of NASH, the leptin-deficient ob/ob mouse.[68] In an uncontrolled clinical trial metformin improved insulin resistance, decreased aminotransferase levels and reduced hepatomegaly[69] but larger randomised controlled studies are needed to prove its effect. The PPARγ ligand troglitazone also showed benefit in NASH patients but later was implicated in severe liver toxicity.[70,71] Rosiglitazone has been tried with success in a small uncontrolled trial. Twenty-five of 30 patients with NASH completed 48 weeks of treatment. Serum aminotransferase levels and necroinflammatory score, steatosis and fibrosis improved but an undesirable weight gain occurred in 67% of these patients.[72] A recent trial showed that pioglitazone may be effective.[73] The thiazolidinediones have a direct effect on PPARγ in hepatic stellate cells. Activation of PPARγ in hepatic stellate cells retards collagen synthesis and fibrosis both *in vitro* and *in vivo*.[74] Thus, PPARα and PPARγ agonists, as well as drugs that stimulate adiponectin release from adipose tissue, may hold promise for treatment of NASH. Activation of PPARδ to stimulate β-oxidation in muscle may be another way to go.[75] Finally, decompensated NASH-related cirrhosis is an indication for liver transplantation[76] and this condition is rapidly becoming a common indication for liver transplantation.

CONCLUSION

NASH is an underdiagnosed disease. The incidence among patients with diabetes mellitus type 2 and obese people is particularly high. NASH may lead to progressive liver

disease, cirrhosis and hepatocellular carcinoma. It also occurs in obese children. For its unequivocal diagnosis a liver biopsy is necessary. Insulin resistance, steatosis, oxidative stress and inflammation play a major role in its pathogenesis and disease progression. Gradual weight reduction and exercise programmes constitute the most rational therapies but are hard to sustain. Medical therapy aims at amelioration of the insulin-resistant state and for this various drugs are under trial. The role of adiponectin and its possible therapeutic implications needs to be investigated.

REFERENCES

1. Clark JM, Brancati FL, Diehl AM. The prevalence and etiology of elevated aminotransferase levels in the United States. Am J Gastroenterol 2003;98:960-7.
2. Bugianesi E, Leone N, Vanni E, et al. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. Gastroenterology 2002;123:134-40.
3. Marchesini G, Bugianesi E, Forlani G, et al. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. Hepatology 2003;37:917-23.
4. Angulo P, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. Hepatology 1999;30:1356-62.
5. Kemper HC, Stasse-Wolthuis M, Bosman W. The prevention and treatment of overweight and obesity. Summary of the advisory report by the Health Council of the Netherlands. Neth J Med 2004;62:10-7.
6. Mofrad P, Contos MJ, Haque M, et al. Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. Hepatology 2003;37:1286-92.
7. Rull R, Vidal O, Momblan D, et al. Evaluation of potential liver donors: limits imposed by donor variables in liver transplantation. Liver Transpl 2003;9:389-93.
8. Yamauchi H, Uetsuka K, Okada T, Nakayama H, Doi K. Impaired liver regeneration after partial hepatectomy in db/db mice. Exp Toxicol Pathol 2003;54:281-6.
9. Petersen KF, Befroy D, Dufour S, et al. Mitochondrial dysfunction in the elderly: possible role in insulin resistance. Science 2003;300:1140-2.
10. Brunt EM. Nonalcoholic steatohepatitis. Semin Liver Dis 2004;24:3-20.
11. Browning JD, Kumar KS, Saboorian MH, Thiele DL. Ethnic differences in the prevalence of cryptogenic cirrhosis. Am J Gastroenterol 2004;99:292-8.
12. Ratziu V, Bonyhay L, Di M, et al. Survival, liver failure, and hepatocellular carcinoma in obesity-related cryptogenic cirrhosis. Hepatology 2002;35:1485-93.
13. Hui JM, Kench JG, Chitturi S, et al. Long-term outcomes of cirrhosis in nonalcoholic steatohepatitis compared with hepatitis C. Hepatology 2003;38:420-7.14.
14. Shimada M, Hashimoto E, Taniai M, et al. Hepatocellular carcinoma in patients with non-alcoholic steatohepatitis. J Hepatol 2002;37:154-60.
15. Ong J, Younossi ZM, Reddy V, et al. Cryptogenic cirrhosis and post-transplantation nonalcoholic fatty liver disease. Liver Transpl 2001;7:797-801.
16. Contos MJ, Cales W, Sterling RK, et al. Development of nonalcoholic fatty liver disease after orthotopic liver transplantation for cryptogenic cirrhosis. Liver Transpl 2001;7:363-73.
17. Ludwig J, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. Mayo Clin Proc 1980;55:434-8.
18. Brunt EM, Ramrakhiani S, Cordes BG, et al. Concurrence of histologic features of steatohepatitis with other forms of chronic liver disease. Mod Pathol 2003;16:49-56.
19. Ludwig J, McGill DB, Lindor KD. Review: nonalcoholic steatohepatitis. J Gastroenterol Hepatol 1997;12:398-403.
20. Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology 1999;116:1413-9.
21. Marchesini G, Brizi M, Bianchi G, et al. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. Diabetes 2001;50:1844-50.
22. Chitturi S, Abeygunasekera S, Farrell GC, et al. NASH and insulin resistance: Insulin hypersecretion and specific association with the insulin resistance syndrome. Hepatology 2002;35:373-9.
23. Pagano G, Pacini G, Musso G, et al. Nonalcoholic steatohepatitis, insulin resistance, and metabolic syndrome: further evidence for an etiologic association. Hepatology 2002;35:367-72.
24. Ratziu V, Munteanu M, Charlotte F, Bonyhay L, Poynard T. Fibrogenic impact of high serum glucose in chronic hepatitis. C J Hepatol 2003;39:1049-55.
25. Hui JM, Kench J, Farrell GC, et al. Genotype-specific mechanisms for hepatic steatosis in chronic hepatitis C infection. J Gastroenterol Hepatol 2002;17:873-81.
26. Ratziu V, Giral P, Charlotte F, et al. Liver fibrosis in overweight patients. Gastroenterology 2000;118:1117-23.
27. Clark JM, Diehl AM. Hepatic steatosis and type 2 diabetes mellitus. Curr Diab Rep 2002;2:210-5.
28. Roberts EA. Nonalcoholic steatohepatitis in children. Curr Gastroenterol Rep 2003;5:253-9.
29. Saadeh S, Younossi ZM, Remer EM, et al. The utility of radiological imaging in nonalcoholic fatty liver disease. Gastroenterology 2002;123:745-50.
30. Talwalkar JA. Motion - all patients with NASH need to have a liver biopsy: arguments for the motion. Can J Gastroenterol 2002;16:718-21.
31. Tarasow E, Siergiejczyk L, Panasiuk A, et al. MR proton spectroscopy in liver examinations of healthy individuals in vivo. Med Sci Monit 2002;8:MT36-MT40.
32. Longo R, Pollesello P, Ricci C, et al. Proton MR spectroscopy in quantitative in vivo determination of fat content in human liver steatosis. J Magn Reson Imaging 1995;5:281-5.
33. Uesugi T, Froh M, Arteel GE, et al. Role of lipopolysaccharide-binding protein in early alcohol-induced liver injury in mice. J Immunol 2002;168:2963-9.
34. Uesugi T, Froh M, Arteel GE, Bradford BU, Thurman RG. Toll-like receptor 4 is involved in the mechanism of early alcohol-induced liver injury in mice. Hepatology 2001;34:101-8.
35. Ijaz S, Yang W, Winslet MC, Seifalian AM. Impairment of hepatic microcirculation in fatty liver. Microcirculation 2003;10:447-56.
36. Kooby DA, Fong Y, Suriawinata A, et al. Impact of steatosis on perioperative outcome following hepatic resection. J Gastrointest Surg 2003;7:1034-44.

37. Boer M den, Voshol PJ, Kuipers F, Havekes LM, Romijn JA. Hepatic steatosis: a mediator of the metabolic syndrome. Lessons from animal models. Arterioscler Thromb Vasc Biol 004;24:644-9.
38. Kim JK, Fillmore JJ, Chen Y, et al. Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. Proc Natl Acad Sci USA 2001;98:7522-7.
39. Kotzka J, Muller-Wieland D. Sterol regulatory element-binding protein (SREBP)-1: gene regulatory target for insulin resistance? Expert Opin Ther Targets 2004;8:141-9.
40. Charlton M, Sreekumar R, Rasmussen D, Lindor K, Nair KS. Apolipoprotein synthesis in nonalcoholic steatohepatitis. Hepatology 2002;35:898-904.
41. Pessayre D, Mansouri A, Fromenty B. Nonalcoholic steatosis and steatohepatitis. V. Mitochondrial dysfunction in steatohepatitis. Am J Physiol Gastrointest Liver Physiol 2002;282:G193-9.
42. Barrero MJ, Camarero N, Marrero PF, Haro D. Control of human carnitine palmitoyltransferase II gene transcription by peroxisome proliferator-activated receptor through a partially conserved peroxisome proliferator-responsive element. Biochem J 2003;369:721-9.
43. Robertson G, Leclercq I, Farrell GC. Nonalcoholic steatosis and steatohepatitis. II. Cytochrome P-450 enzymes and oxidative stress. Am J Physiol Gastrointest Liver Physiol 2001;281:G1135-9.
44. Emery MG, Fisher JM, Chien JY, et al. CYP2E1 activity before and after weight loss in morbidly obese subjects with nonalcoholic fatty liver disease. Hepatology 2003;38:428-35.
45. Reddy JK, Hashimoto T. Peroxisomal beta-oxidation and peroxisome proliferator-activated receptor alpha: an adaptive metabolic system. Annu Rev Nutr 2001;21:193-230.
46. Weltman MD, Farrell GC, Hall P, Ingelman-Sundberg M, Liddle C. Hepatic cytochrome P450 2E1 is increased in patients with nonalcoholic steatohepatitis. Hepatology 1998;27:128-33.
47. Chalasani N, Gorski JC, Asghar MS, et al. Hepatic cytochrome P450 2E1 activity in nondiabetic patients with nonalcoholic steatohepatitis. Hepatology 2003;37:544-50.
48. Ip E, Farrell GC, Robertson G, Hall P, Kirsch R, Leclercq I. Central role of PPARalpha-dependent hepatic lipid turnover in dietary steatohepatitis in mice. Hepatology 2003;38:123-32.
49. Garg R, Tripathy D, Dandona P. Insulin resistance as a proinflammatory state: mechanisms, mediators, and therapeutic interventions. Curr Drug Targets 2003;4:487-92.
50. Tripathy D, Mohanty P, Dhindsa S, et al. Elevation of free fatty acids induces inflammation and impairs vascular reactivity in healthy subjects. Diabetes 2003;52:2882-7.
51. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003;112:1796-808.
52. Yu C, Chen Y, Cline GW, et al. Mechanism by which fatty acids inhibit insulin activation of insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol 3-kinase activity in muscle. J Biol Chem 2002;277:50230-6.
53. Wigg AJ, Roberts-Thomson IC, Dymock RB, McCarthy PJ, Grose RH, Cummins AG. The role of small intestinal bacterial overgrowth, intestinal permeability, endotoxaemia, and tumour necrosis factor alpha in the pathogenesis of non-alcoholic steatohepatitis. Gut 2001;48:206-11.
54. Kugelmas M, Hill DB, Vivian B, Marsano L, McClain CJ. Cytokines and NASH: a pilot study of the effects of lifestyle modification and vitamin E. Hepatology 2003;38:413-9.
55. Yamada S, Iida T, Tabata T, et al. Alcoholic fatty liver differentially induces a neutrophil-chemokine and hepatic necrosis after ischemia-reperfusion in rat. Hepatology 2000;32:278-88.
56. Sheron N, Bird G, Koskinas J, et al. Circulating and tissue levels of the neutrophil chemotaxin interleukin-8 are elevated in severe acute alcoholic hepatitis, and tissue levels correlate with neutrophil infiltration. Hepatology 1993;18:41-6.
57. Yamauchi T, Kamon J, Minokoshi Y, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 2002;8:1288-95.
58. Yamauchi T, Kamon J, Ito Y, et al. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature 2003;423:762-9.
59. Kamada Y, Tamura S, Kiso S, et al. Enhanced carbon tetrachloride-induced liver fibrosis in mice lacking adiponectin. Gastroenterology 2003;125:1796-807.
60. Xu A, Wang Y, Keshaw H, Xu LY, Lam KS, Cooper GJ. The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. J Clin Invest 2003;112:91-100.
61. Haluzik M, Parizkova J, Haluzik MM. Adiponectin and its role in the obesity-induced insulin resistance and related complications. Physiol Res 2004;53:123-9.
62. Clarke SD. Nonalcoholic steatosis and steatohepatitis. I. Molecular mechanism for polyunsaturated fatty acid regulation of gene transcription. Am J Physiol Gastrointest Liver Physiol 2001;281:G865-9.
63. Yahagi N, Shimano H, Hasty AH, et al. Absence of sterol regulatory element-binding protein-1 (SREBP-1) ameliorates fatty livers but not obesity or insulin resistance in Lep(ob)/Lep(ob) mice. J Biol Chem 2002;277:19353-7.
64. Sekiya M, Yahagi N, Matsuzaka T, et al. Polyunsaturated fatty acids ameliorate hepatic steatosis in obese mice by SREBP-1 suppression. Hepatology 2003;38:1529-39.
65. Basaranoglu M, Acbay O, Sonsuz A. A controlled trial of gemfibrozil in the treatment of patients with nonalcoholic steatohepatitis. J Hepatol 1999;31:384.
66. Gilat T, Leikin-Frenkel A, Goldiner I, et al. Prevention of diet-induced fatty liver in experimental animals by the oral administration of a fatty acid bile acid conjugate (FABAC). Hepatology 2003;38:436-42.
67. Harrison SA, Torgerson S, Hayashi P, Ward J, Schenker S. Vitamin E and vitamin C treatment improves fibrosis in patients with nonalcoholic steatohepatitis. Am J Gastroenterol 2003;98:2485-90.
68. Lin HZ, Yang SQ, Chuckaree C, Kuhajda F, Ronnet G, Diehl AM. Metformin reverses fatty liver disease in obese, leptin-deficient mice. Nat Med 2000;6:998-1003.
69. Marchesini G, Brizi M, Bianchi G, Tomassetti S, Zoli M, Melchionda N. Metformin in non-alcoholic steatohepatitis. Lancet 2001;358:893-4.
70. Neuschwander-Tetri BA, Isley WL, Oki JC, et al. Troglitazone-induced hepatic failure leading to liver transplantation. A case report. Ann Intern Med 1998;129:38-41.
71. Menon KVN, Angulo P, Lindor KD. Severe cholestatic hepatitis from troglitazone in a patient with nonalcoholic steatohepatitis and diabetes mellitus. Am J Gastroenterol 2001;96:1631-4.

72. Neuschwander-Tetri BA, Brunt EM, Wehmeier KR, Oliver D, Bacon BR. Improved nonalcoholic steatohepatitis after 48 weeks of treatment with the PPAR-gamma ligand rosiglitazone. Hepatology 2003;38:1008-17.
73. Promrat K, Lutchman G, Uwaifo GI, et al. A pilot study of pioglitazone treatment for nonalcoholic steatohepatitis. Hepatology 2004;39:188-96.
74. Galli A, Crabb DW, Ceni E, et al. Antidiabetic thiazolidinediones inhibit collagen synthesis and hepatic stellate cell activation in vivo and in vitro. Gastroenterology 2002;122:1924-40.
75. Tanaka T, Yamamoto J, Iwasaki S, et al. Activation of peroxisome proliferator-activated receptor {delta} induces fatty acid {beta}-oxidation in skeletal muscle and attenuates metabolic syndrome. Proc Natl Acad Sci USA 2003;100:15924-9.
76. Charlton M, Kasparova P, Weston S, et al. Frequency of nonalcoholic steatohepatitis as a cause of advanced liver disease. Liver Transpl 2001;7:608-14.
77. Palmer M, Schaffner F. Effect of weight reduction on hepatic abnormalities in overweight patients. Gastroenterology 1990;99:1408-13.
78. Ueno T, Sugawara H, Sujaku K, et al. Therapeutic effects of restricted diet and exercise in obese patients with fatty liver. J Hepatol 1997;27:103-7.
79. Harrison SA, Ramrakhiani S, Brunt EM, Anbari MA, Cortese C, Bacon BR. Orlistat in the treatment of NASH: a case series. Am J Gastroenterol 2003;98:926-30.
80. Booth AM, Caldwell SH, Iezzoni JC. Troglitazone-associated hepatic failure. Am J Gastroenterol 2000;95:557-8.
81. Lavine JE. Vitamin E treatment of nonalcoholic steatohepatitis in children: a pilot study. J Pediatr 2000;136:734-8.
82. Chitturi S, George J. Interaction of iron, insulin resistance, and nonalcoholic steatohepatitis. Curr Gastroenterol Rep 2003;5:18-25.
83. Diehl AM. Nonalcoholic steatohepatitis. Semin Liver Dis 1999;19:221-9.
84. Solga SF, Diehl AM. Non-alcoholic fatty liver disease: lumen-liver interactions and possible role for probiotics. J Hepatol 2003;38:681-7.
85. Li Z, Yang S, Lin H, et al. Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease. Hepatology 2003;37:343-50.
86. Laurin J, Lindor KD, Crippin JS, et al. Ursodeoxycholic acid or clofibrate in the treatment of non-alcohol-induced steatohepatitis: a pilot study. Hepatology 1996;23:1464-7.
87. Abdelmalek MF, Angulo P, Jorgensen RA, Sylvestre PB, Lindor KD. Betaine, a promising new agent for patients with nonalcoholic steatohepatitis: results of a pilot study. Am J Gastroenterol 2001;96:2711-7.
88. Egawa T, Toda K, Nemoto Y, et al. Pitavastatin ameliorates severe hepatic steatosis in aromatase-deficient (Ar-/-) mice. Lipids 2003;38:519-23.
89. Merat S, Malekzadeh R, Sohrabi MR, et al. Probucol in the treatment of non-alcoholic steatohepatitis: a double-blind randomized controlled study. J Hepatol 2003;38:414-8.