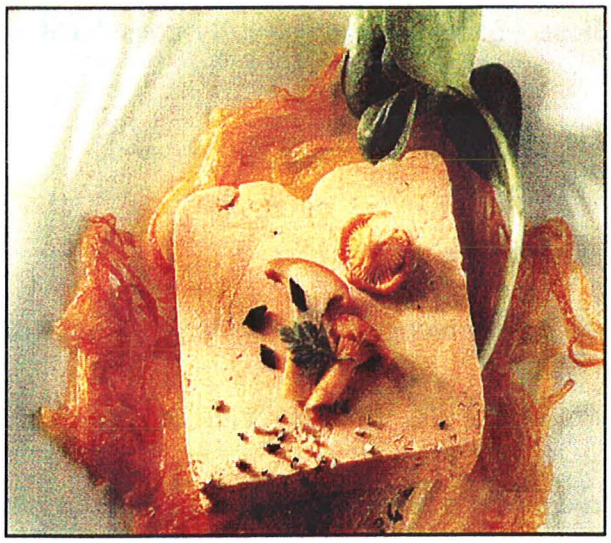
Internal Medicine Grand Rounds

November 1, 2001

Hepatic Steatosis



An Emerging Epidemic

Jay D. Horton, M.D.

This is to acknowledge that Jay D. Horton, M.D., has disclosed no financial interests or other relationships with commercial concerns directly or indirectly related to this program. Dr. Horton will be discussing off-label uses in his presentation.

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Cover: **Foie gras.** The literal translation of foie gras is "fat liver." This specialty of Alsace and Perigord is the enlarged liver from a goose or duck that has been fattened over a period of 4 to 5 months. After the bird is killed, the liver is soaked overnight in milk, water or Port. It is then drained and marinated in a mixture usually consisting of Armagnac Port or Madeira and various seasonings. The livers are then cooked, usually by baking. At its best, it is a delicate rosy color with mottlings of beige. The flavor is extraordinarily rich and the texture silky smooth. Pâté de foie gras is pureed goose liver (by law, 80%) that usually contains other foods such as pork liver, truffles and eggs. Mousse or puree de foie gras must contain at least 55% goose liver. Foie gras should be served chilled with thin, buttered toast slices. Sauternes are generally considered the perfect accompaniment for foie gras.

Steatosis time line (from the Dallas Morning News)

First century B.C. - Sumo wrestling, a sport widely recognized for its large participants, gets its start.

1000 -The English language's first known use of the term *fat* with an unfavorable connotation occurs.

1299 - The first documented use of the word *corset* occurs, in reference to the wardrobe of the household of King Edward I.

1558 -Luigi Cornaro, an aged nobleman who restricted his diet to fight various ailments, writes, "O wretched, miserable Italy! Dost not thou plainly see, that Gluttony deprives thee of more Souls yearly, than either a War, or the Plague itself could have done?"

1742 - "Eat few Suppers," advises Ben Franklin in his annual *Poor Richard's Almanac*, "and you'll need few Medicines."

1863 - The first "popular" diet book appears, a 21-page pamphlet called *A Letter on Corpulence Addressed to the Public* by William Banting. The pamphlet was reprinted worldwide.

1900 -The hamburger (ground lean beef on toast) is reportedly introduced in Connecticut.

1901 - Life insurance data begin to show that excessive weight is associated with shortened life.

End of time - According to the Old Testament (Isaiah 25:6), "The Lord of hosts will make for all peoples a feast of fat things, a feast of wine on the lees, of fat things full of marrow."

Introduction

Hepatic steatosis or "fatty liver" is the accumulation of fat, primarily in the form of triglycerides in liver cells. Nonalcoholic fatty liver disease (NAFLD) is a general term for a clinicopathologic syndrome that ranges from fatty liver alone to steatohepatitis, steatonecrosis, and nonalcoholic steatohepatitis (NASH). NASH is the most severe form of NAFLD and is characterized by fat accumulation plus ballooning degeneration and/or hepatitis-like lesions (sinusoidal fibrosis and polymorphonuclear infiltrates, with or without Mallory hyaline). As the name implies, the liver lesions found in NASH are generally indistinguishable from those found in ethanol-induced hepatitis. Therefore, to definitively make the diagnosis of NAFLD, significant alcohol use (>20 g/day) must be excluded, making NAFLD one of the few diseases that still requires a true clinicopathologic correlation for diagnosis.

NAFLD

NAFLD is associated with a wide variety of insults and metabolic abnormalities, which include obesity, insulin-resistant diabetes, hyperlipidemia, and certain drugs/toxins (Table 1) (1-3).

Table 1. Diseases or Conditions Associated with Hepatic Steatosis (4)

Metabolic Abnormalities	Inborn Errors	Surgical Procedures	Drugs/ Toxins	Miscellaneous
Obesity	Wilson's disease	Jejunoileal bypass	Amiodarone	Acute fatty liver of pregnancy
Diabetes	Abetalipoproteinemia	Biliopancreatic diversion	Glucocorticoids	Jejunal diverticulosis with bacterial overgrowth
Hyperlipidemia	Hypobetalipoproteinemia	Small bowel resection	Synthetic estrogens	Weber-Christian disease
Lipodystrophy	Tyrosinemia	Gastroplasty	Tamoxifen	Tuberculosis
Acute starvation	Glycogen storage disease		Isoniazid	Hepatitis C
TPN	Homocystinuria		Coumadin	ETOH ingestion
Rapid weight loss	Hereditary fructose intolerance		Tetracycline	Reye's syndrome
	Systemic carnitine deficiency		Bleomycin	
	Galactosemia		Methotrexate	
			L-Asparaginase	
			Hydralazine	
			Several metals	

The typical patient with NAFLD as originally described by Ludwig *et al.* (1) in 1980 was an obese diabetic female with abnormal liver function tests who had liver histology consistent with alcoholic hepatitis but denied drinking alcohol. Most of Ludwig's 20 patients were asymptomatic and were initially evaluated for abnormal liver function tests. Eighteen of the twenty patients were obese, 25% had insulin-resistant diabetes, and 25% had hyperlipidemia. Studies conducted during the past 2-5 years have dramatically expanded this patient profile to such an extent that NAFLD is the most common liver abnormality confronted by physicians in the U.S. Despite the high

prevalence of NAFLD, the natural history and clinical consequences of this condition remain poorly understood. The conditions listed in Table 1 are far too numerous to be adequately covered in a single Grand Rounds, therefore, the subsequent discussion will focus primarily on the diagnosis, pathogenesis, and natural history of "Primary" NAFLD, which is associated with obesity, diabetes and hyperlipidemia, as opposed to "Secondary" NAFLD, which is associated with other conditions listed in Table 1.

Prevalence and Demographics of NAFLD

Accurate estimates of the prevalence of NAFLD in the general population have been difficult to obtain. In general, the overall prevalence is probably 16-24% (5, 6). However, the prevalence of NAFLD in certain patient populations is dramatically higher (Table 2).

Table 2. Studies Reporting the Demographics of NAFLD (7)

Source	n	Age (Mean)	Female (%)	Obese (%)	Diabetes (%)	Hyperlipidemia (%)
Ludwig *et al.* (1)	20	54	65	90	50	67
Adler *et al.* (2)	29	46	76	100	2	48
Itoh *et al.* (8)	16	52	75	100	5	63
Diehl *et al.* (9)	39	52	81	71	55	20
Lee (10)	49	53	78	69	51	NA
Powell *et al.* (11)	42	49	83	95	36	81
Laurin *et al.* (12)	40	48	63	70	28	NA
Pinto *et al.*(13)	32	49	75	47	34	28
Bacon *et al.* (14)	33	47	42	39	21	21
Teli *et al.* (15)	40	57	45	30	10	23
Matteoni *et al.* (16)	132	53	53	70	33	92
Angulo *et al.* (17)	144	51	67	60	28	27

NA=not available.

Most cases of NAFLD are detected in the fifth decade of life. Early studies showed a female predominance, however, more recent larger studies have shown an essentially equal sex distribution. The prevalence of hyperlipidemia, diabetes, and obesity in patients with NAFLD varies over a relatively large range. All studies show the highest association with obesity, which is as high as 95% in some studies but ranges from 60-95% in various study populations (1, 6, 9-11, 17). In a literature survey of 41 original articles that contained liver morphology in a total of 1515 obese patients (in adults, a BMI of >25 kg/m^2 is considered overweight and a BMI >30 kg/m^2 is considered obese), liver biopsies were considered normal in only 12% of the cases (18). The distribution of fat is also important. Visceral obesity is an independent predictor of hepatic steatosis, as it is for hyperinsulinemia, decreased insulin clearance by the liver and peripheral insulin resistance (19, 20). Owing to the marked rise in the prevalence of obesity, NAFLD has become a major public health issue, particularly in the U.S. (Fig. 1).

Mokdad *et al.* (21) recently reported that in the U.S. the prevalence of obesity (BMI >30 kg/m^2) in the year 2000 was ~20%, which represents ~38.8 million people (22). This is a 61% increase in the prevalence of obesity compared to figures from 1991

(Fig. 1). As of the year 2000, the majority (56%) of adults in the U.S. were classified as overweight (BMI >25 kg/m^2). In total, ~97 million U.S. adults are currently overweight or obese. Based on these numbers, we can assume that 30-50 million people have some form of NAFLD in the U.S. alone.

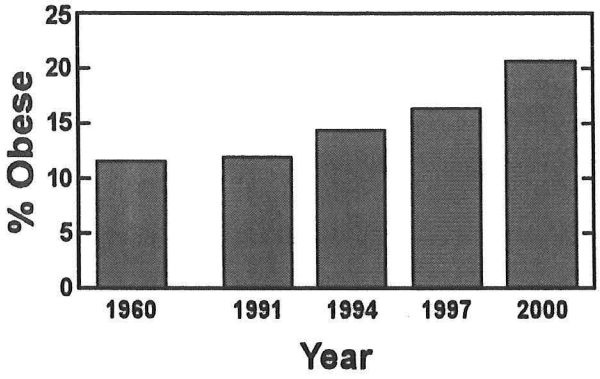


Fig. 1. Percentage of U.S. population defined as obese (21).

The association between type 2 diabetes and NAFLD is more variable (2-55%) than that for obesity (Table 2). Some of this variation is due to patient selection in the various studies and to differences in the criteria used to define type 2 diabetes. However, given the strong association of NAFLD with obesity, it is not surprising that type 2 diabetes is the second most common metabolic abnormality associated with NAFLD. The prevalence of *diagnosed* type 2 diabetes has also increased from 4.9% in 1990 to 7.3% in 2000 (22). If undiagnosed diabetes is taken into consideration, it is estimated that ~10% of the U.S. population currently has type 2 diabetes, which represents ~16 million Americans. This number is expected to rise to ~22 million by 2025, thus providing an even larger NAFLD patient base.

Similarly alarming numbers have been reported in children. Data from the National Health and Nutrition Examination Survey, cycle III, conducted in the U.S. from 1988 to 1994, revealed that approximately 20% of adolescents were overweight and ~10% were obese. Strauss *et al.* (23) reported that 65 of 2450 adolescents had abnormal alanine aminotransferase (ALT) levels. Sixty percent of the adolescents with abnormal ALTs were overweight or obese. Overall, 10% of the obese adolescents had elevated ALTs. The true prevalence of NAFLD in children is not known, however, it is probably the most common cause of liver test abnormalities in adolescents (24).

By definition, primary NAFLD requires the exclusion of excessive ethanol intake. Excessive ethanol ingestion produces histologic liver lesions very similar to those observed in NAFLD. Bellentani *et al.* (6) examined whether the coexistence of obesity and excessive ethanol ingestion increased the prevalence of NAFLD in a large population from northern Italy. The key results from this study are summarized in Table 3. People were excluded if they had evidence of hepatitis B, hepatitis C, type 2 diabetes, hyperlipidemia, or were on any medication. The control group had normal BMIs and had consumed <30 g/d of ethanol and <100 kg throughout their lifetime. The heavy drinker group had normal BMIs and had consumed >100 kg of ethanol over their lifetime and averaged >60 g/d. The obese group had BMIs >30 kg/m^2 and had consumed <30 g/d of ethanol and <100 kg over their lifetime. Finally, the heavy drinker and obese group was the combination of the previous two groups. The prevalence of steatosis as determined by ultrasound was 16.4% in controls. The prevalence of steatosis was 2.8-fold higher in heavy drinkers, 4.6-fold higher in obese individuals and 5.8-fold higher in obese heavy

drinkers. Interestingly, heavy ethanol intake in obese people only increased their risk of having hepatic steatosis by 30% over those that were obese nondrinkers. Other studies have found that moderate alcohol consumption actually reduces the risk of NAFLD in the severely obese, but how much is too much has not been determined (25).

Table 3. Prevalence of Hepatic Steatosis in Northern Italy (6)

Study Population	n	Prevalence (%)
Control (BMI <25 kg/m^2)	67	16.4
Heavy Drinkers (>30 g/day)	69	46.4
Obese (BMI >30 kg/m^2)	60	75.8
Obese+Heavy Drinker	55	94.5

Pathogenesis of Hepatic Steatosis in Obesity and Type 2 Diabetes.

Pure hepatic steatosis, or the mere presence of fat in hepatocytes without evidence of inflammation or fibrosis, is generally considered benign (15). However, steatosis is the first evidence of an alteration in lipid metabolism that can ultimately lead to fibrosis, cirrhosis and liver failure. Studies largely reported over the past 3-4 years have defined some of the metabolic alterations in liver that result in fat accumulation. Hepatic fat accounts for up to 5% of the weight in normal liver. As discussed below, the key metabolic alteration that predisposes a patient to develop primary NAFLD is **insulin resistance** and resulting **hyperinsulinemia**. Insulin resistance is defined as a suboptimal response to any of the biological actions of endogenous or exogenous insulin, with resulting hyperinsulinemia (26).

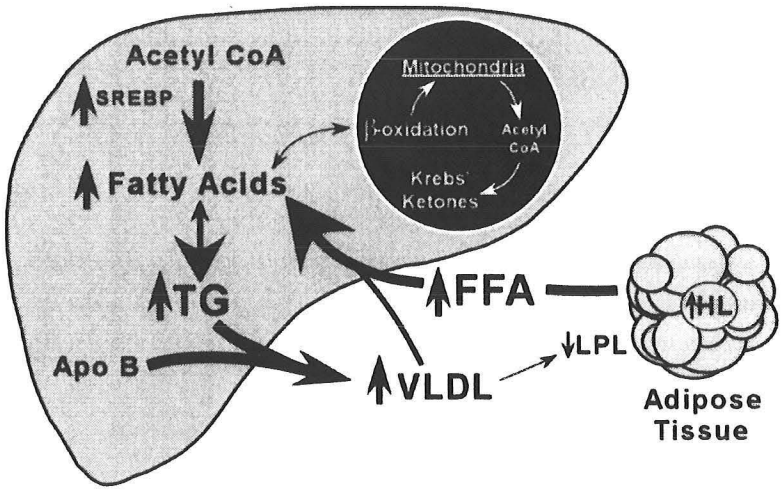
The liver is the principal organ in the body responsible for the intermediary metabolism of carbohydrates, lipids and proteins. In the fed state, all mammals preferentially burn carbohydrates to generate ATP and convert excess carbohydrates into fatty acids, which are stored as triglycerides in adipocytes. Under normal conditions, there is constant cycling of fatty acids between adipose tissue and liver. Fatty acids that enter the liver are derived from one of three sources: 1) hydrolysis of adipose tissue triglycerides; 2) hydrolysis of dietary triglycerides; or 3) direct uptake of chylomicron remnants in the postprandial state. The relative rates of uptake from each of these pathways largely depend on insulin levels and nutritional status.

The release of free fatty acids from adipose triglyceride stores occurs at least partly through the enzyme hormone-sensitive lipase (27). Hormone-sensitive lipase activity is regulated by the intracellular level of cAMP. Insulin normally *inhibits* the release of free fatty acid release from adipose tissue by reducing cAMP formation and, thus, the activity of hormone-sensitive lipase. Conversely, glucagon, catecholamines and other hormones can increase cAMP levels leading to increased free fatty acid release from adipocytes. In most insulin-resistant states, the adipose tissue is resistant to the antilipolytic action of insulin. Therefore, cAMP levels are elevated and FFA release from adipose tissue and subsequent uptake by the liver is enhanced.

In addition to uptake from ingested or exogenously formed fatty acids, the liver is a major organ responsible for the *de novo* synthesis of fatty acids from the precursor acetyl-CoA. Hepatic synthesis of fatty acids occurs principally in the fed state when

insulin and glucose levels are high. Excess glucose is converted to fatty acids and to triglycerides, which is exported from the liver in the form of VLDL for peripheral storage in adipocytes. Regulation of endogenous fatty acid synthesis in liver is mediated, in part, by the transcription factor, sterol regulatory element-binding protein-1c (SREBP-1c) (28). SREBP-1c is positively regulated by insulin, and is one of the transcriptional mediators responsible for increased fatty acid synthesis. In liver, hyperinsulinemia associated with insulin-resistance leads to increased activation of SREBP-1c, which in turn participates in the stimulation of fatty acid and triglyceride synthesis in liver. Increased *de novo* hepatic fatty acid synthesis has the following potentially deleterious effects in liver: 1) increased TG accumulation; 2) increased VLDL production and secretion, which may contribute to the hypertriglyceridemia commonly found in insulin-resistant states; and 3) increased concentrations of malonyl-CoA (as a result of the overall increase in fatty acid synthesis), which can potentially inhibit carnitine palmitoyltransferase I (CPT I) and β-oxidation of fatty acids (Fig. 2) (29).

Fig. 2. Fatty acid flux in insulin-resistance.



The liver has only two mechanisms for disposal of free fatty acids. The first is to esterify the fatty acid to form triglycerides for export in VLDL particles. The second pathway is for fatty acids to undergo oxidation principally in mitochondria and to a lesser extent in peroxisomes. Oxidation breaks fatty acids down to acetyl-CoA, which can be used for energy in the tricarboxylic acid cycle. Alternatively, acetyl-CoA can be converted to ketone bodies that are released into the plasma for use as a source of energy by other tissues. The two pathways of disposal are regulated in large part by a fatty acid synthesis intermediate malonyl-CoA. Malonyl-CoA is the product of the first enzymatic step in fatty acid synthesis and it serves as the 2-carbon donor for subsequent fatty acid elongation. Early work by Drs. McGarry and Foster revealed that CPT I is negatively regulated by malonyl-CoA (29). CPT regulates fatty acid entry into the mitochondria for β-oxidation. Thus, malonyl-CoA-mediated regulation of CPT I prevents a potentially futile cycle of simultaneous fatty acid synthesis and degradation from occurring. Therefore, at least in early insulin-resistant states when hyperinsulinemia is present, all metabolic alterations that could theoretically result in fat accumulation in liver are operating simultaneously (Fig. 2).

Determinants of insulin sensitivity, such as insulin-mediated glucose disposal and insulin-mediated suppression of hepatic glucose output, correlate inversely with increasing obesity (30). Therefore, it has been hypothesized that the strong correlation between obesity and NAFLD may be due to undetected insulin-resistance. Most studies

have used fasting glucose levels as the sole measure of insulin resistance. Recently, more sophisticated methods to measure insulin resistance have been used in patients with NAFLD. Using the homeostasis model assessment method to measure insulin resistance, Marchesini *et al.* (31) reported that the strongest predictor of NAFLD was insulin resistance, *irrespective* of BMI, fat distribution, or glucose tolerance. Limitations of this study were that they selected only patients with abnormal LFTs and used ultrasound as the criterion for the presence of steatosis. One additional interesting finding in this study was that 57% of the 46 subjects with NAFLD had insulin resistance and *normal* BMIs.

These studies were recently extended to NAFLD patients with chronically elevated ALTs but with BMIs <30 kg/m^2 and normal fasting glucose levels (32). Of the 30 patients studied, 21 (70%) had a histologic evidence of NASH and 9 had pure steatosis. Fasting plasma insulin levels were increased 3-fold on average (124 *vs.* 44 pmol/L) in the 30 patients despite normal fasting and postload glucose levels. Euglycemic clamp studies demonstrated that NAFLD patients had a 50% reduction in glucose disposal (Fig. 3). In addition, NAFLD patients had moderately elevated fasting basal levels of plasma free fatty acids and *reduced* insulin-induced suppression of lipolysis. Finally, the normal ability of insulin to suppress glucose output from the liver was also attenuated in individuals with NAFLD (Fig. 3). These studies showed that even in normal weight individuals with normal glucose tolerance, NAFLD was associated with physiologic hallmarks of insulin resistance.

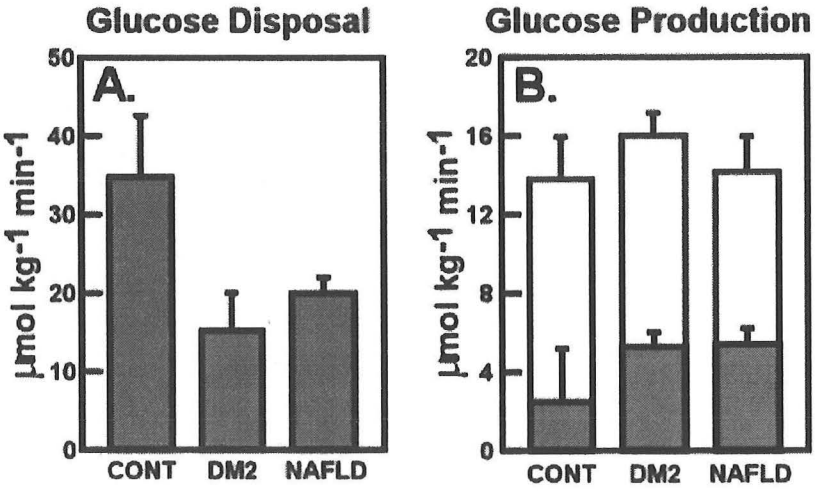


Fig. 3. Glucose disposal (A) and hepatic glucose production (B) during the course of the clamp study in controls (CONT), patients with type 2 diabetes (DM2) and patients with NAFLD. Shaded bars in B represent the hepatic glucose production at the end of the clamp study (32).

The strong link between insulin resistance and hepatic steatosis has led investigators to ask whether hepatic steatosis is another manifestation of Syndrome X or the metabolic syndrome. This syndrome is typically associated with obesity, diabetes, hyperlipidemia and hypertension. Marceau *et al.* (33) examined the prevalence of hepatic steatosis and individual components of the metabolic syndrome in 580 patients undergoing biliopancreatic diversion for severe obesity. Liver biopsies from these patients showed that 86% had hepatic steatosis and 74% had fibrosis. Steatohepatitis (NASH) was found in 24% of the biopsies and frank cirrhosis in 2%. The percentage of patients with cirrhosis was lower than other estimates probably because the average age of the patients in the study was only 36. The addition of each component of the metabolic syndrome increased the risk of steatosis from 1- to 99-fold. Fasting plasma

glucose levels were the only measure of insulin resistance in this study, but the findings do support the concept that hepatic steatosis is an additional manifestation of the metabolic syndrome.

Non-alcoholic Steatohepatitis (NASH)

A consensus has emerged that steatosis is required, but not sufficient, for the development of necrosis, inflammation and fibrosis; the key components of NASH (34). Although primary NAFLD is strongly associated with diabetes and obesity, only a relatively small percentage of patients ultimately progress to clinically significant disease (35). In this respect, NAFLD is similar to ethanol-induced liver disease in which less than 10% of heavy drinkers ultimately develop cirrhosis (36). Clearly, other factors must play a role for NASH to develop from mere hepatic steatosis. This supposition has led investigators to propose a "2-hit hypothesis" for the development of NASH (34). The "first hit" is the underlying metabolic process that results in fat accumulation and the "second hit" is a cellular event that leads to inflammation, fibrosis and ultimately cirrhosis. The stimulus that causes steatosis to progress to steatohepatitis and fibrosis is not defined. Study in this area is hindered by the lack of a clear consensus regarding the nomenclature and histologic categorization of NAFLD and NASH. However, most experts in the field do agree that in addition to steatosis and inflammation, ballooning degeneration and fibrosis are required features of NASH. The liver histology of NASH is characterized by the following:

1) **Macrovesicular fat deposits.** Cytoplasmic lipid droplets composed principally of triglycerides and some cholesteryl esters that stain positively with Oil red-O stain.
2) **Ballooning degeneration.** Hepatocellular injury results in two different morphologic manifestations, either ballooning degeneration or acidophilic degeneration. The ballooning results from intracellular fluid accumulation and the cells are typically located in zone 3 (pericentral).
3) **Evidence of focal necrosis with mixed polymorphonuclear inflammatory cells**. The inflammation of NASH is typically mild and is predominantly lobular rather than portal. Neutrophilic cells in the lobular inflammatory infiltrates are a distinguishing feature from other forms of acute and chronic liver injury.
4) **Sinusoidal fibrosis.** The patterns of fibrosis are one of the characteristic findings in NASH. The deposition of collagen initially occurs in the perivenular and perisinusoidal spaces of zone 3. In some areas the collagen envelopes single cells in a pattern that is commonly referred to as chicken-wire fibrosis. This pattern of fibrosis distinguishes NASH and alcohol-induced fibrosis from other forms of chronic liver diseases in which the fibrosis is initially periportal.
5) **Mallory bodies**. Mallory's hyaline is an intracytoplasmic inclusion that consists of many aggregated cytoskeletal peptides, some of which include cytokeratins 7, 18, 19 and ubiquitin. It is generally located in ballooned hepatocytes in zone 3. If found in setting of #'s 1-4, it is very supportive, but not required, for the diagnosis of NASH. In adult studies, the incidence of Mallory's hyaline ranges from 9.5-90% (10, 11).

A recently proposed classification system that separates NAFLD into 4 types, which represents the full histologic spectrum of NAFLD lesions is listed below.

Type 1: Fat alone
Type 2: Fat and inflammation
Type 3: Fat and ballooning degeneration
Type 4: Fat and fibrosis and/or Mallory bodies

In this classification system, only types 3 and 4 typically progress to advanced liver disease. It is clearly important to precisely categorize NAFLD patients in future studies since the clinical course and management of the defined NAFLD groups may significantly differ. This is illustrated in a recent study that showed over a 10-year period, approximately 25% of patients with Type 3 and Type 4 histology on their initial biopsy developed cirrhosis and 12% died a liver-related death, whereas only 4% with Type 1 and Type 2 histology developed fibrosis and 2% suffered a liver-related death (Table 4) (16).

Table 4. Clinical Outcome as a Function of Liver Histology

Outcome	Type 1 (n=49)	Type 2 (n=10)	Type 3 (n=19)	Type 4 (n=54)
Cirrhosis, n (%)	2 (4)	0	4 (21)	14 (26)
Liver-related death, n (%)	1 (2)	0	1 (5)	1 (13)

Prevalence and Demographics of NASH

The overall prevalence of NASH is probably 2-3% in the general population (5, 6). However, in a large autopsy series, NASH was found in 6.3% of all patient autopsies (3). In the U.S. and Canada, NASH is the histologic diagnosis in 7-11% of all patients undergoing a liver biopsy (1, 37). In patients with unexplained elevations in liver function tests, NASH is found in at least 26% of all biopsies (38). Overall, NASH is probably the third most common liver disease in adults in the U.S. (after hepatitis C and alcohol) and it is the most common liver disease among U.S. adolescents.

Potential Mediators of the "Second Hit" Leading to NASH

1) Role of the Mitochondria

Increased reactive oxidation species (ROS) production and lipid peroxidation have received the greatest attention as mediators of the "second hit" in NASH. Livers from humans with NASH exhibit ultrastructural mitochondrial lesions (megamitochondria) that have decreased activity of respiratory chain complexes, which can result in the accumulation of ROS (39). The mitochondria constitute the greatest source of ROS since the mitochondrial electron transport system consumes about 90% of the oxygen used by the cell (40). Ultimately, the state of oxidative stress in a cell depends on the balance between the production of ROS and the capacity of cellular detoxification. ROS are derived mainly from the ubiquinone site of complex III of respiration, which activates molecular oxygen to superoxide anion (O_2^-). Superoxide

anions can then lead to the formation of other oxygen-derived free radicals (41). ROS, such as hydrogen peroxide (H_2O_2) and hydroxy radicals (•OH), damage cellular lipids, DNA and proteins (42). Free radical attack on unsaturated lipids initiates a chain reaction of lipid peroxidation. Lipid peroxidation products can alter mitochondrial DNA and react with mitochondrial proteins to inhibit the transfer of electrons along the respiratory chain (43, 44). ROS can also increase the synthesis of several cytokines possibly through the activation of NF-κB (45). Clinical support for the role of ROS in the development of NASH comes from a single clinical trial in 11 children with presumed NASH that were given the antioxidant, vitamin E, for 4 to 10 months (46). Liver function tests improved in all of the children during the treatment period, however, no liver histologic correlation was obtained.

2) Role of Microsomal Proteins

Alternative cellular sources other than the mitochondria can contribute to the formation of ROS in NASH. The most publicized is a microsomal cytochrome P450 enzyme, CYP2E1. One function of CYP2E1 is to hydroxylate long chain fatty acids at the ω-1 position (47). The hydroxy fatty acids produced by these enzymes are ultimately dehydrogenated to a dicarboxylic acid, converted to the CoA derivative and metabolized by undergoing oxidation in the peroxisome. The expression of CYP2E1 is markedly enhanced in most animal models of fatty livers as well as in humans with NASH (48, 49). Interestingly, fatty acids are apparently both substrates and inducers of CYP2E1. Specifically, CYP2E1 expression is increased in response to exposure to high fat diets, high carbohydrate diets, ethanol, fasting, and insulin-resistant diabetes (50-52). CYP2E1 may generate ROS because it has a relatively low affinity for oxygen and may "leak" electrons to form ROS (47). If the antioxidant pathways are overwhelmed, ROS can react with unsaturated carbonyl bonds of long chain fatty acids to initiate the autocatalytic process of lipid peroxidation. Recently, the overall importance of CYP2E1 has been called into question because of studies in knockout mice lacking Cyp2e1 and still develop NASH-like histology when fed diets that induce NASH (50, 53). It should be noted, however, that an alternative family of cytochrome P450 genes (Cyp4a) was induced in these mice. The CYP4A family also has ω-1 hydroxylation activities and could explain the lack of a phenotype in these mice. Whether similar compensatory regulation of CYP4A expression exists in humans is not known.

3) Role of the Peroxisome

β-Oxidation of very long chain fatty acids (fatty acids with carbon chain lengths >18) preferentially occurs in peroxisomes (54). Therefore, peroxisomes are also capable of producing ROS through the β-oxidation of very long chain fatty acids and subsequent generation of H_2O_2 (55). In NAFLD, peroxisomal generation of ROS may become quantitatively important because hepatic fatty acid accumulation and availability likely exceed the mitochondria's β-oxidation capacity, thus increasing peroxisomal β-oxidation. Two mouse models that lack key genes involved in peroxisomal β-oxidation illustrate the potential importance of peroxisomes in the development of NASH. The first is knockout mice lacking PPARα (56) and the second is knockout mice lacking fatty acyl-CoA oxidase (AOX) (57). PPARα is a nuclear receptor that positively regulates genes involved in peroxisomal β-oxidation. AOX is the first enzyme in the classical

peroxisomal β-oxidation system. Both mouse models develop NASH and have increased hepatic production of H_2O_2. The elevated H_2O_2 production in the livers from these knockout mice was attributed to some combination of the following: 1) peroxisomal proliferation; 2) induction of peroxisomal β-oxidation; and 3) microsomal Cyp4a-mediated fatty acid oxidation.

A proposed sequence of events that results from the production of ROS is shown in Fig. 4. Lipid peroxidation, regardless of its source, has the following multiple deleterious effects in the hepatocyte, including:

1) Lipid peroxidation results in the release of malondialdehyde and 4-hydroxy-nonenal, which can cause direct toxicity to cells by causing the expression of FAS ligand, resulting in FAS-mediated cell death.

2) Malondialdehyde and 4-hydroxy-nonenal can covalently bind proteins and this cross-linking action may contribute to the formation of Mallory bodies (58).

3) Malondialdehyde and 4-hydroxy-nonenal induce collagen synthesis (59).

4) 4-hydroxy-nonenal has chemotactic activity for neutrophils, which may be responsible for the neutrophilic infiltrate observed in NASH (59).

5) 4-hydroxy-nonenal up-regulates TGF-β1 expression in macrophages, which could be an additional link between lipid peroxidation and fibrosclerosis (60).

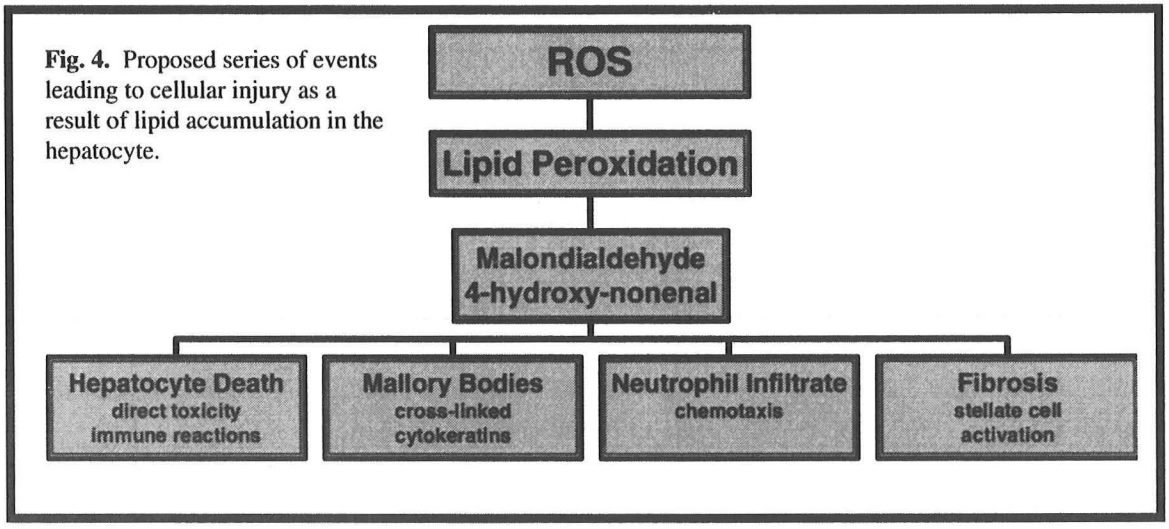


Fig. 4. Proposed series of events leading to cellular injury as a result of lipid accumulation in the hepatocyte.

4) Role of Cytokines

In alcohol-induced liver disease, endotoxin and endotoxin-inducible cytokines, including tumor necrosis factor alpha (TNFα) and certain TNF-inducible cytokines, such as interleukins-6 and -8 (IL-6, IL-8), have been incriminated in the pathogenesis of steatohepatitis and cirrhosis. Several lines of evidence suggest that, at least in rodents, these cytokines could be involved in the progression of liver disease to NASH. The leptin-deficient *ob/ob* mouse develops severe obesity, insulin resistance and fatty livers. Endotoxin (lipopolysaccharide) injection into *ob/ob* mice results in increased TNFα

release and induces necroinflammatory changes in liver (61). Basal TNFα expression levels are also increased in livers and adipose tissue of *ob/ob* mice as well as in adipose tissue from obese humans (62). Finally, TNFα may contribute to NAFLD by interfering with insulin receptor mediated signal transduction, which is important for the development of insulin resistance in mice inasmuch as *ob/ob* mice that lack TNFα are protected from insulin resistance (63).

5) Activation of Stellate Cells

In all forms of liver disease, the final road leading to cirrhosis travels through the stellate cell (also referred to as Ito cells, fat-storing cells, or lipocytes) (64). As in other parenchymal tissues, normal liver contains an epithelial component (hepatocytes), an endothelial lining, tissue macrophages (Kupffer cells), and a perivascular mesenchymal cell, the stellate cell. Stellate cells comprise ~15% of the total number of cells in liver. They have long cytoplasmic processes that facilitate their interactions with neighboring cell types. Following hepatic injury, stellate cells undergo a process referred to as "activation." This process transforms the quiescent vitamin A storing cells into proliferative, fibrogenic, and contractile myofibroblasts (64) (Fig. 5).

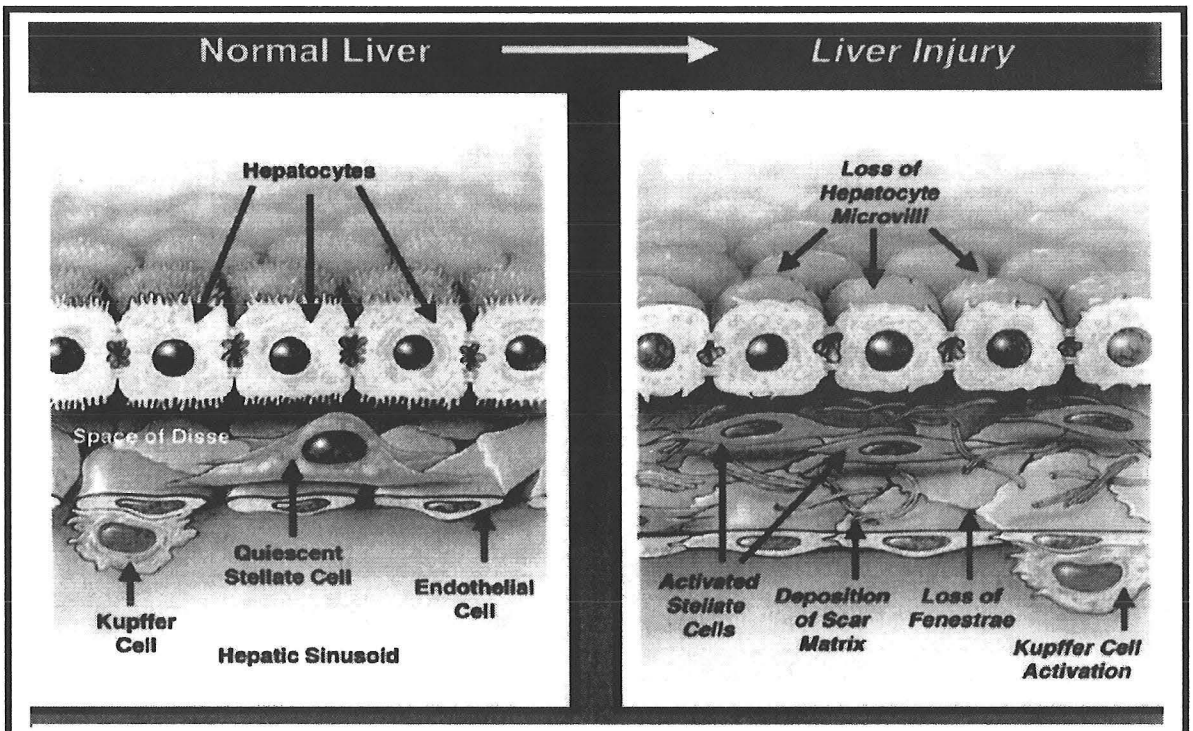


Fig. 5. Liver injury activates stellate cells. Activated stellate cells produce fibril-forming matrix (scar). This contributes to the loss of hepatocyte microvilli and sinusoidal fenestrae. Illustration from Ref. 65.

The stimuli that initiate stellate cell activation in NASH are very poorly characterized. Injury to all cell types can ultimately result in the production of substances that may initiate the activation of stellate cells. Hepatocytes and Kupffer cells are capable of producing ROS, which makes them leading candidate cells responsible for

stellate cell activation in NASH. The major lipid peroxidation products, malondialdehyde and 4-hydroxy-nonenal can activate cultured stellate cells (64). Whether endothelial cells participate in stellate cell activation in NASH is not clear. However, injured endothelial cells do produce a splice variant of cellular fibronectin (EIIIA isoform) that can also activate stellate cells and they can convert latent transforming growth factor-β1 (TGF-β1) to the fibrogenic form through the activation of plasmin (64, 65).

During the initiation of stellate cell activation, rapid changes in gene expression occur that change the phenotype of the cell so that it can respond to extracellular signals. A cascade of events within the cell results in an increase in extracellular matrix (ECM) synthesis, expression of growth factors, cytokine receptors, contractile structures and metalloproteinases (see Fig. 6). This results in a cell that has proliferative, synthetic and contractile properties. The proteins produced by activated stellate cells remodel the ECM in the subendothelial space, changing it from the normal low-density basement membrane matrix to an interstitial type matrix containing fibril-forming collagens (64). These events do not seem to be specific to NASH, but seem to be a general wound healing response by the liver.

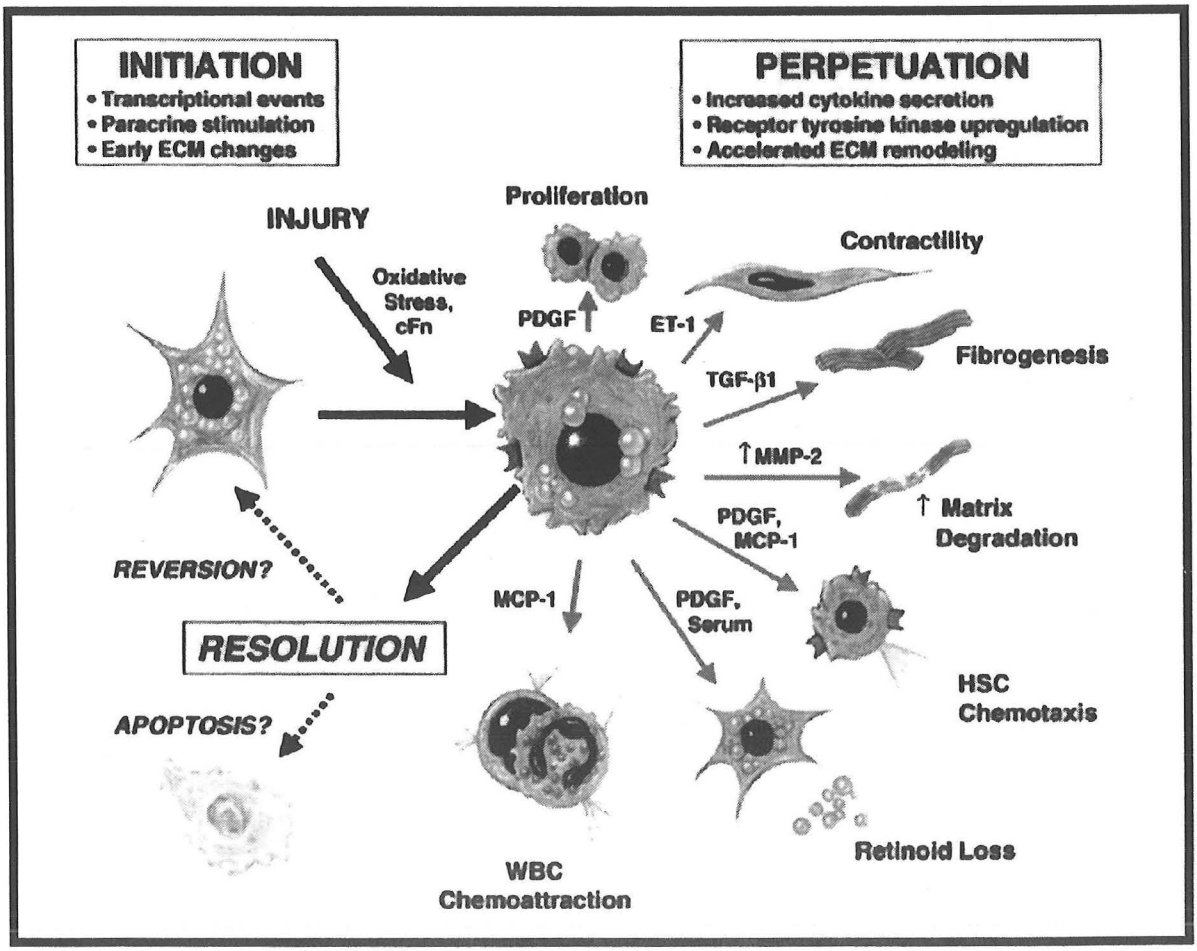


Fig. 6. Key mediators of proliferation, contractility, fibrogenesis, matrix degradation, chemotaxis, and WBC chemoattraction produced by activated stellate cells. The fate of the activated stellate cell is not known, but is felt to be either reversion to the quiescent phenotype or clearance through apoptosis. Illustration adapted from Ref. (65).

Progression to Fibrosis and Cirrhosis

Chronic stellate cell activation ultimately results in cirrhosis. Cirrhosis occurs in a minority of patients with NASH, although the overall incidence may be as high as 26% (9, 16). Only four small studies have attempted to determine the rate of progression of fibrosis in patients with NASH lesions. A total of 42 patients have been studied if the results from Powell *et al.* (11), Lee (10), Bacon *et al.* (14) and Ratziu *et al.* (35) are combined. Of these, 4 (9.5%) improved, 21 (50%) remained unchanged, and 17 (40%) had histologic progression during follow-up periods of 1 to 15 years. Larger controlled studies are obviously required, however the results from these studies do suggest that once NASH is present, subsequent progression to cirrhosis can occur in a significant number of patients. Therefore, NASH may be a major contributor to the 10-15% of liver patients that have "cryptogenic cirrhosis." Several studies have suggested that this is indeed the case. In one large series of 70 patients who received liver transplants for cryptogenic cirrhosis, the incidence of diabetes and/or obesity was 73% (66). This incidence was very similar to that found in 50 patients who received a transplant for NASH, and was much higher than the 28-33% incidence of diabetes and obesity found in patients who received transplants for other disease processes.

Is diabetes an independent risk factor for fibrosis and cirrhosis?

The current consensus is that type 2 diabetes confers an increased risk for the subsequent development of fibrosis and cirrhosis in NAFLD. NAFLD occurs in up to 75% of patients with type 2 diabetes. A number of studies have shown that hepatic fibrosis is more common and prominent in obese patients that have hyperglycemia (Table 5) (16, 67). In a large autopsy series, Wanless and Lentz (3) found that type 2 diabetes was associated with a 2.6-fold increased risk of steatohepatitis.

Table 5. Type 2 Diabetes and the Incidence of Cirrhosis and Liver-related Deaths (16)

Characteristic	Diabetes (n=42)	Normoglycemic (n=84)
Age at diagnosis	57 ± 11	54 ± 14
Female (%)	67	47
Triglycerides (mg/dl)	489 ± 312	226 ±115
Development of cirrhosis (%)	24	1
Liver-related deaths (%)	19	2

Evidence is emerging that may provide insight into the mechanisms by which hyperglycemia promotes fibrosis. First, cells incubated in a high glucose medium have increased production of ROS due to activation of the tricarboxylic acid cycle. This activates phospholipase C and NF-κB, which can lead to cytokine production (68). Second, glucose and insulin stimulate the expression of connective tissue growth factor (CTGF) in stellate cells (69). CTGF is a peptide that belongs to a family of intermediate early growth responsive genes and it stimulates the expression of several extracellular

matrix proteins as well as collagen. Elevated levels of CTGF have also been found in human livers with NASH and its expression correlates with the degree of fibrosis (69).

Evaluation of Patients with Suspected NAFLD and/or NASH

Typically, NAFLD patients initially present for evaluation of other conditions and are found to have abnormal LFTs or hepatomegaly incidentally (1, 10, 14, 16). The majority of patients with NAFLD are asymptomatic (45-100%) (1, 10, 11, 14) but occasionally, right upper quadrant pain, fatigue and malaise are reported (Table 6) (70). The most common liver function test abnormalities are 2- to 5-fold elevations of ALT and aspartate aminotransferase (AST) (7). In 60-90% of NAFLD patients, the ratio of AST/ALT is <1, which is typically the reverse of that measured in serum from patients with alcohol-induced liver injury. Unfortunately, this ratio commonly reverses in NAFLD if the liver disease progresses to cirrhosis. Serum gamma-glutamyltranferase (GGT) and alkaline phosphatase levels are elevated in less than 50% of the cases. Therefore, the laboratory abnormalities are rather non-specific and cannot be used exclusively to provide the diagnosis of NAFLD.

Table 6. Symptoms and Signs Associated with NAFLD

Symptoms	Signs	Laboratory Values
Fatigue Malaise RUQ pain	Hepatomegaly (~75%) Splenomegaly (~25%) Rarely portal hypertension	2-5 fold increase in ALT and AST +/- Increased Alk. Phos. and GGT Increased Cholesterol and Triglycerides Hyperglycemia

The diagnosis of NAFLD is ultimately made by exclusion after ruling out chronic viral hepatitis, autoimmune chronic active hepatitis, hemochromatosis, inherited metabolic abnormalities, alcohol use and reactions to medications. Laboratory testing, including serologic tests for viral hepatitis, iron studies, ceruloplasmin levels, phenotype and levels of α-1 antitrypsin, antimitochondrial and antinuclear antibodies should be done to detect potentially treatable causes of chronic liver diseases. No combination of laboratory tests is definitive in making the diagnosis. Van Ness and Diehl (71) showed that the predictive value of a diagnosis of nonalcoholic fatty liver before biopsy was only 56% for NASH compared with 86% for alcoholic liver disease.

Radiologic Studies in NAFLD

Radiologic studies can be very suggestive of NAFLD using the available modalities as briefly described below.

1) **Sonography**-Liver with fatty change is often described as a "bright liver" because of the increased echogenicity and sound attenuation. These findings are very difficult to distinguish from other disease processes that present with diffuse increased echogenicity since fibrosis from any cause can have similar sonographic appearance. This has led some to use the term "fatty-fibrotic pattern" to emphasize the fact that sonography cannot distinguish these two types of liver pathology (72). Unfortunately, no recent studies have been performed to assess the sensitivity and specificity of ultrasonography for

detecting hepatic steatosis using state of the art equipment. Older reports show that ultrasonography is 89-95% sensitive and 84-93% specific for steatosis, but only 57-77% sensitive and 85-89% specific for fibrosis (73, 74).

2) **Computed Tomography**-The most accurate CT method to characterize hepatic steatosis is unenhanced CT. The difference in attenuation values between the spleen and liver are measured and, if greater than –10 Hounsfield units, the criteria for hepatic steatosis are met. Normal liver has greater attenuation than spleen.

3) **Magnetic Resonance Imaging**-MR characteristics of fat can be used to assess hepatic steatosis, however, the diagnosis is often more easily made by using other imaging modalities. There does appear to be a reasonably close correlation between MRI assessment and histological evaluation of hepatic steatosis (75).

4) **Magnetic Resonance Spectroscopy**-Chemical shift-sensitive MR is emerging as the most sensitive and specific non-invasive test for the presence of fatty liver. The measurement of fat is robust because it merely requires the evaluation of two dominant peaks (water and lipid) within the MR spectrum. The correlation of MRS with CT and histologic fatty liver specimens has been reported but MRS has the clear advantage of giving a more quantitative measurement (76). This method will undoubtedly become the gold standard for noninvasive measurements of fat content and will be an invaluable tool for longitudinal studies in patients with hepatic steatosis.

Unfortunately, no laboratory or radiologic criteria from any of the listed modalities can distinguish NASH from steatosis alone (77), relegating the physician to make the decision whether to proceed with a liver biopsy.

Can a Liver Biopsy be Avoided?

It is not feasible or cost effective to perform liver biopsies in all overweight people who have evidence of hepatic steatosis on an imaging study. Determining whether a liver biopsy is helpful or necessary would be easier if the true natural history of the disease was known. The natural history of NAFLD does vary according to the histologic type. The presence of only hepatic fat seems to be a benign condition, and progression to fibrosis and cirrhosis is reportedly very rare (15, 16). It should be noted, however, that only four relatively small studies, each containing ~40 patients, have looked specifically at the natural history of NAFLD using serial liver biopsies in *some* of the reported cases (10, 11, 15, 35). In addition, the follow-up period for the vast majority of patients was <10 years. Nevertheless, the conclusions from these very limited studies were that if the initial biopsy is free of fibrosis and inflammation (i.e. pure hepatic steatosis), <10-20% will develop fibrosis and none have reportedly developed cirrhosis or liver failure (11, 15). The problem is that a large longitudinal study that includes serial liver biopsies has not yet been performed. Therefore, the rate of progression (or lack thereof) of simple steatosis to NASH is really not known.

Inasmuch as a majority of patients with NAFLD do not have NASH, there is a clear need for indicators that could predict which patients are more likely to progress to fibrosis and cirrhosis. No convincing strategies have been identified, although one has

been proposed but not validated (35). In this study, 93 obese patients with abnormal LFTs and varying degrees of NAFLD as determined by liver biopsy were evaluated. Thirty percent of the patients had fibrosis and 11% had cirrhosis. They found that 4 variables were significantly correlated with septal fibrosis: 1) age >50 years; 2) BMI $\geq$ 28 kg/m^2; 3) ALT $\geq$ 2 times normal; and 4) triglycerides $\geq$ 1.7 mmol/L. No patients <50 years of age, with BMIs <28 kg/m^2 and ALTs <2-fold had any evidence of septal fibrosis or cirrhosis.

Angulo *et al.* (17) performed a multivariate analysis of 144 patients and found that older age, obesity, diabetes, and AST/ALT >1 were all statistically correlated with biopsy findings of bridging fibrosis or cirrhosis. Several additional studies have also identified age as a significant predictor for cirrhosis (16, 17, 35). This most likely reflects the duration of the disease and is likely to be less useful as the prevalence of obesity rises in adolescents (78).

Dixon *et al.* (25) studied 105 consecutive patients undergoing weight reduction surgery (all with BMIs > 35 kg/m^2). In these obese patients, hyperinsulinemia, systemic hypertension, and elevated ALTs were all independent predictors of NASH. The association between NASH and type 2 diabetes was the strongest in this study (Fig. 7).

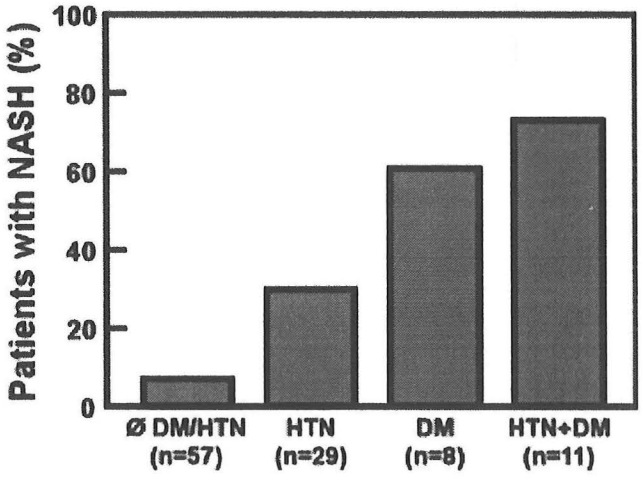


Fig. 7. Association between NASH and type 2 diabetes (DM) and hypertension (HTN) in 105 obese patients undergoing adjustable gastric band surgery. The percentages of patients with NASH in each group are indicated.

Although most studies have identified elevated ALTs as an independent risk factor for cirrhosis, the discriminatory value is relatively low. This is illustrated in a study of 46 consecutive patients undergoing gastroplasty for weight reduction (79). The characteristics of the patients were not significantly different from studies discussed in the preceding paragraphs. However, in this study ~50% of the obese patients had *normal* liver function tests and liver biopsies consistent with NASH (Table 7).

Table 7. LFTs and Liver Histology in Obese Patients (79)

Liver Histology	Normal LFTs n (%)	Abnormal LFTs n (%)
Normal Liver	3 (75%)	1 (25%)
Isolated steatosis	4 (40%)	6 (60%)
Mild NASH	9 (47%)	10 (50%)
Severe NASH	6 (46%)	7 (54%)

Therefore, the results from the current body of literature are not very satisfying since the predictive factors for fibrosis and cirrhosis identified (obesity, diabetes, hypertension and elevated ALTs) are those most commonly associated with NAFLD. Therefore, their use would identify and eliminate very few patients from further evaluation. Whether patients with suspected NAFLD require a liver biopsy is debated. As will be discussed in the following section, no treatment is currently available for NAFLD, so one could argue the results will not significantly impact the patient's course. On the other hand, the results of a liver biopsy in NAFLD do have some predictive value and could identify those patients most likely to benefit from future clinical trials and for treatment once available,

Treatment

Currently, there are no recommended or clearly beneficial treatments available for NAFLD. As presented above, most patients with NAFLD have a relatively benign course. Therefore, treatment when available should be restricted to those with more severe histologic lesions (i.e. NASH). In addition, since the true natural history of NAFLD remains largely unknown, it is difficult to recommend routine treatment strategies. Several small trials in highly selected populations have been performed and are discussed below.

Weight loss-NAFLD may resolve with weight loss but the actual benefits seem to be inconsistent and most studies have reported mixed results. One example is a study of 41 morbidly obese patents with NASH who were placed on a severe 388 kcal/d diet (80). The mean weight loss was 34 kg during the treatment. All patients had improvements in liver biochemistries and the degree of fat infiltration. However, those who had a more pronounced weight loss (~one-fifth of the patients) developed portal inflammation or fibrosis. This study is typical in that most studies have shown some improvement in liver histology with weight loss, particularly if the weight loss is gradual. Rapid weight loss is in itself associated with NAFLD. Therefore, the rate and degree of weight loss seems very important but the optimal amount and rate required for normalization of the liver histology have not been determined.

Pharmacologic therapies- Table 8 contains all of the published therapeutic trials for NAFLD. All studies to date have included a very small number of patients and most have been uncontrolled open-label trials. A summary of the more promising therapeutic agents is provided on the following page.

Table 8. Therapeutic Trials for the Treatment of NAFLD

Study	Drug	# of Pts	Type of Study	Duration (months)	LFTs	Histology
Laurin (12)	UDCA	24	Open label	12	Yes	Yes
Laurin (12)	Clofibrate	16	Open label	12	Yes	Yes
Basaranoglu (81)	Gemfibrozil	46	Randomized	1	Yes	No
Abdelmalek (82)	Betine	8	Open label	12	Yes	Yes
Lavine (46)	Vitamin E	11*	Open label	4-10	Yes	No
Caldwell (83)	Troglitazone	10	Open label	6	Yes	Yes
Assy (84)	Orlistat	8	Open-label	6	Yes	Yes
Marchesini (85)	Metformin	20	Open label	4	Yes	No

* The 11 patients were children.

1) **Ursodeoxycholic acid** is a hydrophilic bile acid that may protect cells from apoptosis (86). The only published trial (not including abstracts) in adults using ursodeoxycholic acid (UDCA) was in 24 patients with NASH who received UDCA at a dose of 13-15 mg/kg/d for 12 months (12). The treatment led to a significant improvement in LFTs and in the amount of steatosis. However, there was no change in the degree of inflammation or fibrosis. A large-scale placebo-controlled trial is currently underway.

2) **Clofibrate and gemfibrozil** are fibric acid derivatives used as lipid lowering drugs. The mechanisms by which they reduce plasma lipids are multiple but at the molecular level, they activate the nuclear receptor PPARα. PPARα transcriptionally activates several genes involved in oxidation of fatty acids. Clofibrate reduced the liver triglyceride content in rats with ethanol-induced steatosis (87), however, results in human studies have not shown any beneficial effect in NAFLD. The longest trial with Clofibrate was in 16 patients that were treated with 2 g/d for 1 year (12). No significant improvements in LFTs or liver histology were found at the end of the 12 month treatment period.

3) **Betaine** is a normal component of the methionine metabolic cycle. Previous studies have shown that ethanol feeding to rats alters methionine metabolism by decreasing the activity of methionine synthetase, the enzyme that converts homocysteine to methionine. This causes a reduction in S-adenosylmethionine, which is the activated form of methionine. Betaine administration to rats significantly increased S-

adenosylmethionine and protected them from ethanol-induced fat accumulation (88). Abdelmalek *et al.* (82) recently reported a pilot study in which 8 patients with NASH were treated with betaine (20 g/d) for 12 months. Seven patients completed the study and 3 of these had a 50% reduction in their LFTs. Overall, 50% of the patients had histologic improvement. The authors concluded that the results were encouraging enough to warrant a large controlled trial.

4) **Troglitazone** is a thiazolidinedione that is a peroxisome proliferator-activated receptor gamma (PPARγ) ligand previously used to treat type 2 diabetes prior to its withdrawal from the market. Caldwell *et al.* (83) studied 10 female patients with histological NASH. All but two were obese and one had type 2 diabetes. Troglitazone was given at a dose of 400 mg/day for < or = 6 months. Seven of ten patients had normal ALTs at the end of treatment. In the responders, ALTs fell from 87 +/- 38 before to 39 +/- 9 at the end of treatment ($p = 0.01$), and ASTs decreased from 77 +/- 23 to 30 +/- 8 ($p = 0.002$). Biopsy comparisons from before and after therapy showed persistent steatohepatitis in all cases, although four of seven showed a one-point improvement in the necroinflammatory grade. The authors concluded that normalization of the liver enzymes in patients with NASH who are treated with thiazolidinediones should be viewed with reservation and that follow-up biopsies are essential to evaluate the efficacy of these agents, inasmuch as the histologic benefits appeared to be relatively modest.

5) **Metformin** is a biguanide that is an attractive candidate for study in NAFLD because it improves hepatic and peripheral sensitivity to insulin and suppresses gluconeogenesis, but does not cause overt hypoglycemia. The first indication that metformin may be useful in the treatment of NAFLD came from studies by Lin *et al.* (89) in the mouse model of fatty liver, the *ob/ob* mouse. *Ob/ob* mice and lean controls were treated with metformin (350 μg/kg/d) for 4 weeks. The treatment resulted in a marked improvement of LFTs, a normalization of liver weight and a markedly improved liver histology (Fig. 8). The mice were administered much higher doses of metformin than is typically used in humans. However, none of the mice developed lactic acidosis, a known side effect in humans.

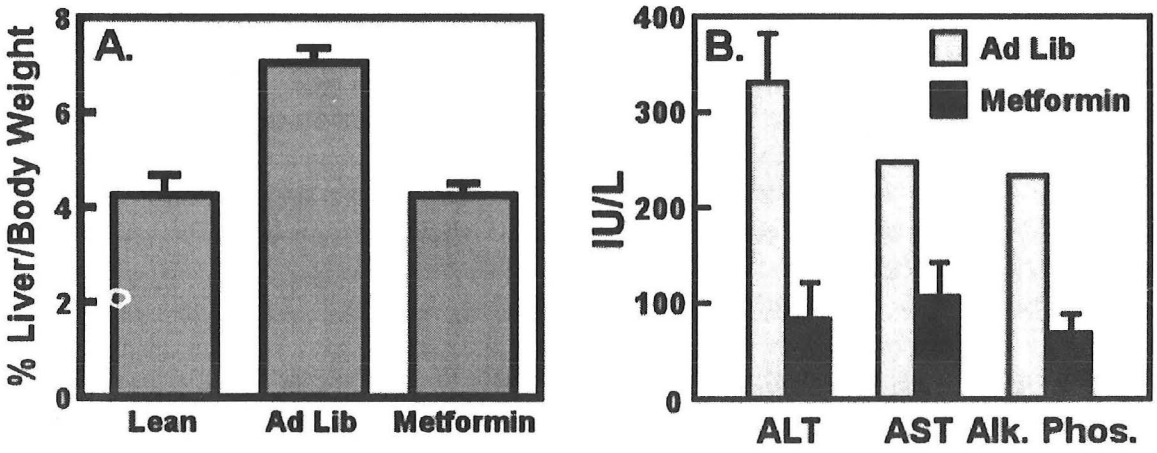


Fig. 8. A) Effect of metformin on the liver to body weight ratio in *ob/ob* mice fed chow *ad libitum* or metformin (350 μg/kg/d). Lean littermates were fed chow and are shown as a control. B) Liver function tests in non-treated *ob/ob* mice and in *ob/ob* mice treated with metformin for 4 weeks (92).

The encouraging results in mice led to a pilot study with metformin in humans reported this year. Marchesini *et al.* (85) treated 14 patients with histologic evidence of steatohepatitis with metformin (500 mg TID) for 4 months. All patients in the study had normal fasting glucose levels and oral glucose tolerance tests. When compared with the six individuals not complying with treatment, 50% of the actively treated patients had a complete normalization of their transaminase levels (Fig. 9). Insulin sensitivity (measured by euglycemic and a hyperinsulinemic glucose clamp) improved significantly and liver volume was decreased on average by 20%. A randomized-controlled study with liver biopsies is clearly needed to confirm the results from this pilot study. In addition, because lactic acidosis is a rare complication of metformin treatment, liver disease is frequently considered a contraindication for metformin therapy (90, 91).

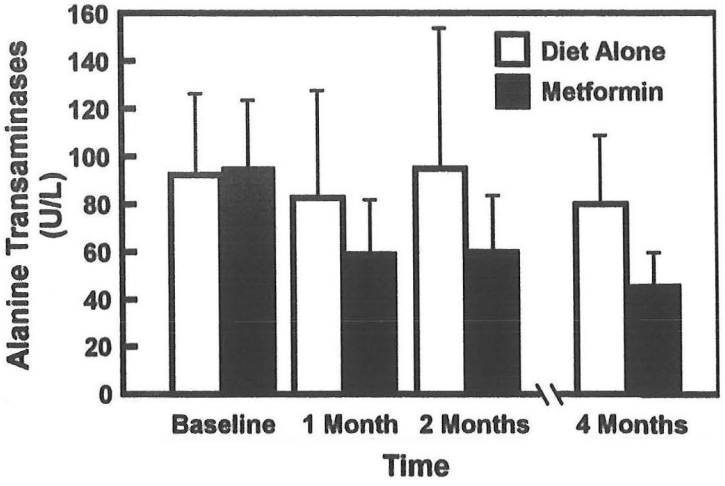


Fig. 9. ALT levels as a function of time in patients with NASH. Six patients were in the diet alone group and 14 patients were treated with metformin 500 mg TID for 4 months.

Conclusions

Hepatic steatosis is the most common liver abnormality encountered in the U.S. and the prevalence is expected to increase. The initial fat accumulation in primary NAFLD is likely the result of insulin resistance. In general, there is a clear lack of data regarding the natural history of this condition. However, it is apparent that a relatively small percentage of patients eventually develop significant liver disease. Studies designed to identify those individuals at risk for histological progression are required so that those most likely to benefit from potential therapies can be targeted for further investigation and possible treatment. Although several therapeutic pilot studies are encouraging, solid therapeutic recommendations must await the results of well-controlled randomized clinical trials with clinically relevant end-points. Based on the known physiologic alterations responsible for fat accumulation in liver, therapies targeted to increase insulin sensitivity seem to be the best candidates for future study. Patients who develop end-stage liver disease from NASH should be evaluated for liver transplantation. The overall outcome of liver transplantation in these patients seems to be good, although NASH can recur in the transplanted liver (92).

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