NASH (Non-Alcoholic Steatohepatitis)... Some Answers, Many Questions

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- Clinical hepatology
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Glossary of frequently used abbreviations...

- ALT (SGPT)... aspartate aminotransferase
- AST (SGOT)... alanine aminotransferase
- ASH... alcoholic steatohepatitis
- CYP2E1... cytochrome P450 2E1
- FFA... free fatty acids
- FL... fatty liver
- LPS... lipopolysaccharide (endotoxin)
- OLT... orthotopic liver transplantation
- ROS... reactive oxygen species
- TNFa... tumor necrosis factor alpha

NASH (Non-alcoholic steatohepatitis)

Non-alcoholic steatohepatitis (NASH) is not an uncommon liver disease, but it is an under-appreciated one. Some of the reasons for the low level of awareness are understandable and include: the relatively recent coining of the term "NASH" in 1980, the requirement of a liver biopsy for a definitive diagnosis, the relatively indolent nature of the disease in most cases which does not prompt a rapid nor extensive diagnostic evaluation and, lastly, the lack of a consensus regarding diagnosis and management of NASH.

This situation is likely to change as NASH becomes more recognized as a fairly common liver disease that may affect otherwise lean healthy persons and may result in cirrhosis, necessitating liver transplantation. Advances in techniques for studying hepatocellular mechanisms of injury and the refinement of animal models of steatohepatitis are also stimulating increased interest in NASH. The recent NIH-sponsored NASH Symposium held December 10-11, 1998, the proceedings of which are to be published (1), was a potentially watershed event in the study of NASH and will be referred to frequently.

This presentation will attempt to address many questions concerning NASH and the caveat that the knowledge base is far from complete will be stated at the onset. Most of the data concerning NASH is based on small numbers of observations or patients and there are few prospective studies. There are very few human observations regarding pathogenesis; animal models of fatty liver/NASH have been used as well as extrapolations of data concerning alcoholic liver disease (ASH). Thus, while there are many intriguing preliminary observations or hypotheses, both basic and clinical, concerning NASH, confirmation is necessary through future studies.

Definition of NASH

The words non-alcoholic steatohepatitis (NASH) were used for the first time (Table 1) by Ludwig et al (2) in 1980 in describing 20 non-alcoholic patients at the Mayo Clinic with liver biopsy changes interpreted as **"diagnostic for or compatible with alcoholic liver disease"**. These patients drank alcohol only on "rare occasions"; "most had less than one drink per week"; no other potential cause of liver disease was identifiable. **"The biopsy specimens were characterized by the presence of striking fatty change with evidence of lobular hepatitis, focal necroses with mixed inflammatory infiltrates, and in most instances, Mallory bodies. Evidence of fibrosis was found in most [70%] specimens, and cirrhosis was diagnosed in biopsy tissue from three [of 20] patients".** 90% of these patients were overweight, but it is unclear how many were actually obese (BMI $\geq$ 30). 25% had diabetes mellitus and 25% hyperlipidemia.

NASH IS LIVER DISEASE IN NON-ALCOHOLICS THAT IS IDENTICAL TO THAT SEEN IN ALCOHOLIC LIVER DISEASE

It is of interest to note that the description of the liver histology stated that "the inflammatory changes were characterized by the presence of lymphocytes and other mononuclear cells and of neutrophils." Neutrophils alone were, therefore, not a *sine qua non*. In addition, "Portal and periportal inflammation usually was mild." Was this a true feature of NASH or did it represent a feature of chronic hepatitis C, the serologic tests for which was still more than a decade away? All studies pre-1990 need to be considered in view of hepatitis C, since HCV infection can result in some fatty changes (although will not look exactly

like NASH). Lastly, 3 patients had mild iron deposition, the potential significance of which will also be addressed below.

This retrospective case series, with the usual weaknesses of any such retrospective study, introduced NASH to the medical nomenclature. It was an important recognition that such patients were not closet drinkers or alcoholics, as they had frequently been accused of. The diagnostic description has a fair degree of latitude to it; subsequent case series have used similar but slightly modified diagnostic criteria, thus impairing comparison amongst reports. Herein lies one of the main problems with studying NASH, that is, the variability of mainly the alcohol consumption and histologic criteria used to include or exclude patients. This lack of a uniform histologic grading system continues today, although proposals for a grading and staging system have been made by an NIH sponsored NASH Pathology Working Group, as described below.

From 1980 to now, the clinical concept of NASH has been overly-simplified in some quarters; for example, to a busy clinician, NASH may equate to an elevated ALT + "fat" (increased echogenicity) seen on liver ultrasonography in a patient in whom "everything else has been ruled out". Also, since liver biopsy (the gold standard for NASH) is often not performed, persons with NASH may be mistakenly labeled as having simple fatty liver or *vice-versa*.

TABLE 1... NASH histologic features; original description in 1980 (Ludwig et al, ref 2)

moderate-severe macrovesicular fat (in 100%)
- either diffuse or primarily in zone 2 or 3

lobular inflammation (100%)
- lymphocytes, other mononuclear cells, neutrophils
- focal necrosis
- mild portal and periportal inflammation

Mallory bodies [hyaline] (70%)
- in zone 3

Councilman bodies (25%)

Fibrosis (70%)

Cirrhosis (15%)

Fatty Liver... differences vs NASH

Simple (also termed bland or pure) fatty liver (FL) is histologically characterized by hepatic steatosis without ballooning degeneration, inflammation, necrosis, Mallory's hyaline, fibrosis or cirrhosis. It is uncertain how to classify FL biopsies when mild inflammation or spotty necrosis or ballooning degeneration is also seen, except to describe the changes and allow the clinician to make the clinical correlation.

FL is fairly commonly encountered in clinical practice and is associated with many conditions (Tables 2 & 3). The great majority of FL is macrovesicular. Serum ALT & AST are usually only mildly elevated.

An example of how often FL or NASH may be seen in practice was published in 1996 (5); this was a profile of an urban, hospital-based hepatology practice. 1226 referrals made to the Liver Unit at the Winnipeg Health Science Center in Canada were analyzed and FL or NASH (not separated out) was diagnosed in 11%. Acute and chronic viral hepatitis (47%),

drug-induced liver disease (6%), alcoholic liver disease (5%), PBC (4%), PSC (3%), cryptogenic cirrhosis (3%) and miscellaneous or undiagnosed liver disease (20%) comprised the remainder.

TABLE 2... SELECTED CONDITIONS ASSOCIATED WITH MACROVESICULAR STEATOSIS (adapted from Burt et al, ref 3 and Sheth et al, ref 4)

Some, but not all, may also be associated with NASH.

Medications/toxins
- alcohol
- corticosteroids
- bleomycin
- methotrexate
- synthetic estrogens
- zidovudine
- tamoxifen
- amiodarone
- perhexilene

Systemic disorders
- obesity
- diabetes mellitus
- hyperlipidemia
- rapid weight loss
- starvation
- total parental nutrition
- jejuno-ileal bypass surgery
- gastroplasty for morbid obesity
- extensive small bowel resection
- inflammatory bowel disease
- chronic hepatitis C
- HIV infection
- panhypopituitarism
- Wilson's disease
- abetalipoproteinemia
- tyrosinemia
- homocystinuria
- galactosemia
- a variety of other rare inherited metabolic disorders

TABLE 3... SELECTED CONDITIONS ASSOCIATED WITH MICROVESICULAR STEATOSIS (adapted from Burt et al, ref 3)

A feature common to all is severe impairment of mitochondrial ß-oxidation; none are known to be associated with NASH

- acute fatty liver of pregnancy
- Reye's syndrome
- inherited urea cycle disorders
- inherited disorders of fatty acid metabolism
- mitochondrial cytopathies
- Medications/toxins
  - Na valproate
  - salicylates
  - ketoprofen
  - acute Fe toxicity
  - didanosine
  - MDMA (ecstasy)

Natural history of fatty liver

Non-alcoholic FL is thought to be a fairly benign condition (depending on the associated disorders, if any, which may have their own over-riding influence on outcome) and a recent report (6) confirms this. 40 patients with non-alcoholic FL were followed for a mean of 11 years. 12 had repeat liver biopsies; 1 patient had developed mild fibrosis 9.8 years after her index biopsy. 14 patients had died during the follow-up period, but none of a liver-related cause and none had clinical evidence of liver disease before death.

Data presented by A. McCullough of the Cleveland Clinic at the NIH NASH Symposium (1) confirms these findings. After 10 years of follow-up, 49 patients with FL alone had a 2.5% liver-related death rate while 11 patients with FL and inflammation had no liver-related deaths. This contrasted with a 15% liver-related death rate observed in 57 patients with NASH.

NASH Background... timeline of important events

Pre-1980

"Fat people have fat livers" (7). This statement describes what has been known for many decades (or centuries). Only in the 1960's was it appreciated that some fatty livers in non-alcoholics had inflammatory features as well and the overall histologic picture could resemble that of alcoholic hepatitis. Often, such persons were suspected of underestimating their alcohol intake.

However, this awareness of an inflammatory type of FL was stimulated by the performance of jejuno-ileal (J-I) bypass surgery for morbid obesity; postoperatively, a significant number of patients developed what is now recognized as NASH (8, 9). Some (≤5%) of these J-I bypass patients died from liver failure; others (5-10%) developed cirrhosis within a year or so and most had a milder degree of liver disease. Interspersed with the reports of liver disease due to J-I bypass were scattered reports in the 60's and 70's of what would now be termed NASH (8, 10)

Post-1980

the term "NASH" coined by Ludwig et al in 1980. Thereafter, sporadic retrospective reports of steatohepatitis, as it was still called; few insights into pathogenesis.

Post-1990

introduction of 1st serologic test for hepatitis C in 1990 results in straightforward identification of chronic hepatitis C, thus eliminating this as a confounding influence on liver biopsy interpretation. Increasing number of basic science reports of potential NASH pathogenic mechanisms in humans and in animal models.

Post-1994

expansion of the clinical spectrum of NASH... significance of Bacon et al (11) data in 1994 with 58% male, 61% non-obese, 79% non-diabetic and 79% normal lipid levels. More sophisticated basic science reports concerning mitochondrial function, cytokines and oxidative stressors in NASH pathogenesis; animal models of NASH... fa/fa rats and ob/ob mice.

Post-1998

December 10-11, 1998... NIH Symposium on NASH... the 1st international symposium on NASH--- a coalescence of world authorities/report of NASH Pathology Working Group on uniform histologic terminology. Malet gives Grand Rounds at UTSW.

Selected NASH Clinical Associations

Obesity

The single most consistent association with NASH is overweight/obesity. Although not nearly all patients exhibit this feature, the majority of patients with NASH are either overweight (BMI≥25) or obese (BMI≥30), although body mass index (weight in Kg/height in m^2) has not been used as the determinant of obesity in hardly any studies. %ideal body weight (IBW) has most often been used. in the past, but BMI is the preferred parameter for judging obesity in future studies. In the United States, in 1961, 10% of men were obese and 15% of the women. In 1991, the percentages increased dramtically: 20% of men were obese while 25% of women were obese (12).

Total body fat and % body fat do not correlate with steatosis on liver biopsy nearly as well as does central body fat (waist:hip circumference).

Drug-associated

Numerous medications may cause fatty liver, but very few result in a true NASH-like histologic picture. Amiodarone, which is known to inhibit mitochondrial ß-oxidation and cause phopholipidosis, can result in true NASH; similarly for perhexilene maleate. Molecular insights into amiodarone-induced NASH have been recently reported (13). A NASH-like picture has also been described with tamoxifen (14-16) and, recently, NASH with cirrhosis has been described in 2 tamoxifen-induced cases (17).

Lipodystrophies

An interesting aspect of NASH is its occurrence in severe insulin resistance (IR) syndromes such as limb lipodystrophies, insulin receptor mutations and others (18). The liver disease in these syndromes can be severe and has not been well-characterized in the past as NASH. However, with the increasing awareness of the diagnosis of NASH, it is becoming apparent that NASH is, in fact, found in many insulin resistance syndrome patients.

DW Herion, of the Diabetes Branch, NIDDK, presented his group's findings at the NIH NASH Symposium (1) on severe insulin resistance syndromes and liver disease. Of 43 patients with various forms of severe IR (23 with lipodystrophies, 20 with others; mean fasting insulin 61±47 uU/ml & insulin sensitivity index 2.6±2.1 uU/ml/min) evaluated at the NIH, 15 (9 with lipodystrophies, 6 with other) had elevated ALT; the ALT ranged from 44 to 331. 10 of the 15 had overt DM and 13 had hyperlipidemia. 11 of the 15 had liver biopsies with all of them demonstrating NASH; 8 of the 11 had fibrosis and none cirrhosis.

It is easy to speculate that the severe insulin resistance in these patients with elevated insulin levels, hyperlipidemia, elevated free fatty acid (FFA) levels and their subsequent effects on intra-hepatocellular metabolism, is the major pathophysiologic disturbance resulting in the development of NASH. However, the precise mechanisms of hepatic injury remain unknown as does optimal treatment for these patients.

Prevalence of NASH

The true prevalence of NASH is unknown and may never be known because of the inherent selection bias in patients scheduled for liver biopsy, the gold standard for diagnosis. Other potentially confounding aspects of prevalence studies are the exact histologic definition of NASH used and the amount of alcohol use allowable; are small amounts of alcohol use significant? Autopsy studies provide some limited degree of cross-sectional information but also suffer from selection bias.

Wanless & Lentz (19) reported the autopsy prevalence of NASH in 351 apparently non-alcoholic patients. 22 (6%) of the 351 were found to have NASH; the prevalence ranged from 2.7% of those who were not overweight to 18.5% of those who were >40% above IBW. Type 2 diabetics had a 12.2% prevalence rate; interestingly, no type 1 diabetic had NASH.

The prevalence of NASH in liver biopsies from obese patients has ranged from 1 to 9% (20, 21), although both these studies are from the early 80's.

Liver Histology

NASH = fatty change + hepatocyte injury (inflammation or necrosis) ± fibrosis

This statement oversimplifies the diagnostic difficulties involved, particularly in milder cases. The main issue revolves around differentiating simple FL with some mild patchy inflammation or a ballooned cell or two from true NASH (3, 22). Where does the transition occur? How much inflammation is required to make the diagnosis of NASH vs FL? What is "true NASH" anyway?

Features of hepatocyte injury, like Mallory bodies (hyaline), may also be seen in NASH. **Mallory bodies** are eosinophilic inclusions within the cytoplasm of injured hepatocytes; this is a condensation of the cytoskeleton and may be stained with an immunostain for ubiquitin. **Ubiquitin** is a cellular stress protein that coats the surface of filamentous tangles.

The term **ballooning degeneration** is often used to describe hepatocytes undergoing necrosis. Ballooned cells have become swollen and lost their usual polygonal shape (due to disruption of cytoskeleton?); the cytoplasm becomes pale and Mallory bodies may be seen within.

Councilman bodies or eosinophilic/acidophilic degeneration are older terms used to describe what is now called apoptotic bodies. These small more-eosinophilic hepatocytes undergoing apoptosis with eventual loss of its nucleus.

NASH is mainly a disease of acinar zone 3 (peri-central vein or centrilobular), although all the zones may be involved in severe disease.

A significant problem with interpreting liver histology in suspected cases of NASH is interobserver variation among pathologists. The intra- and interobserver variation in reading liver biopsies from patients with fatty liver (FL) and NASH was recently reported (23). 19 histologic parameters compartmentalized into steatosis, inflammation, liver cell injury and fibrosis were evaluated on 53 liver biopsy specimens. Moderate to substantial concordance for both intra- and interobserver was present for only 6 parameters: extent of steatosis, sinusoidal location of fibrosis, perivenular fibrosis, grade of fibrosis, ballooning degeneration and presence of vacuolated nuclei. Parameters of inflammation were not scored as reliably as those of fibrosis and cell injury; this has been previously noted with chronic viral hepatitis liver biopsy readings.

Proposals for Standardized Diagnostic Criteria for NASH

In an attempt to standardize the histologic criteria for NASH, several proposals have been made:

A. Zachery Goodman, of the Armed Forces Institute of Pathology, has proposed (1) the following, quite practical, system for classification of the spectrum of FL/NASH:

1. fatty liver
2. fatty liver + minimal spotty parenchymal inflammation
3. suspected NASH
4. diagnostic NASH

This is a readily understandable system, but suffers from vagueness.

B. Kleiner, of the NCI, spokesperson for the NIH NASH Pathology Working Group, presented their proposal at the NIH NASH Symposium (1):

1. NASH consists of diffuse involvement of the liver with fat, with hepatocellular injury centered in zone 3 **PLUS** 1 of either Mallory bodies or pericellular fibrosis in zone 3.
2. Staging of NASH:
   - stage 0... no fibrosis
   - stage 1... mild fibrosis (limited to zone 3)
   - stage 2... moderate fibrosis
   - stage 3... severe fibrosis with bridging
   - stage 4... cirrhosis
3. Grading of NASH

grading reflects disease activity, rate of decline of function or rate of progression; there are no criteria available and, thus, no grading system can be devised at this time.

For future studies (not for clinical practice), the NIH NASH Pathology Working Group proposed the following scoring system with 7 features, each scored from 0 to 4:

1. fibrosis
2. hepatocellular injury/ballooning degeneration
3. steatosis
4. parenchymal inflammation (lymphocytes, macrophages, PMN's)
5. portal inflammation
6. Mallory bodies
7. hepatocellular iron

C. A group from the Cleveland Clinic (1) has proposed the following classification system for the spectrum of FL and NASH, which they term NAFL (non-alcoholic fatty liver):

1. fatty liver
2. fatty liver + inflammation
3. fatty liver + ballooning degeneration
4. fatty liver + ballooning degeneration + one of: PMN's, Mallory bodies or fibrosis (NASH)

As evidence that their proposed system had clinical relevance, they (retrospectively) identified 136 patients with NAFL and were able to obtain 10 year follow-up data on 98 of them and reported the liver-related deaths in each category:

1.	n=49	2.5% liver-related death rate after 10 years
2.	n=11	0%
3.	n=19	5%
4.	n=57	15%

D. Another proposal has been put forth by the Hepatology-Pathology group from St. Louis Univ. (24).

Grading is based on degree of fatty change, ballooning degeneration and inflammation:

Grade	steatosis	ballooning deg.	inflammation
grade 1 (mild)	1-2+	minimal	1-2+
grade 2 (moderate)	2-3+	present-zone 3	2+
grade 3 (severe)	3+	marked-zone 3	3+

Staging:

stage 0	no fibrosis
stage 1	zone 3 sinusoidal fibrosis
stage 2	fibrosis involving entire zone 3 and other zones

stage 3	bridging fibrosis
stage 4	cirrhosis

Which of the proposed classification systems, if any, gains acceptance over the next decade remains to be seen; the correlation of any system with disease progression and outcome will be a major factor in its applicability.

Clinical Features of NASH

TABLE 4
SELECTED MAJOR SERIES OF PATIENTS WITH NASH
(adapted from Bacon et al, ref 11 and James et al, ref 25)

		n	%female	%obesity	%DM	%incr. lipids	%fibrosis /cirrhosis
Ludwig et al	1980 (ref 2)	20	65	90	50	67	70
Itoh et al	1987 (ref 26)	16	75	100	5	63	19
Diehl et al	1988 (ref 27)	39	81	71	55	20	39
Lee	1989 (ref 28)	49	78	95	51	---	34
Powell et al	1990 (ref 29)	42	83	95	36	81	50
Bacon et al	1994 (ref 11)	33	42	39	21	21	39
Laurin et al	1996 (ref 30)	40	73	70	28	---	---
Pinto et al	1996 (ref 31)	32	75	47	34	28	55
Sanyal	1998 (ref 1)	62	65	85	40	---	58

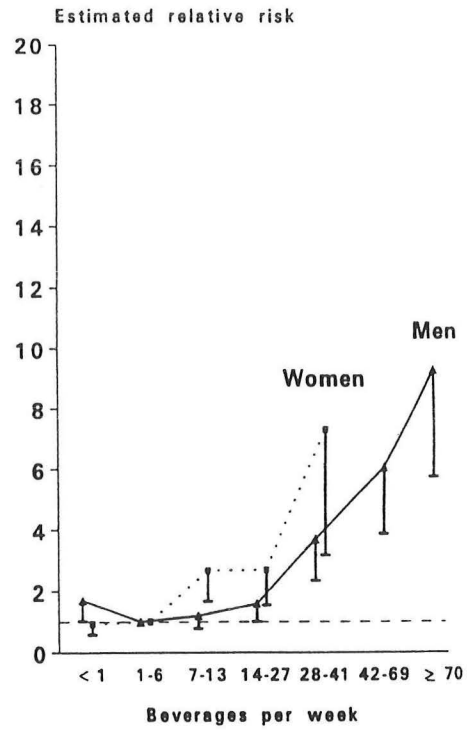
NOTE... there is wide variability among these studies in terms of definitions used (especially for obesity and DM), population sampled, etc. and this greatly limits comparability among studies.

Alcohol use and NASH

How much is too much? Underestimation of alcohol use is, of course, a potential problem in diagnosing NASH vs ASH. Alcohol use has been variously defined in the series of NASH patients, in terms such as "non-alcoholics, less than 20 gms/day of alcohol, lack of excessive alcohol consumption, denial of alcohol abuse", and in the study from Japan by Itoh et al (26), "a lack of any evidence of drinking". That some under-reporters of alcohol use are included in the series on NASH is quite possible, even probable, and in clinical practice the question of whether or not a particular patient overuses alcohol sometimes is a difficult issue to resolve.

Along these lines, a prospective population-based study of 13, 285 men and women with 12 year follow-up demonstrated that the (self-reported) level of alcohol intake (Figure 1) above which the relative risk of developing liver disease was significantly greater than 1 was 7 to 13 drinks per week (1-2/day) for women and 14 to 27 drinks (1 drink = 12gm alcohol) per week (2-4/day) for men (32). Women had a significantly higher risk of developing alcohol-related liver disease than men for any given level of alcohol intake.

Figure 1
(from Becker et al, ref 32)



Symptoms, Signs, Laboratory Studies in NASH

The diagnosis of NASH cannot be made with certainty without a liver biopsy... the gold standard. The crux of the matter is that simple fatty liver can give a similar appearance on radiologic studies and no clinical or laboratory parameter can accurately distinguish fatty liver from NASH (33).

Most patients with NASH are asymptomatic; some may have fatigue or malaise or right upper quadrant discomfort. Hepatomegaly may or may not be present, and, if so, it is usually mild in degree. Very few patients have stigmata of chronic liver disease.

Laboratory studies are, therefore, non-diagnostic but are essential in excluding other potential causes of liver disease. There are no reliable distinguishing laboratory features of NASH vs FL, nor for NASH vs ASH (31, 34, 35).

Generally, patients have elevated ALT &/or AST, usually both; most frequently, the elevation is modest (in the 2 to 3-fold range) but elevations of 10-fold or so may occasionally be seen. Only about 1/3 have elevated alkaline phosphatase, virtually always modest in degree; only about 10-15% have elevated serum bilirubin and very few have a low serum albumin or elevated PT.

Evaluation of Patients with Suspected NASH

The diagnostic evaluation of patients with elevated liver enzymes is complex and always starts with a history and physical with attention to obvious factors like alcohol, illicit drugs, medications, as well as concurrent diseases like IBD, CHF and so forth. Presuming that obvious potential causes has been considered, in evaluating patients with persistently elevated serum transaminases who are suspected of having NASH, the following studies should generally (using your clinical judgment) be obtained:

1.	for chronic hepatitis C	hepatitis C antibody
2.	for chronic hepatitis B	HBsAg, anti-HBs, anti HBc

3.	for hemochromatosis	ferritin, Fe/TIBC
4.	for autoimmune hepatitis	ANA, anti-smooth muscle antibody
5.	for alpha-1-antitrypsin def.	AIAT level
6.	for Wilson's disease	ceruloplasmin
7.	for PBC (if elev. alk P)	AMA

If these studies are unrevealing, my practice is then to obtain, if not already done so, fasting serum cholesterol and triglycerides, TSH, glucose (and Hgb A1c, if glucose elevated, [although note that NASH has been reported to precede the diagnosis of DM]) and an ultrasound examination of the liver/spleen to document primarily whether increased liver echogenicity is present or not, a finding that may indicate steatosis or NASH. Increased liver echogenicity on ultrasonography is not specific for steatosis; it may also represent fibrosis or a combination of the two (fibro-fatty changes).

While CT-scanning may reveal diffuse or localized hepatic hypodensity consistent with fatty infiltration and T1-weighted MR scanning may reveal "brightness" also consistent with fat, neither can distinguish between FL and NASH (with the exception of the infrequent finding of focal fatty change in the liver which is typical for FL, not for NASH). Whether to proceed with either CT or MR scanning in this type of diagnostic evaluation is made on a case-by-case basis, but in the majority of cases it is not necessary.

Liver biopsy... to do or not to do?

The major clinical decision point reached after the above evaluation reveals only a patient with elevated serum transaminases and perhaps a hyperechoic liver is whether or not to proceed with liver biopsy Table 5). At this point in the evaluation , the patient appears likely to have FL or NASH. Is it important to distinguish between the two? Will establishing a precise diagnosis change the treatment recommendations or the "aggressiveness" of the treatment? Would it be important for a patient to know he/she had NASH with bridging fibrosis? Theoretically, establishing an exact diagnosis would be highly preferable; in practice, performance of liver biopsy depends heavily on the clinician's own bias, something that is highly variable but the general tendency is not to biopsy unless some unusual feature is present such as highly elevated transaminases, etc. The lack of treatment which has been proven to alter the course of NASH dissuades many clinicians from recommending liver biopsy as does the perceived (true or not) generally indolent nature of the disease.

TABLE 5... PROS & CONS OF PERFORMING LIVER BIOPSY IN SUSPECTED NASH VS FATTY LIVER

PROS	CONS
establishes diagnosis	expense
aids in prognosis... fibrosis/cirrhosis	risk
serial biopsies... progression or not	most patients will have FL, not NASH; many unnecessary biopsies
measure effect of therapy	no proven therapy except perhaps weight loss
required for entry into clinical trials	same rx recommendations for NASH & FL
staging can motivate patient to lose weight, etc.	

NASH in Children

NASH is not a disease solely of adults, nor is obesity. The prevalence of obesity in children is rising as fast as if not faster than in adults. In 1984, A Japanese study (36) of 299 obese children found that 36 (12%) had elevated serum tranaminases. Liver biopsy was performed in 11 of the 36 and 8 had NASH, the other 3 fatty liver alone; 5 of the 8 had fibrosis and 1 cirrhosis.

A more recent multicenter study (37) from Massachusetts found 14 children (mean age 13.5 years) had NASH out of 82 total with hepatic steatosis seen over a 3 year period. All 14 were obese (mean 159% IBW) and had a mean ALT of 129±73 IU; 10 of the 14 had bridging fibrosis. **Thus, NASH is not rare in children and significant fibrosis may be found at an early age.**

The elevated serum transaminases in such children have been demonstrated to decrease or normalize with weight loss (38); some such children have sustained normalization of transaminases despite weight re-gain. What effect maturation has on NASH is children is not known.

A very intriguing treatment study with vitamin E, an anti-oxidant, is being carried out by J. Lavine at UCSD (1, 39). 10 children (mean age 12 years, mean ALT 136±74 IU, mean BMI 32) with NASH were given 400 U vitamin E daily for 1 month; if their ALT did not normalize or near-normalize, 800 IU was given the 2nd month and 1200 IU, the 3rd (only 1 patient required this later dose). The children were also advised regarding weight loss. After 4 to 10 months follow-up, the cohort had no change in BMI (still 32) but the mean ALT had fallen to 41±27 ($p<0.0003$). The children tolerated the vitamin E well and are being continued on it for follow-up observations.

Natural History of NASH

The true natural history of NASH is unknown. The major series reporting follow-up with liver histology (11, 28, 29) have each been retrospective in nature with just a total of 28 patients combined with serial liver biopsies over a 1 to 7 year period. ***Among these 28 patients, 1 improved, 15 remained unchanged and the other 12 (43%) progressed histologically (4 of the 28 [14%] developed cirrhosis). Therefore, the available data shows that NASH is progressive in a substantial proportion of patients.***

A small **prospective** study of 12 patients with NASH is underway at Walter Reed Medical Center (40) and follow-up liver biopsies in 7 patients after 18 to 45 months showed disease progression in 4 of the 7 (***3 with worse fibrosis [43%], the same as from the combined series above,*** 1 with worse steatosis).

In the Powell et al series (29), 2 patients were of particular interest. One of the patients with cirrhosis died 4 years later from hepatocellular carcinoma (HCC). The incidence of HCC in NASH is completely unknown. Another of the patients developing cirrhosis after 2 years lost all previous evidence of NASH over the next 8 years; if this patient had been biopsied only at the later point in time, the diagnosis most likely would have been "cryptogenic" cirrhosis. This loss of fat and inflammation as NASH progresses has been observed sporadically and reported anecdotally and deserves further attention in future studies.

NASH & cryptogenic cirrhosis

As noted above, NASH may progress to an inactive, non-fatty cirrhosis after a number of years and may, thus be termed as "cryptogenic" if the index biopsy had not been performed. It has been observed that many of the patients with "cryptogenic" cirrhosis have similar characteristics as those with NASH. A recent report (41) characterizing patients with

cryptogenic cirrhosis which comprised 18% of all patients with cirrhosis in their series of over 600. Analysis of data from 71 patients with cryptogenic cirrhosis revealed that the mean age at diagnosis was 60 years, 69% were female, a history of DM &/or obesity was present in 72% and 37% presented with ascites, 25% with variceal bleeding. The authors hypothesized that given the known potential evolution of NASH to a state of relatively bland cirrhosis, that some of their cases of cryptogenic cirrhosis may have progressed in such a manner given that the majority had similar characteristics to many patients with NASH. Clearly, better prospective studies are needed to test the validity of this hypothesis.

Pathogenesis of NASH

The visible fat in the liver is triglyceride within vacuoles in the hepatocytes. This hepatic fat accumulation (steatosis) results from 1 or more of the following:

- increased mobilization & delivery of FFA to the liver

  (normally, 99% of FA's are preformed, 1% *de novo* synthesized)
- increased hepatic synthesis of FFA

  (with glucose feeding & subsequent hypernsulinemia, approx. 15% of hepatic FA's are synthesized *de novo*, 85% pre-formed)
- impaired hepatic oxidation of FFA
- increased formation of triglycerides from FFA
- decreased export of triglycerides from the liver (decreased VLDL assembly or secretion)

The key question is what is responsible for the transition from a liver with excess triglycerides (i.e., fatty liver) to one in which an ongoing inflammatory process has been established (i.e., NASH)? The major hypotheses center around the roles of oxidative stress, lipid peroxidation and cytokine release.

Day and James (42) have hypothesized that the development of steatohepatitis from simple steatosis (the 1st "hit") requires some other perturbation (a 2nd "hit"). The 2nd hit would tend to overcome the normal cellular defenses by increasing the normal mild level of oxidative stress that is constitutively present. Increased oxidative stress as a result of some drugs like amiodarone would be a good example. Another source of oxidative stress in the form of increased reactive oxygen species (ROS) would be increased expression of cytochrome P450 2E1 (CYP2E1). Besides CYP2E1, other potential sources of free radicals are mitochondrial respiration with increased NADH/NAD, xanthine and aldehyde oxidases and peroxisomal ß-oxidation of FFA which generates hydrogen peroxide, which in turn, can interact with free iron to form more ROS. Interindividual differences in the severity of NASH could be explained by the magnitude of the 2nd hit or may be genetically determined (such as having a more readily inducible form of CYP2E1) or by environmental factors such as the dietary intake of antioxidants or exposure to pro-oxidant stresses.

Free Fatty Acids

The visible fat (triglyceride) is inert in NASH; "invisible" FFA's and/or ketones have been hypothesized to be a key initiating factor in NASH pathogenesis (43). The normal fate of FFA in the liver is either mitochondrial oxidative production of ATP or ß-oxidation via the TCA cycle. One or more of the first 3 disturbances above may result in excessive FFA in the liver (44). **FFA can induce CYP2E1 which can result in increased generation of ROS and**

lipid peroxidation. If the increased levels of FFA saturate the mitochondrial ß-oxidation pathway, then peroxisomal ß-oxidation can provide a further source of free radicals by generating H_2O_2, which can in turn react with free iron to form hydroxyl radicals. While these hypotheses are far from proven in humans, various pieces of evidence from animal models support the basic theories.

Hyperinsulinemia results in fat deposition and may contribute to elevated levels of hepatic FFA. There are several case reports of solely subcapsular hepatic steatosis forming in patients receiving chronic ambulatory peritoneal dialysis in which insulin was added to the dialysate (45, 46).

Lipid Peroxidation

Lipid peroxidation results from the action of ROS and other free radicals on FFA's or fatty acid side chains of membrane phospholipids, including mitochondria. **It has been shown in mice that hepatic steatosis, regardless of cause, is associated with lipid peroxidation (47). It has also been demonstrated in human livers with NASH that liver fat content is correlated with the degree of lipid peroxidation (48).** One of the products of lipid peroxidation, malodialdehyde, activates hepatic stellate cells (49) and is also a pro-inflammatory mediator, as is another product of lipid peroxidation, 4-hydroxylnonenal. These products can also stimulate neutrophil chemotaxis (50).

The NASH-causing drugs, amiodarone and perhexilene, have been shown to cause mitochondrial dysfunction (ATP depletion) and lipid peroxidation (5-10-fold increase) in cultured hepatocytes through the formation of ROS in mitochondria (13). This mechanism for drug-induced NASH supports the oxidative stress hypothesis.

Stellate Cell Activation

There are 4 types of non-parenchymal (hepatocyte) cells in the liver: 1. sinusoidal endothelial cells, 2. stellate cells (formerly called Ito cells or lipocytes), 3. Kupfer cells and 4. pit cells. Stellate cells comprise approximately 13% of all liver cells, are primarily periportal and normally store retinoids (51). They can be activated to a phenotypically different type of cell with a prominent protein-secretory apparatus and are capable of secreting many different substances including various cytokines and collagen. The activation of stellate cells can result from stimulatory cytokines released from Kupfer cells and from sinusoidal endothelial cells.

In rat hepatic stellate cells, oxidative stress (by malondialdehyde or Fe-generated free radicals plays an essential role in cell activation through the induction of c-*myb* nuclear expression and activation of NFkB (52). These effects can be blocked by anti-oxidants.

Kupfer cell activation

Kupfer cells are liver macrophages and are about as numerous as stellate cells; they are primarily periportal in location. Kupfer cells can be activated by endotoxin (LPS) or various cytokines and are capable of releasing a variety of substances, for example, TNF-a, interleukins, eicosanoids and also ROS (51).

Kupfer cells are hypothesized to be a mediator of alcohol-induced liver injury, being activated either directly or via another pathway such as endotoxin, and producing some of the secretory products noted above. It has been postulated that in NASH, FFA or ketones may play a similar role as alcohol in activating Kupfer cells.

Cytochrome P450 2E1 (CYP 2E1)

Among the large family of enzymes in the cytochrome P450 system, CYP2E1 catalyzes the metabolism and, in many cases, the bioactivation of many low MW lipophilic compounds such as aromatic hydrocarbons, halogenated hydrocarbons, alcohols, ketones and nitrosamines. Normally, CYP 2E1 is expressed in zone 3 in a two to three cell thick rim around the terminal hepatic venule.

INCREASED CYP2E1 ACTIVITY IS A POTENTIAL SOURCE OF ROS (FREE RADICALS)

In alcohol-induced liver disease CYP2E1 appears to play an important role in the generation of free radicals resulting in lipid peroxidation and hepatocyte damage (53). In alcohol-fed animals, there is a direct correlation between liver CYP2E1 content and liver injury. CYP2E1 is capable of generating superoxide, hydroxyl and hydroxymethyl radicals.

CYP2E1 is also up-regulated in animal models of 2 of the key clinical settings associated with NASH, obesity and diabetes mellitus (54-56). CYP2E1 can also be induced by FFA and ketones (43, 57) with increased production of ROS as a result. In addition, the increased activity of CYP2E1 results in increased oxygen consumption and, thus, demand for oxygen.

RATS FED A DIET DEFICIENT IN METHIONINE & CHOLINE ARE AN ANIMAL MODEL FOR NASH DEVELOPMENT.

Rats fed a diet devoid of methionine-choline (MCD diet) develop fatty liver with inflammation and cellular injury, resembling human NASH. After 4 weeks, **the MCD diet rats have steatohepatitis in zone 3 and increased immunostaining of CYP2E1 corresponding to the extent of the steatohepatitis**; the CYP2E1 has been shown to be catalytically active (58-59).

[This is analogous to what is observed in rats fed alcohol with resultant liver injury. In the rat model of intragastric alcohol feeding with a corn oil diet normally results in a moderate increase in CYP2E1 immunostaining with histologic changes of 4+ FL, 2+ inflammation and 1+ fibrosis (60). Inhibiting CYP 2E1 with a chemical inhibitor (diacyl sulfide) results in only a slight increase in CYP2E1 with 2+ FL, 1+ inflammation and no fibrosis, thus implicating CYP2E1 in the actual pathogenesis of the steato-inflammatory changes seen in this model.]

This MCD diet rat model results (59) in depleted hepatic GSH stores and an increase in hepatic lipid peroxidation, thus documenting the oxidative stress is present and may result from CYP2E1 induction. The lipid peroxidation seen in this model can be almost completely blocked by chemical inhibitors of CYP2E1.

Rats on the MCD diet have mildly increased TNFa levels; TNFa can cause hepatocyte injury. When the rats are administered endotoxin (LPS), the levels of TNFa are greatly elevated compared to baseline (61). The rats become SAM (S-adenosyl methionine) depleted on the MCD diet; SAM is an important anti-oxidant. When LPS and SAM are concurrently administered, TNFa levels do not rise much above baseline.

INCREASED HEPATIC CYP2E1 IS THE 1ST POTENTIAL PATHOPHYSIOLOGIC DEFECT TO BE DEMONSTRATED IN HUMANS WITH NASH

In a landmark study (62), CYP 2E1 has been documented by specific immunostaining to be increased in 31 humans with NASH (Table 6) compared with 10 controls. The distribution of the CYP2E1 corresponds to that of the steatosis and, in severe cases, both extend into zones 2 and 1. This does not appear to be a generalized increase in cytochrome P450 proteins since CYP3A distribution is decreased in the NASH livers. CYP3A is involved in antipyrine metabolism, which has previously been documented to be decreased in NASH patients (63). The increase in CYP 2E1 was seen whether or not there was associated obesity, DM or hyperlipidemia. The pattern of CYP 2E1 distribution in the liver was similar to that of 6 patients with ASH. **Therefore, evidence is accumulating that CYP2E1 plays an important role in NASH pathogenesis in humans.**

TABLE 6... IMMUNOREACTIVITY FOR CYP2E1 IN LIVERS FROM PATIENTS WITH NASH & IN CONTROLS (adapted from Weltman et al, ref 62)

Group	n	extent of reactivity			intensity of reactivity			
		zone1	zone 2	zone 3	0	1+	2+	3+
Controls	10	10	---	---	---	10	---	---
NASH no cirrhosis	27	27	27	18	---	---	8	19
NASH with cirrhosis	4	[N/A due to loss of normal zonal architecture]			---	---	---	4

Leptin

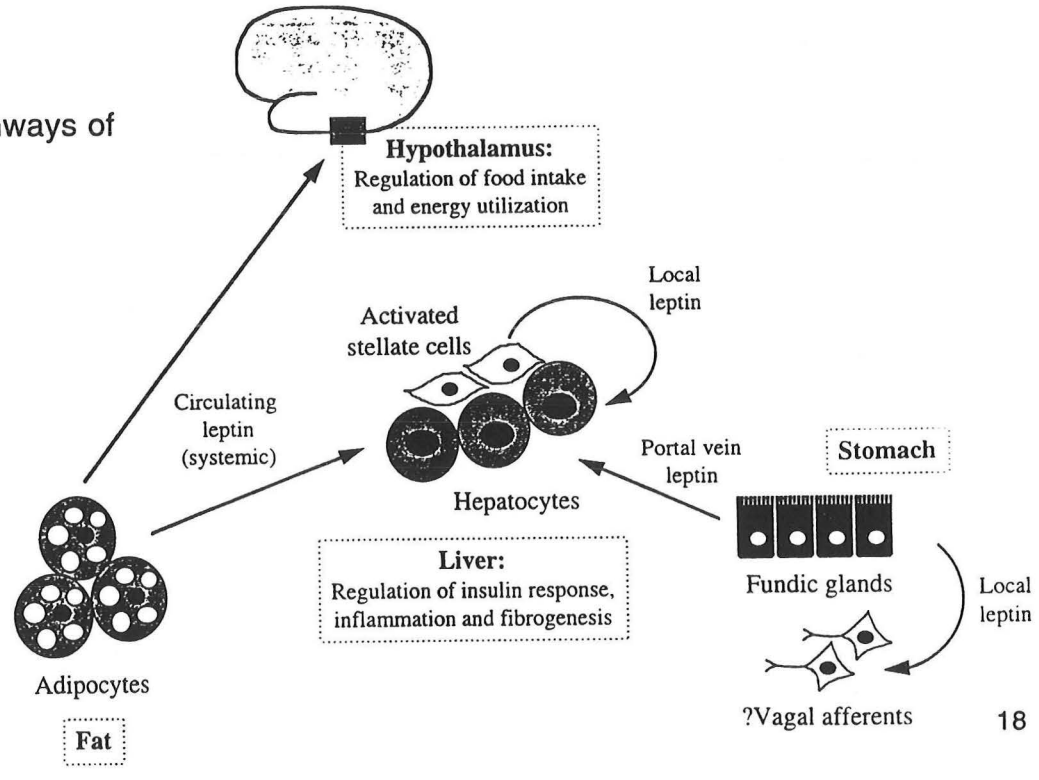


Figure 2
Sources & potential pathways of leptin action in the liver.
(from Kaplan, ref 65)

Leptin was identified in 1994 and is the product of the mouse*obese* gene (64). Plasma levels of leptin are correlated with the % body fat and virtually all obese persons have elevated leptin levels. Leptin thus appears to be important in weight regulation. Leptin is produced by adipocytes; its secretion is affected by a variety of physiologic processes - its major secretogues are: TNFa, IL-1, insulin and corticosteroids (65). Plasma leptin concentration decreases with fasting and increases with eating. Leptin regulates insulin secretion and tissue insulin responsiveness and down-regulates gluconeogensis (Figure 2). **Thus, it is possible that the high circulating leptin levels in obese persons contributes to hepatic steatosis by promoting insulin resistance and elevated plasma insulin levels and by altering insulin signaling in hepatocytes with promotion of increased free fatty acids.**

Leptin is considered a cytokine and results in the transcription of many target organ genes. There is increasing data to indicate that leptin can influence metabolic, inflammatory and fibrogenic responses in the liver. For example, in rodents, it appears to up-regulate selected inflammatory immune responses in the liver (66). In activated cultured hepatic stellate cells, leptin expression and synthesis was observed (67), suggesting the possibility that locally produced leptin may help initiate or propagate inflammatory activity. Leptin levels (as % of body fat) are elevated in alcoholic cirrhotics (68), more pronounced in women than in men, but the physiologic significance of this finding is not yet clear. Clearly, leptin is a potentially important area for future research in NASH patients

Hepatic Iron and NASH

It has been noted in most series that some patients with NASH either have elevated transferrin saturation, serum ferritin or stainable iron on liver biopsy. In the Bacon et al series (11), in addition to the findings noted in Table 7, 65% had 1+ or 2+ liver iron staining; none an hepatic iron index >1.9 or 3+ or 4+ liver iron staining.

TABLE 7... IRON STUDIES IN 31 PATIENTS WITH NASH (adapted from Bacon et al, ref 11)

	normal range	# abnormal	range of abnormal
transferrin saturation	10-55%	2/31 (6%)	58-78%
ferritin (ng/ml)	10-195	17/31 (55%)	218-1060
hepatic iron conc. (ug/g dry wgt)	<1500	4/10 (40%)	1608-2230
hepatic iron index (umol/g/age)	<1.9	0/10	

The significance of these findings has been uncertain, but **it now appears that iron may play a role in pathogenesis in some patients by promoting oxidative stress. In addition, the reason why some patients with NASH may have increased iron is related to their status for the hemochromatosis gene (HFE).**

Iron is a potent catalyst of oxidative stress and may act synergistically with other promoters of lipid peroxidation by catalyzing these reactions. Iron reacts with oxygen to generate hydroxyl free radicals and ferryl radicals. **Iron overload can directly cause lipid peroxidation**, and one of the subsequent products, malondialdehyde, in turn, has been

shown to activate hepatic stellate cells in vitro (52) and increased collagen production by activated stellate cells (69). Activated stellate cells are the major source of fibrogenesis in liver injury (70).

An important study (Table 8) from Brisbane, Australia (71) studied 51 patients with NASH and found that 31% were either homozygous (7.9%) or heterozygous (23.5%) for the C282T mutation of the HFE gene vs 12% of a control group. There was no difference in the frequency of the H63A mutation.

TABLE 8... Prevalence of Cys282Tyr and His63Asp mutations in 51 patients with NASH compared with an Australian control population (adapted from George et al, ref 71)

Mutation	NASH	Controls	Relative Risk	95% Confidence Interval
Cys282Tyr				
homozygous	4/51 (7.9%)	16/2375 (0.67%)	14.9	4.7-46.8 (p<0.0005)
heterozygous	12/51 (23.5%)	274/2375 (11.5%)	2.6	1.3-5.1 (p<0.005)
wild type	35/51 (68.6%)	2085/2375 (87%)	1	
His63Asp				
homozygous	2/51 (3.9%)	2/90 (2.2%)	1.9	0.3-14.4 (NS)
heterozygous	14/51 (27.5%)	20/90 (22.2%)	1.4	0.6-3.0 (NS)
wild type	35/51 (68.6%)	68/90 (75.6%)	1	

The presence of the C282T mutation (analyzed either with or without the 4 homozygotes) was significantly associated with hepatic iron staining, hepatic iron concentration and transferrin saturation. Increased hepatic iron (either concentration or by staining) was significantly correlated with the severity of fibrosis. Interestingly, those with the C282T mutation had significantly less steatosis. Not every patient with this mutation had elevated iron indices and not all patients with increased iron stores had the mutation

There are some discordant results in this regard. In a preliminary report from Massachusetts (72), 29 patients with NASH were genotyped for HFE and 5 (17%) were heterozygous for C282Y vs 6.7% of a control population; 1 of the NASH patients was homozygous The heterozygotes had significantly higher transferrin saturations, but did not have more fibrosis.

The effect of heterozygosity for the HFE gene on hepatic fibrosis is not specific for NASH; patients with chronic hepatitis C who are heterozygous for HFE have more fibrosis on liver biopsy also (73). This effect is independent of HCV RNA levels or degree of inflammation.

A final note on Fe and NASH is that there is also evidence (H Bonkovsky, NIH NASH Symposium) that hyperinsulinemia upregulates Tf (transferrin) receptors expressed on hepatocyte cell membranes and may thus lead to increased iron content intracellularly.

Genetic animal models (ob/ob mice & fa/fa rats) of fatty liver & steatohepatitis

ob/ob mice, homozygous for the obese/obese gene, fail to produce leptin and exhibit increased food intake and obesity; they develop hyperglycemia, hyperlipidemia, insulin resistance and fatty liver. fa/fa rats, homozygous for the fatty/fatty gene exhibit a similar obese phenotype.

In ob/ob mice or fa/fa rats, NASH quickly develops after exposure to low doses of LPS which has been shown to induce TNFa expression. The constitutive Kupfer cell activation that is a feature of these mice, with increased production of H_2O_2 in mitochondria, may promote the development of NASH by sensitizing hepatocytes to LPS (74). H_2O_2 levels (and other ROS and cytokines) are further increased after LPS exposure.

Induction of mitochondrial uncoupling proteins (UCP's) may reduce the efficiency of ATP synthesis, thereby increasing hepatocyte susceptibility to injury when demand for ATP increases. Leptin interacts with UCP's and UCP2 in hepatocytes is inducible by LPS and TNFa (75). Hepatocytes in these mice have adapted to the baseline oxidant stress by up-regulating expression of UCP2, which may be maladaptive by decreasing ATP production, as noted above. LPS further increases UCP2 expression above its baseline levels. This effect is blocked by pre-administration of neutralizing antibodies to TNFa, implicating TNFa as a mediator. In primary rat hepatocyte cultures, TNFa induces UCP2 m-RNA accumulation (76).

The genetically obese mice and rats provide good models to study the transitional events in the progression of from fatty liver to steatohepatitis, a phenomenon that is exceedingly difficult to study in humans.

Reduced capacity of human fatty livers to compensate for increased oxidative stress in vivo

It is interesting to note that when severe fatty livers are used as donors for liver transplantation (due to donor shortage, suboptimal donor livers are sometimes used), there is a high rate of primary and delayed donor nonfunction (77). This illustrates the increased susceptibility of fatty livers to the potentially injurious triggers of oxidative stress, endotoxin and hypoxia associated with graft reperfusion after transplantation.

Treatment of NASH

A single treatment may not be feasible due to the probable multiple mechanisms involved and perhaps different predominant mechanisms in different patients (such as lean vs obese patients). There is no correlation between serum tests and disease activity (liver histology), so liver biopsy is the only true measure of treatment efficacy.

Establishing NASH vs Fatty Liver and Stage of NASH

It is important to define the disease one is dealing with; it has already been noted that fatty liver is indistinguishable from NASH using all non-invasive parameters. If NASH is present, the degree of fibrosis present, if any, would be of importance to know in tailoring how aggressive to be with various aspects of treatment. Having said this, there is wide variability in how often liver biopsies are performed in patients with presumed fatty liver or NASH. The opposite point of view to the statement above would be that since there is no effective therapy for NASH and the patient will be advised to loss weight anyway, why is the biopsy really needed?

Alcohol Restriction

How much is too much? In a patient with NASH, is any alcohol too much? Since many of the presumed pathophysiologic mechanisms involved in alcoholic and non-alcoholic steatohepatitis are similar (oxidative stress, lipid peroxidation, increased cytokine production, etc.), is it not reasonable to restrict all alcohol intake in NASH? The answers are, of course, all unknown.

These questions assume that a diagnosis of NASH (vs FL) has been made by biopsy and the disease has been staged in terms of fibrosis; knowing that a patient had bridging fibrosis would be very helpful in advising the patient regarding prognosis and the potential effects of alcohol, whereas in a patient with mild NASH and no fibrosis, a more liberal attitude towards any alcohol consumption could be taken. Of course, one could take the stance that all alcohol consumption should be stopped in all patients with NASH or FL, but is this really necessary and is it practical---will a patient comply?

Weight Control

Modest gradual weight loss can result in improvement of serum transaminases and in the liver histology (78). As with most other aspects of NASH, weight reduction has not been systematically and prospectively studied and, thus, only case reports and very small series exist. It is, of course, the most reasonable advice to offer patients with NASH.

Although it seems almost axiomatic that weight loss will result in improvement in NASH, this is not necessarily the case. Rapid weight loss can actually be detrimental. Andersen et al (79) studied the effect of weight loss using a very low calorie liquid diet on liver histology in 41 NASH patients and found that lower weight (median loss 34kg) was associated with less steatosis and inflammation, but 24% of patients had less steatosis but increased inflammation and fibrosis.

A major problem with weight reduction as a means of treatment is that obesity (or "overweight-ness", if not true obesity) is now being increasingly recognized as a chronic disease, analagous to hypertension or diabetes, difficult to "cure". "Diets" routinely fail, since the perception of the dieter is that this one episode of markedly reduced caloric intake will solve "the problem", when in fact, "the problem" is life-long. Permanent weight loss is a vanishing ephemeral goal that is rarely achieved unless the patient has a significant change in "lifestyle" in terms of the ratio of caloric intake vs expenditure. The effect of weight cycling, with loss, then re-gain, on the progression of NASH is not known and deserves further study.

Diabetes Mellitus

Treatment of DM is a standard recommendation when present in NASH, but there is no evidence that it affects disease activity or progression. Nonetheless, good control of DM in such patients appears reasonable.

A preliminary report (80) on the use of troglitazone for 2 to 4 months in 6 patients with NASH (3 with mild DM) has found that 4 of the 6 normalized their ALT and this effect was sustained 3 months post-treatment.

Serum Lipids

Similar to treatment of DM, treatment of any hyperlipidemia present in NASH has not been shown to decrease disease activity, but does appear to be reasonable.

Clofibrate has been shown not to be effective in a study (30) of 16 patients with NASH and elevated triglycerides; after 1 year of therapy, there was no change in ALT or liver biopsy findings.

Gemfibrazole has been studied in 46 patients with NASH in a controlled randomized study (81). Preliminary results have shown that after 4 weeks, 17 of the 23 patients on gemfibrazole have decreased ALT (mean decreased from 72±37 to 50±28 IU/L) while 7 of 23 on placebo have (mean showed no change... 76±35 pre- and 77±25 post-); final results of this ongoing study are awaited.

There have been no studies of statins in NASH yet reported. Interestingly, simvastatin has been shown (82) to decrease the growth of human hepatic stellate cells, independently of its effect on cholesterol synthesis.

Ursodiol

In a pilot study (30) of ursodiol in 24 patients, investigators at the Mayo Clinic found that treatment with 13-15 mg/kg/day ursodiol resulted in decreased ALT values and less steatosis on repeat liver biopsy in 12 of 19 patients. In the ongoing prospective randomized controlled trial of ursodiol at the Mayo Clinic (1), 65 patients have now been randomized to ursodiol or placebo; liver biopsy will be performed after 2 years to assess the results.

In an Italian study (83) of 31 patients with NASH, 16 received ursodiol 10 mg/kg/day + a "low fat diet" (?gms) and 15, the low fat diet alone. After 6 months of treatment, 14/16 patients in the ursodiol group had normal ALT's while 4/15 in the other group did. After discontinuation of ursodiol, 6/14 patients who had normalized their ALT had a recurrence of ALT elevation.

Recently, ursodiol was shown in vitro to raise the apoptotic threshold to ROS in hepatocytes by preventing mitochondrial membrane damage (83a,83b)

Vitamins

With virtually unrestrained vitamin and herbal therapy the latest fad, *caveat medicatum.* In a study (84) of 4 patients with NASH who were given a regimen of 20 gm lecithin, 250 mg vitamin C, 50 IU vitamin E, 2500 IU beta carotene, 50 ug selenium and 300% RDA vitamin B complex, repeat liver biopsies after 12 weeks showed reduced steatosis in 2 of the 4 but increased fibrosis in 3 of the 4. Whether this result reflects intraobserver error or a true finding remains to be seen; the results do emphasize the potential hazards of polypharmacy outside of a clinical trial..

Phlebotomy

A preliminary report (85) studied the effect of phlebotomy in 8 patients with NASH , all with ≤1+ iron staining on biopsy, with mean ferritins of 300±90 and transferrin saturations of 27±6%. After a mean of 8 units (range 3-14) blood removed over 3 to 24 months, the patients' mean ALT fell from 179±39 u/L to 67±13 ($p<0.01$); the effect on liver histology of phlebotomy still needs to be evaluated with repeat liver biopsy.

Discontinuation of exposure to environmental toxins

A very intriguing preliminary report (86) implicates industrial exposure to petrochemical toxins as a cause of NASH. 20 non-obese, non-diabetic patients with biopsy-proven NASH were removed from not only the job but also the city in which the industrial plant was located. Repeat liver biopsies 1 year later in 9 of these patients revealed significant improvement in NASH (both inflammatory and fibrotic components) in all. Job exposure to toxins is an ill-studied aspect of NASH and deserves further consideration.

NASH & Liver Transplantation

There are increasing reports (87) of recurrence of NASH after OLT, sometimes remarkably quickly. One such report from the Mayo Clinic (88) found that of 5 patients transplanted for NASH, it recurred in 2 at 4 and 6 weeks post-OLT. One of these required re-transplantation and NASH recurred again at 3 weeks. In another report from Wisconsin (89), NASH also recurred in 2 of 5 patients post-OLT; 1 of these had NASH with fibrosis, the other with cirrhosis which developed 1.5 years post-OLT. Lastly, 3 of 5 patients transplanted at the U of Nebraska for NASH re-developed the disease post-transplant; none had significant weight gain post-operatively (M. Sorrell, NIH NASH Symposium). These cases illustrate the concept that a systemic derangement of fat metabolism that is not "cured" by OLT is responsible for NASH.

Conclusions incorporating known data and hypotheses...

NASH is a metabolic disease whose prevalence is higher than currently thought. Liver biopsy is the only certain method of diagnosis. NASH is caused by multifaceted disturbances of hepatic lipid homeostasis. It should not be considered a disease seen only in obese diabetic women; whether the pathophysiology is the same in lean, otherwise healthy, men remains to be seen.

Elevated FFA levels in the liver are probably essential for disease initiation. Oxidative stress and lipid peroxidation are important intracellular disturbances that result in propagation of inflammation and cell injury. Increased expression of hepatic CYP2E1 is involved in NASH pathogenesis. Cytokine production, stellate and Kupfer cell activation are also involved.

The course of NASH is highly variable but it is well underestimated as a cause of cirrhosis. Gradual sustained weight loss appears to improve NASH, but is not easily attainable. In the future, mutli-targeted therapy will be needed to reverse the multiple mechanisms of cell damage. NASH may recur after liver transplantation if the underlying pathophsiologic disturbances are not addressed.

Future Research

The quality of most of the clinical research concerning NASH is suboptimal, particularly that performed before the 90's when hepatitis C could be identified with some certainty. The lack of uniform histologic diagnostic criteria have been a major stumbling block since 1980 but this is now being seriously addressed by the NIH Pathology NASH Working Group. Tremendously important advances have been made recently in potential cellular mechanisms of NASH pathogenesis in the past decade. Well-conducted clinical studies on prevalence in the general population, on the prospective natural history and controlled treatment trials are all needed.

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