SPECIAL ARTICLE

Nonalcoholic Steatohepatitis: Summary of an AASLD Single Topic Conference

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Fatty liver disease that develops in the absence of alcohol abuse is recognized increasingly as a major health burden. This report summarizes the presentations and discussions at a Single Topic Conference held September 20-22, 2002, and sponsored by the American Association for the Study of Liver Diseases. The conference focused on fatty liver disorders. Estimates based on imaging and autopsy studies suggest that about 20% to 30% of adults in the United States and other Western countries have excess fat accumulation in the liver. About 10% of these individuals, or fully 2% to 3% of adults, are estimated to meet current diagnostic criteria for nonalcoholic steatohepatitis (NASH). Sustained liver injury leads to progressive fibrosis and cirrhosis in a fraction, possibly up to one third, of those with NASH, and NASH may be a cause of cryptogenic cirrhosis. NASH is now a significant health issue for obese children as well, leading to cirrhosis in some. The diagnostic criteria for NASH continue to evolve and rely on the histologic findings of steatosis, hepatocellular injury (ballooning, Mallory bodies), and the pattern of fibrosis. Generally recognized indications for biopsy include establishing the diagnosis and staging of the injury, but strict guidelines do not exist. Liver enzymes are insensitive and cannot be used reliably to confirm the diagnosis or stage the extent of fibrosis. Older age, obesity, and diabetes are predictive of fibrosis. The pathogenesis of NASH is multifactorial. Insulin resistance may be an important factor in the accumulation of hepatocellular fat, whereas excess intracellular fatty acids, oxidant stress, adenosine triphosphate (ATP) depletion, and mitochondrial dysfunction may be important causes of hepatocellular injury in the steatotic liver. Efforts are underway to refine the role of insulin resistance in NASH and determine whether improving insulin sensitivity pharmacologically is an effective treatment. An altered lifestyle may be a more effective means of improving insulin sensitivity. The research agenda for the future includes establishing the role of insulin resistance and abnormal lipoprotein metabolism in NASH, determining the pathogenesis of cellular injury, defining predisposing genetic abnormalities, identifying better noninvasive predictors of disease, and defining effective therapy. (HEPATOLOGY 2003;37: 1202-1219.)

The following is a summary of talks presented at the American Association for the Study of Liver Diseases (AASLD) Clinical Single Topic Conference on Nonalcoholic Steatohepatitis (NASH) held in Atlanta, Georgia on September 20-22, 2002. The syllabus and lecture materials prepared by the conference's 25 presenters form the basis of this summary. As course organizers, the authors of this summary would like to acknowledge the presenters for their substantial contributions. These individuals are listed in the Appendix.

The objectives of AASLD Single Topic Conferences are to bring together researchers and practitioners with a focused interest in a specific area of liver disease to review current thinking and identify areas needing further discovery. The objective of this particular conference was to bring together active clinical and basic science researchers in the field of fatty liver disease to critically analyze the latest developments and share ideas with respect to pathogenesis, diagnosis, and treatment. A focus of the meeting was the role of insulin resistance and hyperinsulinemia in the pathogenesis of nonalcoholic fatty liver disease (NAFLD) and NASH. Because of the focused nature of

Abbreviations: AASLD, American Association for the Study of Liver Diseases; NASH, nonalcoholic steatohepatitis; NAFLD, nonalcoholic fatty liver disease; AST, aspartate aminotransferase; ALT, alanine aminotransferase; IRS-1, insulin receptor substrate 1; TNF-α, tumor necrosis factor α, VLDL, very low density lipoprotein; apoE, apolipoprotein E; MTTP, microsomal triglyceride transfer protein; SREBP, sterol regulatory element binding protein; ATP, adenosine triphosphate; UCP, uncoupling protein.

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the conference, many key aspects of fatty liver disease could not be discussed in depth. The purpose of this report is to provide an overview of the conference and not summarize all aspects of NAFLD and NASH.

Defining NAFLD, NASH, and the Meaning of "Nonalcoholic"

The term *NASH*, coined by Ludwig et al.[1] in 1980 to describe the biopsy findings in patients with steatohepatitis in the absence of significant alcohol consumption, has served the field well by bringing attention to this entity and promoting further research. However, this inclusive term has become problematic because it requires a pathologist to make a clinical statement about alcohol consumption. As recently proposed,[2,3] an alternative name—metabolic steatohepatitis—was discussed but not uniformly accepted. Another alternative under consideration by pathologists is to further simplify the nomenclature by reporting only steatohepatitis using specific criteria (see later) and leaving it up to the clinicians to assign causality and risk factors.

NAFLD is defined currently as fat accumulation in the liver exceeding 5% to 10% by weight, but it is estimated practically as the percentage of fat-laden hepatocytes observed by light microscopy.[4] Whether NAFLD with the minimum amount of fat is truly a disease, hence the D in the acronym, or simply a benign condition, was debated. Progression to cirrhosis is rare in mild NAFLD, yet progression has been observed and any amount of fat may sensitize the liver to injury from other causes. Clearly, there is wide agreement on the need for a consensus regarding the precise criteria for classifying, grading, and staging histologic injury in these disorders collectively known as NAFLD (see later).

Inherent to defining NAFLD and NASH is the threshold at which steatohepatitis becomes alcohol related. This is not a sharply demarcated distinction. Many centers accept up to 14 to 28 units of ethanol per week (up to 20-40 g/d in men and 20 g/d in women) whereas other investigators have used a cut-off level of 7 units/wk (10 g/d) or less. One report has suggested that limited alcohol intake is protective against NASH (as well as diabetes).[5] Given the health benefits derived from modest ingestion of ethanol, this problem is unlikely to be resolved easily. As Dr. Oliver James has suggested, a reasonable compromise is to accept a working figure of 14 units/wk (20 g/d or roughly the equal of 2 glasses of wine per day) with acknowledgment that there will be uncertainty in the gray areas of this limit. This cut-off level is well below the traditional threshold for alcohol-induced liver disease.

The spectrum of histologic abnormalities defined by NAFLD includes simple steatosis (steatosis without other injury) and NASH as its more extreme forms. How NASH is best defined remains unsettled because there is significant diversity of opinion among expert pathologists regarding the necessity and character of specific findings. These include the amount and types of fat (macrovesicular and microvesicular), lobular inflammation (acute and/or chronic), and fibrosis (zone 3 and portal). In addition, the histologic findings in the pediatric population may differ from those in adults.[6,7] One of the key histopathologic features of NASH in adults (Table 1) is the presence of perisinusoidal fibrosis (Fig. 1), whereas in children portal fibrosis may be more characteristic. A proposed method of grading and staging NASH based on the presence and degree of the major histopathologic findings is shown in Table 2.

A NAFLD classification system also has been proposed that correlates certain histologic features with the long-

Table 1. Histopathologic Abnormalities in NASH

Necessary components
Steatosis, macro > micro; accentuated in zone 3
Mixed, mild lobular inflammation; scattered polymorphonuclear leukocytes as well as mononuclear cells
Hepatocellular ballooning; most apparent near steatotic liver cells, typically in zone 3
Usually present; but not necessary for diagnosis
Zone 3 perisinusoidal fibrosis
Zone 1 hepatocellular glycogenated nuclei
Lipogranulomas in the lobules; of varying size, but usually small
Occasional acidophil bodies or periodic acid-Schiff–stained Kupffer cells
Fat cysts
May be present but not necessary for diagnosis
Mallory's hyaline in ballooned hepatocytes
Usually zone 3 in NASH, may be zone 1 in diabetes, amiodarone
Typically poorly formed, may require immunostaining for ubiquitin, p62, or cytokeratins 7, 18, 19 to confirm
Mild (1+) granular periportal (zone 1) hepatocellular iron or scattered iron granules in sinusoidal lining cells (best detected by Prussian blue stain)
Megamitochondria in hepatocytes
Unusual for NASH; recommend consideration of other causes of liver test abnormalities
Macrovesicular steatosis: <30% of parenchyma involved or nonzonal distribution
Purely or predominantly microvesicular steatosis
Sclerosing hyaline necrosis, veno-occlusive lesions, perivenular fibrosis, phlebosclerosis
Portal inflammation greater than lobular inflammation, lymphoid aggregates, numerous plasma cells
Significant eosinophils in portal or lobular inflammation; epithelioid granulomata
Portal/periportal fibrosis in absence of, or markedly greater than, zone 3 perisinusoidal fibrosis; portal-based bridging fibrosis without concurrent perisinusoidal fibrosis
Lobular disarray, marked inflammation or confluent or bridging necrosis, endophlebitis
Acute cholestasis: bile plugs
Chronic cholestasis +/− bile duct lesions (florid duct lesions), bile duct loss, ductular proliferation; copper granules in periportal hepatocytes
Periodic acid-Schiff–stained globules (α_1AT) in periportal hepatocytes
Significant granular iron in hepatocytes +/− zone 1 to zone 3 gradient

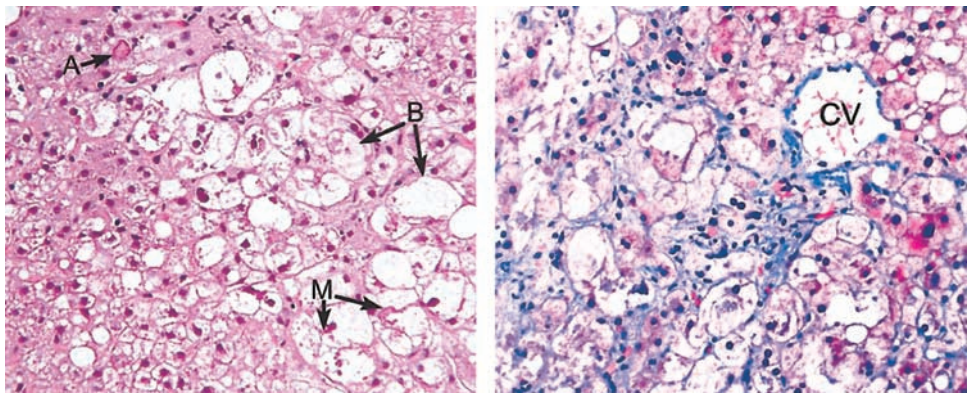

Fig 1. (**Left panel**) Characteristic hepatocellular abnormalities of NASH. Mixed micro- and macrovesicular steatosis is present and examples of acidophil bodies (A), ballooning (B), and Mallory's hyaline (M) are identified by **arrows**. The ballooned cells are enlarged, have pale to clear cytoplasm, and fragments of cytoskeletal aggregates within. Ballooned hepatocytes are markers of cell injury, most commonly noted in alcoholic and nonalcoholic steatohepatitis (hematoxylin-eosin stain, original magnification ×400). (**Right panel**) The characteristic initial pattern of fibrosis in steatohepatitis is the perisinusoidal collagen deposition in zone 3 around the central vein (CV), as identified by blue staining. This is the portion of the acinus predominantly affected in steatohepatitis (Masson's trichrome stain, original magnification ×400). Photographs courtesy of Elizabeth Brunt.

term prognosis[8] (these groups are identified variably as class or type). Class 1 constitutes simple steatosis, class 2 is steatosis with lobular inflammation, class 3 requires the additional presence of ballooned hepatocytes, and class 4 requires the presence of either Mallory's hyaline or fibrosis. Within this system, class 3 and 4 NAFLD are similar and might be considered as a single group constituting NASH.[9] Class 2 NAFLD is more controversial[10]; it may be benign and includes relatively more men, often with a normal body mass index.[11]

Table 2. Proposed Grading and Staging of NASH

Grade 1, mild
Steatosis: predominantly macrovesicular, ranges from less than 33% to up to 66% of the lobules
Ballooning: occasionally observed; zone 3 hepatocytes
Lobular inflammation: scattered and mild acute (polymorphs) and chronic inflammation (mononuclear cells)
Portal inflammation: none or mild
Grade 2, moderate
Steatosis: any degree, usually mixed macrovesicular and microvesicular
Ballooning: present in zone 3
Lobular inflammation: polymorphs may be noted associated with ballooned hepatocytes, and/or pericellular fibrosis; ± mild chronic inflammation
Portal inflammation: none, mild to moderate
Grade 3, severe (florid steatohepatitis)
Steatosis: usually >66% (zone 3 or panacinar); commonly mixed steatosis
Ballooning: predominantly zone 3; marked
Lobular inflammation: scattered acute and chronic inflammation; polymorphs may appear concentrated in zone 3 areas of ballooning and perisinusoidal fibrosis
Portal inflammation: mild or moderate; not predominant or marked
Staging (requires Masson trichrome or equivalent stain): a separate portal-based process with sparing of zone 3 has been proposed but remains to be established or refuted
Stage 1: Zone 3 perivenular, perisinusoidal, or pericellular fibrosis; focal or extensive
Stage 2: As for stage 1 plus focal or extensive portal fibrosis
Stage 3: Bridging fibrosis, focal or extensive
Stage 4: Cirrhosis with or without residual perisinusoidal fibrosis

Modified from Brunt et al.[221]

Class 3-4 NAFLD or NASH is described further by using 4 stages of fibrosis. In addition to the stages shown in Table 2, a separate group of adult patients with primarily periportal fibrosis has been described, but this variant is not yet established as a distinct entity (V. Ratziu, unpublished data). Stage 4 NASH has been suggested to include NASH with cirrhosis, cirrhosis with features of NASH, and cryptogenic cirrhosis.[12] It is now accepted that cryptogenic cirrhosis may represent a late phase of NASH that has lost the typical necroinflammatory and steatotic features in up to 80% of patients.[13-16]

Clinical Aspects

Prevalence and Prognosis. NAFLD is perhaps the most common of all liver disorders.[17-23] Wanless and Lentz[24] found steatosis in 70% of obese and 35% of lean patients and NASH in 18.5% of obese and 2.7% of lean patients in a consecutive autopsy study. Among obese patients, the prevalence of class 1 NAFLD (simple steatosis) is about 60%, whereas NASH is found in 20% to 25% and 2% to 3% have cirrhosis.[5,25-31] Among type 2 diabetic patients, it is estimated that 75% have some form of fatty liver.[32-35]

A number of reports have addressed clinical predictors of more advanced histology on the initial diagnostic biopsy. Among these, age greater than 40 to 50 years, and the severity of obesity, diabetes, or hyperlipidemia (especially hypertriglyceridemia) are among the most reliable.[5,14,26-29,36,37] The role of female gender has been more

variable in reported series, but the relatively increased prevalence of women in patients with more advanced disease supports female gender as a risk for progression.[8,11,27,28,36] Other reported predictors of advanced disease include elevation of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and an AST: ALT ratio greater than 1.[5,8,20,26,27,38] However, it is well known that significant liver disease may exist with liver enzymes in the normal range among NAFLD patients. This could represent upward drift in the normal range, but treatment with antidiabetic medications also may produce normalization of the aminotransferase levels despite pre-existing liver disease. Elevated serum immunoglobulin A level is under study as a potential predictor of disease activity.[39] It is important to note that all of these factors have not been studied adequately as predictors of progression over time but rather indicate the likelihood of finding more advanced disease on the initial biopsy. It is, however, likely that they also carry long-term prognostic significance.

The 5- and 10-year survival in NASH has been estimated at 67% and 59%, respectively, although death often may be from comorbid conditions.[40] In Japan, the ratio of observed versus expected deaths was actually higher for cirrhosis than for heart disease in people with diabetes (2.67 vs. 1.81).[41] Longitudinal studies of NASH are few in number. Compiling figures from several reports (n = 32 patients), the risk in class 3 and 4 NAFLD for developing increased fibrosis over approximately 5 years is 25% and for developing cirrhosis is 15%.[11,13,27,31] Preliminary studies suggest a more benign course for class 2 NAFLD,[8] although even simple steatosis also has been shown to progress occasionally to cirrhosis.[8,42] Raw survival figures fail to reveal the morbidity among obese diabetic patients who develop cirrhosis and recent studies have indicated marked risk in such patients for complications of portal hypertension and a significant risk for hepatocellular cancer in the subset who progress to cirrhosis.[43-46]

NAFLD is reported increasingly in pediatric patients. Sixty percent of adolescents with elevated liver enzyme levels are obese or overweight[47] and it is estimated that greater than 1% to 2% of adolescents have NAFL. The spectrum of histologic injury in this group clearly includes cirrhosis.[6] Lavine presented data showing the predictive power of the degree of insulin resistance and elevation of the aminotransferase levels. Unfortunately, no longitudinal studies yet exist.

Gender, Ethnic, and Familial Considerations. It is now suspected that there is an even distribution of NASH among men and women although there may be gender variation among the specific classes. Series of patients with more advanced disease have generally had more women, suggesting a more aggressive course.[10] Surveys have suggested ethnic variation with relative paucity among African Americans compared with European and Hispanic Americans. This may represent variation in referral patterns or genetic differences in body fat distribution or metabolic thermogenesis.[48-55] Clustering within kindreds also has been described, further suggesting that genetic factors predispose to the development of NASH.[56,57]

History. A history of obesity, diabetes, or hyperlipidemia is common but not invariable. An increasing number of patients have been described with normal body mass index, although these individuals may have central adiposity and occult insulin resistance.[58-61] Clinical findings include other features of metabolic syndrome such as hypertension, hyperuricemia, and polycystic ovarian syndrome (hirsutism, oligomenorrhea, or amenorrhea).[62-65] The importance of eliciting a previous history of features of the metabolic syndrome has been emphasized because changes in body composition due to aging and cirrhosis may mask prior severe and long-standing obesity.[66]

Associated Conditions. Because of their associations with metabolic syndrome, NAFLD and NASH are associated commonly with obesity, diabetes, and hyperlipidemia, as well as hypertension, hyperuricemia, and polycystic ovarian disease. Other conditions associated with these primary problems such as sleep apnea in obesity may be observed. In addition, an association with lipodystrophy has been observed although the exact mechanism is not clear.[67,68] Other noted associations include peroxisomal diseases,[69] mitochondrialopathies,[70-73] Weber-Christian disease,[74] Mauriac Syndrome,[75] Madelung's lipomatosis,[76] Wilson's Disease,[77] industrial solvent exposure,[78-80] medications[81] (amiodarone,[82] tamoxifen,[83,84] nucleoside analogues,[85] and methotrexate[86]), celiac disease,[87] and abetalipoproteinemia.[88] Many of these disorders have in common either abnormal fat metabolism and/or mitochondrial injury or dysfunction.

Symptoms, Signs, Laboratory, and Imaging. Referral is often precipitated by abnormal liver enzyme levels detected at routine evaluation or during antihyperlipidemic drug therapy (Tables 3 and 4). Variation in the reference ranges among obese patients may partially explain normal levels despite histologic disease.[89] It is not uncommon for patients to present with a complication of previously unrecognized cirrhosis despite long-standing medical care because these patients often lack the classic nutritional changes of cirrhosis. Because of the association between NAFLD and insulin resistance, laboratory evaluation of insulin sensitivity may be reasonable during the evaluation of patients with NAFLD (Table 4).

Table 3. Symptoms, Signs, Biochemistry and Imaging in NASH

Symptoms and physical findings
Fatigue (correlates poorly with histologic stage)
Right upper quadrant pain (usually mild but may be mistaken for gallstone disease)
Hepatomegaly
Bowel dysmotility and small bowel bacterial overgrowth[222,223]
Constipation (especially in children [J.E. Lavine, personal communication])
Anthropometric (waist circumference indicates central adiposity[136,137])
Acanthosis nigricans (especially in children[6])
Lipomatosis
Lipoatrophy/lipodystrophy (may be underrecognized in its focal or partial form)
Panniculitis (rare, feature of Weber-Christian)
Neurologic deficits (ocular muscle palsy,[224] also, deafness may be part of maternally inherited deafness and diabetes[225])
Palmer erythema, spider angiomata, and splenomegaly (cirrhosis)
Subacute liver failure[226]
Laboratory
Mild elevation of aspartate AST and ALT levels; levels seldom exceed 10× the upper level of normal and more typically are <1.5× the upper normal level[5,28]
ALT > AST; AST > ALT suggests significant fibrosis or cirrhosis (altered with antidiabetic therapy)[20,36,38]
γ glutamyltransferase and alkaline phosphatase level elevation[1,11]
Hyperglycemia (caused by the association with diabetes present in about one third of patients)
Hyperlipidemia (usually triglycerides) in approximately 20% to 25%[1,11,227]
IgA deposition has been described in histologic sections in NASH patients and serum IgA level is elevated in about 25%[39,228]
Antinuclear antibody in about one third of patients[229,230]
Abnormal iron indices (common but generally do not indicate genetic hemochromatosis[36,231-233])
Imaging[234]
Ultrasound, computed tomography, or magnetic resonance imaging: all insensitive to degrees of steatosis less than 25% to 30%
None of these modalities are able to reliably identify fibrosis and stage the disease[9]
Magnetic resonance spectroscopy: fat content and ATP levels in fatty liver[124]

Liver Biopsy. Liver biopsy is the only means of assessing the presence and extent of specific necroinflammatory changes and fibrosis. However, firm recommendations of when to perform a liver biopsy in the routine clinical setting have not yet been developed and care will continue to require individualization. A pragmatic approach in younger patients without clinical evidence of more advanced disease is a trial period of increased exercise and improved dietary habits. The role of baseline or serial biopsy in patients with liver abnormalities while using statin drugs has yet to be established. The use of surrogate markers such as aminotransferases and fibrosis markers may have a limited role in pilot studies but are not adequate end points for definitive investigations. The degree of sampling error and the significance of occasional apoptotic bodies are 2 areas that have not been studied adequately. Likewise, the use of ubiquitin for the detection of Mallory hyaline has yet to be explored fully. Megamitochondria with crystalline inclusions are seen commonly at electron microscopy[70,71] and form a part of the pleomorphic mitochondrial changes in NAFLD, which likely represent varied degrees of injury (free radical damage) and adaptation (uncoupling).

Pathophysiology

The development of steatosis, steatohepatitis, progressive hepatic fibrosis, and cirrhosis is most likely the result of multiple metabolic abnormalities taking place in the right (or wrong) genetic environment. The field has yet to develop a unifying framework that successfully organizes and reconciles the many diverse observations made to date. In lieu of such a framework, this conference summary will work from the simple model shown in Fig. 2.

Insulin Resistance: Mechanisms and Role of Cytokines and Fatty Acids. Insulin resistance is common in patients with NAFLD and NASH. Based on the hypothesis that insulin resistance and hyperinsulinemia are pri-

Table 4. Tests of Insulin Homeostasis

Fasting insulin
A simple approach to assess insulin resistance or latent diabetes. Fasting insulin per se provides an approximate measure of insulin sensitivity but is not felt to be very precise.
QUICKI and HOMA
The Quantitative Insulin Check Index (QUICKI) and Homeostasis Model Assessment of Insulin Resistance (HOMA) are mathematic models based on the product of the fasting insulin and glucose levels that provide similar measures of insulin sensitivity. The HOMA is calculated as insulin (mU/L) × glucose (μmol/L)/22.5; the QUICKI is calculated as 1/log(insulin [mU/L] × glucose [mg/dL]).
Hyperinsulinemic euglycemic clamp
The clamp technique is the gold standard. The insulin infusion rate can be tailored to examine hepatic sensitivity to insulin and/or peripheral glucose use. The index of insulin sensitivity (SIClamp) is defined as M/(G × ΔI) corrected for body weight (where M = steady-state glucose infusion rate (mg/min), G is the steady-state blood glucose concentration (mg/dL), and ΔI is the difference between basal and steady-state insulin concentrations (mU/L)).
Frequently Sampled Intravenous Glucose Tolerance Test (FSIGT)
A less labor-intensive estimation of insulin sensitivity that provides the minimal model index of insulin sensitivity (SIMM). It correlates with glucose clamp measurements in normal and obese subjects. The minimal model can generate an inaccurate index of insulin sensitivity in patients with impaired insulin secretion (*i.e.,* in overt diabetes).
C-peptide/insulin ratio
Hepatic degradation of insulin can be assessed by simultaneous measurement of fasting plasma insulin and C-peptide. A reduced C-peptide-to-insulin molar ratio therefore indicates impaired hepatic degradation of insulin. Renal impairment alters this relationship.
Oral Glucose Tolerance Test (OGTT)
Fasting plasma glucose (FPG) ≥ 110 and <126 mg/dL indicates impaired fasting glucose and FPG ≥ 126 mg/dL constitutes a provisional diagnosis of diabetes. Using a 75-g oral glucose tolerance test, the corresponding categories are used: 2 hour postload glucose < 140 mg/dL is normal; ≥ 140 and < 200 mg/dL is impaired glucose tolerance; and ≥ 200 mg/dL is consistent with diabetes. A firm diagnosis of diabetes requires repeat testing on another day.

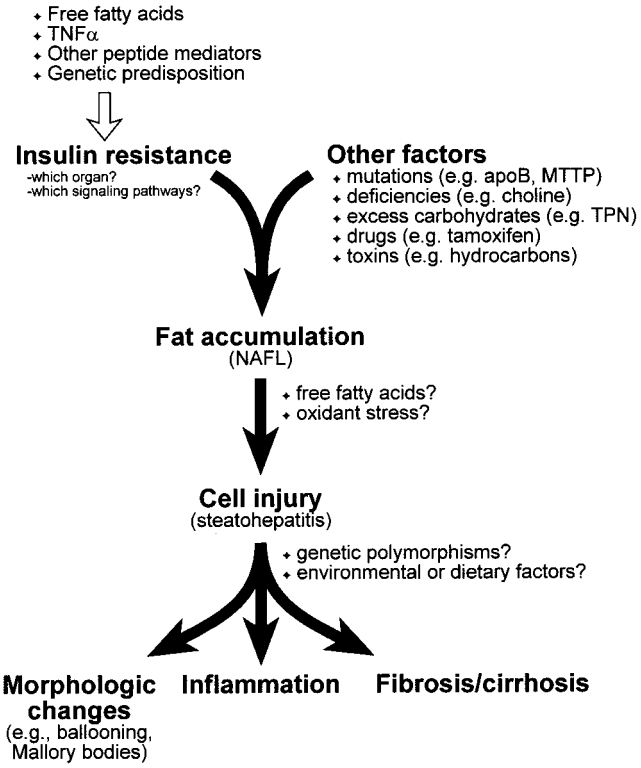

Fig. 2. A parsimonious model for the development of NAFLD and NASH. This model places insulin resistance at the top as an important antecedent metabolic abnormality in many patients. In what fraction of patients this is true is yet to be established. Other abnormalities, alone or with insulin resistance, also may contribute to hepatic fat accumulation. Excess fat in the liver predisposes to hepatocellular injury in some individuals. This may be caused by the direct cellular toxicity of excess free fatty acids, oxidant stress and lipid peroxidation, or other mechanisms. Hepatocellular injury may cause an inflammatory response with progressive fibrosis in a subset of patients; the extent of this adverse outcome most likely depends on a variety of environmental and genetic influences. Portions of this conceptual model have been referred to as the 2-hit hypothesis, accounting for the accumulation of fat as the first hit and hepatocellular injury in the fatty liver as the second hit.[237] Some investigators also have used a taxonomic distinction of secondary NASH, or that attributable to readily identifiable drugs, toxins, or genetic abnormalities, and primary NASH, or that which is not secondary and is probably related to insulin resistance. The value of these simplifications is uncertain.

mary abnormalities in NAFLD (rather than being caused by fatty liver), much of the conference discussion was focused on understanding the causes and consequences of insulin resistance in the hope that this understanding could lead to more effective treatment strategies to prevent progressive liver disease.

Insulin modulates intracellular signaling by activating at least 9 postreceptor pathways through the tyrosine kinase activity of the occupied insulin receptor.[90] Primary defects in the insulin receptor are uncommon, whereas defects in one or more of the postreceptor pathways appear to underlie the preponderance of insulin resistant states. It follows that, paradoxically, although some signaling pathways are impaired, others may be overactivated as a result of a hyperinsulinemic state. The significance of this excessive signaling has not been explored with respect to liver disease.

A major mechanism of insulin resistance is the down-regulation of insulin receptor substrate 1 (IRS-1) signaling by excess free fatty acids. Several decades ago, the Randle hypothesis was used to explain the inhibitory effect of fatty acids on muscle glycogen formation by invoking strictly biochemical mechanisms. However, a series of elegant experiments over the past decade using ^{1}H-NMR and ^{13}C-NMR in humans have shown that fatty acids impair the tyrosine phosphorylation of IRS-1.[91-93] Tyrosine phosphorylation of insulin receptor substrates is a general mechanism of insulin action; in contrast, impaired tyrosine phosphorylation, accelerated dephosphorylation, and phosphorylation of serine residues all have the effect of deactivating insulin receptor substrates such as IRS-1, leading to insulin resistance.

Most of what is known about the mechanisms of insulin resistance is based on studies in muscle. In muscle, activated IRS-1 promotes translocation of the glucose transporter protein GLUT4 to cell membranes. As a result, myocyte glucose uptake by GLUT4 increases glucose disposal from blood and a reduced need for insulin. The focus is now on which kinases or phosphatases are responsible for altering insulin receptor substrate activation; in muscle, PKC-θ is a likely candidate as a serine kinase regulated by fatty acids that can impair the activation of IRS-1.

Insulin sensitivity also is regulated by peptide mediators. Adipose tissue, especially mesenteric fat with its venous blood flowing directly to the liver, is a rich source of cytokine and peptide hormone production that regulates downstream metabolic activity. Examples include tumor necrosis factor α (TNF-α), angiotensinogen, plasminogen activator inhibitor-1, leptin, and complement components.

TNF-α is derived primarily from adipose tissue in the absence of active infections or inflammatory conditions and, under normal conditions, its plasma levels correlate with body fat mass. A major role of TNF-α in the link between adipose tissue mass and insulin resistance is suggested by several important animal studies and observations in humans.[94] Most convincing is the TNF-α knockout mouse, which fails to develop insulin resistance after induction of obesity.[95] Down-regulation of IRS-1 signaling caused by serine phosphorylation of IRS-1 appears to be the underlying mechanism. It is uncertain how TNF-α stimulates serine phosphorylation, but JNK, several PKC isoforms, and IκKβ all may have important roles in this process. Although it is probably not clinically

relevant, important mechanistic insights were obtained by treating patients with high doses of aspirin to inhibit IκKβ, an intervention that improved insulin sensitivity and glucose disposal.[96,97]

The relative roles played by increased serum free fatty acids levels and cytokines such as TNF-α in mediating insulin resistance remains a major unresolved issue. On the one hand, circulating free fatty acid levels remain high in patients after obesity surgery but insulin sensitivity improves. On the other hand, circulating TNF-α levels tend to be lower than would be expected if the cytokine is to exert a physiologic effect.

Leptin may play an important role in regulating the partitioning of fat between mitochondrial β-oxidation and triglyceride synthesis.[98] Defects in leptin signaling, such as the deficiency that characterizes the ob/ob mouse, are associated with preferential accumulation of fat and impaired β-oxidation of fat in the liver. Defects in humans are related more commonly to acquired states of leptin resistance rather than a deficiency of the hormone. New data relevant to the development of fibrosis in NASH has suggested that leptin is necessary for the development of liver fibrosis.[99,100] Other potentially important adipocyte-derived mediators of insulin resistance include resistin (in the mouse, yet to be shown in humans) and adiponectin (Acrp30, AdipoQ).[101,102] Adiponectin has a cytokine structure and, unlike other peptide mediators, appears to improve hepatocyte insulin sensitivity.[103]

Oxidant Stress and Liver Injury. Oxidant stress frequently is stated to be a central mechanism of hepatocellular injury in NASH. This conclusion is drawn primarily from models of fatty liver disease in animals and studies in humans that correlate markers of oxidant stress with the presence of NASH. Although these studies have not established convincingly a causal relationship, one study did suggest a benefit of the antioxidant vitamin E in NASH.[104] Multiple possible sources of oxidant stress in the fatty liver have been identified and include cytochrome P450,[105] peroxisomal β-oxidation, mitochondrial electron leak, and recruited inflammatory cells. Reactive lipid peroxidation products can further potentiate the oxidant stress that led to their initial formation.

Fatty Acids and Liver Injury. Increased levels of free fatty acids, besides mediating insulin resistance, can be directly toxic to hepatocytes (Table 5). Therefore, simply the increased flux of free fatty acids through the liver in states of exuberant peripheral lipolysis may play a direct role in hepatocellular injury. However, one of the difficulties in understanding the role of free fatty acids in hepatocellular injury is the lack of reliable methods to measure their intracellular levels. Interestingly, polyunsaturated fatty acids are highly facile substrates for lipid peroxidation in animal models of alcohol-induced liver injury whereas saturated fatty acids exert a protective effect.[106]

Table 5. Mechanisms of Free Fatty Acid Toxicity

Membrane disruption (detergent effect) at very high concentrations
Inhibition of Na^+/K^+ ATPase
Inhibition of glycolysis
Uncoupling of mitochondrial β-oxidation
Overall disruption of mitochondrial function (dicarboxylic fatty acids)
Protein kinase C activation
Dysregulation of intracellular Ca^{++} homeostasis
Sustained PPARα activation
Promiscuous nuclear receptor activation (*e.g.,* THR, SSHR, Fos/Jun)
Genotoxicity of lipid-peroxidation–derived reactive aldehydes
Formation of toxic fatty acid ethyl esters
MAP kinase activation?

Abbreviations: THR, thyroid hormone receptor; SSHR, sex steroid hormone receptor; MAP, mitogen-activated protein.

If free fatty acids are agents of destruction in the pathogenesis of liver disease, abundant and overlapping protective mechanisms against this toxicity would be expected. Indeed such mechanisms exist. Hepatocytes in particular are well endowed with mechanisms to bind, transform, catabolize, and export excess free fatty acids through the concerted actions of fatty acid binding proteins, triglyceride synthesis, and secretion as very low density lipoprotein (VLDL), mitochondrial β-oxidation, and enzymatic removal of lipid peroxidation products. The nuclear receptor PPARα (peroxisomal proliferator activated receptor α) plays a central role in sensing excess free fatty acids and up-regulating the genetic program of fatty acid disposal. The flip side of this protective mechanism, at least in rats, may be a predisposition to carcinogenesis.

VLDL and the Disposal of Hepatic Fat. A major route of free fatty acid disposal in the liver is the secretion of triglycerides by hepatocytes into the space of Disse as VLDL. Hepatic VLDL assembly is a complex process and, as such, is subject to impairment at multiple sites.[107] VLDL synthesis requires the protein apoB100, a 550-kd protein translated from the liver-specific splice form of the *APOB* transcription product. Apolipoprotein E (apoE) is also an important component of circulating VLDL metabolism and polymorphisms of apoE such as apoE3-Leiden are associated with hepatic steatosis in mice.[108]

The production of apoB100 protein is highly regulated. Transcriptional regulation plays a relatively minor role whereas cotranslational and posttranslational steps play dominant roles in regulating protein abundance.[109] The nascent protein cotranslationally inserts into the endoplasmic reticulum where it undergoes essential disulfide bond formation and association with triglycerides. Necessary for these latter steps are protein disulfide

isomerase and microsomal triglyceride transfer protein (MTTP).[110] Severe defects in MTTP expression cause abetalipoproteinemia, a condition associated with NASH and cirrhosis. In fact, the knockout of MTTP in mice is embryonically lethal. Lesser defects, such as a G/T polymorphism at position −493 of the human MTTP promoter, have been linked to an increased risk for hepatic steatosis in diabetes[111] and increased sensitivity of the liver to endotoxin.[112]

Even with normal processing of the nascent apoB100 protein, most of the translational product undergoes degradation in the endoplasmic reticulum and only a small fraction reaches the circulation, an observation that suggests ample overproduction of apoB100 in the basal state. Nascent VLDL particles also can be derailed from their usual path from the endoplasmic reticulum into the Golgi and degraded in a separate compartment. Interestingly, this process is stimulated by phosphatidylinositol (PI)-3 kinase activation, a pathway that also mediates insulin signaling. Experimentally, insulin treatment of cultured hepatocytes induces VLDL degradation and the intracellular accumulation of fat. This insulin-stimulated VLDL degradation is blocked by wortmannin, an inhibitor of PI-3 kinase. Therefore, one of the many links from hyperinsulinemia to NAFLD may be increased intracellular VLDL degradation and impaired secretion of fat from the liver. This conclusion necessitates that the liver PI-3 kinase pathway is not down-regulated as it is in insulin-resistant muscle. Such organ-specific data are currently lacking.

Minimal data are available on VLDL trafficking in NASH, although one study suggested synthesis was impaired.[113] Another important step regulating net apoB100 appearance in the circulation is reuptake of newly secreted VLDL back into the liver mediated by the LDL receptor. This immediately recycled fat may serve as a sensing mechanism of the extracellular milieu for the regulation of lipid trafficking in the hepatocyte.

Lipodystrophies and the Role of Peripheral Fat. One mechanism for the control of circulating lipids is the uptake and storage of fat in peripheral stores. Disorders of peripheral fat deposition, the lipodystrophies, are defined by partial or complete inability to form adipose tissue. Hepatic steatosis, NASH, and cirrhosis are established sequelae of these disorders, with the amount of hepatic steatosis being proportional to the extent of adipose tissue loss.[114] Congenital generalized lipodystrophy is characterized by nearly absent peripheral fat, severe hepatic steatosis, and a significant risk for cirrhosis.[115] More provocative with respect to putative mechanisms of NASH are some of the mutations associated with partial lipodystrophies. Mutations of gene-encoding PPAR-γ, *PPARG*, are associated with partial lipodystrophies, although liver disease has not been explored in these patients.[116] Mutations in the gene for the nuclear envelope protein lamin A, *LMNA*, also have been identified in partial lipodystrophies.[117] Lamin A may have a role in regulating sterol regulatory element binding proteins 1 and 2 (SREBP-1 and -2). These transcription factors are key mediators of insulin signaling that lead to a genetic program of increased lipid synthesis in hepatocytes. In fact, overexpression of a dominant-positive SREBP-1 in transgenic mice is associated with severe hepatic steatosis,[118] whereas SREBP-1 knockout mice are resistant to the development of fatty liver.[119]

The absence of peripheral fat in the lipodystrophies also impairs leptin signaling because of a deficiency of adipocyte-derived leptin. A clinical trial of leptin administration to hypoleptinemic patients with partial lipodystrophy reduced liver volume and liver triglyceride content.[120] It has not been established whether the response to leptin was due to improved leptin signaling in the liver or a central nervous system effect of leptin leading to diminished food intake.

Altered Energy Homeostasis and Mitochondrial Dysfunction. Because adenosine triphosphate (ATP) is critical for maintaining cellular integrity, its depletion may predispose to hepatocellular injury. Studies as early as Dianzani's[121,122] work in the 1950s have shown that hepatic ATP levels are depleted in experimental models of fatty liver. These observations have been corroborated by more recent animal studies using the choline-deficient diet model.[123] Human investigations of energy homeostasis have been aided by the development of ^{31}P NMR spectroscopy, which can noninvasively distinguish phosphate triesters (*e.g.*, ATP) from diesters (*e.g.*, ADP), monoesters (*e.g.*, AMP, fructose-1-phosphate), and inorganic phosphate. Hepatic ATP typically is depleted after an intravenous fructose load. A study of 8 patients with NASH compared with 7 control patients showed similar depletion of hepatic ATP in both groups, but the recovery of ATP levels was delayed significantly in the NASH patients.[124] The magnitude of delay in hepatic ATP recovery correlated with body mass index in both groups. The inability to recover intracellular ATP levels after a stress in obesity may explain the predisposition to liver injury in the obese caused by ischemia/reperfusion (*e.g.*, donor liver preservation).

Mitochondrial injury may be one cause of reduced hepatocellular ATP stores in NASH.[70] Electron microscopy studies have identified crystalline structures of uncertain composition with the mitochondrial matrix.[71] Although these structures have been called paracrystalline, further studies confirmed that they are true crystals.

Not all mitochondria are affected in NASH: about 5% to 15% of hepatocytes contain these abnormalities and, in afflicted cells, about 5% to 10% of mitochondria contain crystalline structures. Similar hepatocellular mitochondrial abnormalities also have been identified in Wilson's disease, alcoholic steatohepatitis, and experimental hepatic steatosis, all diseases that share certain pathologic features with NASH. The mitochondrial crystals of NASH disappear as the disease progresses to bland cirrhosis and the characteristic histologic features of steatohepatitis also resolve. Although these abnormalities are associated with mitochondrial dysfunction, the relationship between the two is unknown.

Mitochondria also can produce less ATP for a given amount of oxygen consumed through a process of uncoupling of oxidative phosphorylation (*i.e.*, production of ATP) with the reduction of oxygen. The net result is oxygen consumption without ATP production. Members of the uncoupling protein (UCP) family facilitate this process. One isoform, UCP-2, is up-regulated in fatty livers, although its role in liver disease is uncertain.[125] Experimentally, the expression of UCP-2 is up-regulated in animal models of steatohepatitis. However, a direct role of UCP-2 in the development of liver injury could not be confirmed in experimental models of fatty liver by using a UCP-2 knockout mouse.[125]

Mitochondrial injury can lead to mutation and loss of mitochondrial DNA. Although the mitochondrial genome encodes only 17 of the many essential mitochondrial proteins, integrity of its genome and function of these proteins are essential for mitochondrial (and hence cellular) viability. Mitochondrial DNA damage or loss is profound in nucleoside hepatotoxicity and alcohol-induced liver injury, but also occurs with aging and Wilson's disease. Limited data suggest that mitochondrial DNA damage also occurs in NASH.

Cytokines. The response to chronic hepatocellular injury varies dramatically among individual patients with liver disease, possibly explaining why steatohepatitis is relatively well tolerated in some yet associated with rapidly accumulating fibrosis in others. This variability may in part be explained by a variety of polymorphisms of peptide mediators of the inflammatory cascade and their receptors. The finding of familial clustering of NASH and cryptogenic cirrhosis supports a role for genetic polymorphisms in the factors that predispose to NASH.[56,57] Studies of genetic predispositions to NASH have been hampered by the lack of a sensitive, noninvasive means of identifying the disease in relatives of a proband short of a liver biopsy. Nonetheless, preliminary studies have suggested an increased frequency of a specific TNF-α promoter polymorphism in NASH patients[126,127] and a number of other TNF-α promoter polymorphisms have been described recently that need similar evaluation for their relevance to NASH. A gain-of-function promoter polymorphism of the endotoxin receptor, CD14, also has been found more often in NASH patients,[128] as well as in alcoholic steatohepatitis.[129] As the human genomic data regarding single nucleotide polymorphisms and other polymorphisms rapidly increase over the coming decade, a better understanding of how these genetic variations contributes to diseases such as NASH should emerge.

Animal Models. Several animal models have provided useful information with respect to the complex behavioral, metabolic, and genetic factors that lead to NASH.[130] One such model is the ob/ob mouse, an animal deficient in leptin. Because leptin plays a key role in mediating satiety at the level of hypothalamic neurons, absence of this adipocyte-derived peptide hormone results in excessive food consumption. The resulting phenotype simulates the human condition of the metabolic syndrome in many respects with the exception of the associated leptin deficiency. Leptin has biologic effects that include interaction with stellate cells, macrophages, lymphocytes, and vascular endothelium. This pluripotent effect raises the question of whether the steatohepatitis in ob/ob mice is related strictly to overeating or if other key processes that regulate inflammation and fibrosis are dysfunctional. This question appears to have been answered by the report of a similar phenotype of insulin resistance and fatty liver in mice specifically lacking only neuronal leptin receptors.[131]

One consequence of leptin deficiency in animal models is a chronic low-grade activation of the hypothalamic pituitary axis mimicking chronic physiologic stress.[132] How this affects corticosteroid levels in NASH is uncertain. Local overproduction of active corticosteroids in adipose tissue also can occur by the enzyme 11β hydroxysteroid dehydrogenase type I. This enzyme is overexpressed in adipose tissue of obese humans, and mice overexpressing this enzyme in adipose tissue develop the phenotype of obesity, insulin resistance, and diabetes.[133] Conversely, mice lacking this enzyme are resistant to the development of insulin resistance and diabetes.

Another major animal model of steatohepatitis is the depletion of S-adenosylmethionine with a diet deficient in the methyl donors methionine and choline. Methyl donors are required for the synthesis of phosphatidyl choline, a necessary component of VLDL for hepatic fat secretion, and methionine is required for the transsulfuration pathway of glutathione precursor synthesis. Severe steatohepatitis also develops in mice lacking the enzyme required for S-adenosylmethionine synthesis, MAT1-A.[134] These animal models have provided an opportunity

Table 6. Reported Therapy for NASH

Reference	Therapy	N	Study Type	Duration	Liver Enzyme Level	Histology
Diet						
Drenick,[152] 1970	Fasting	11	Open label	1.5-3.5 mo	Not performed	Variable
Drenick, 1970	Diet	7	Open label	2-7 mo	Not performed	Variable
Eriksson,[147] 1986	Diet	3	Case series	12 mo	Improved	Improved (S,F,I)*
Andersen,[153] 1991	Diet	41	Open label	4-23 mo	Improved	Variable
Rozental,[151] 1967	Severe diet	5	Open label	1-4 wk	No change	Variable
Diet, exercise						
Ueno,[148] 1997	Diet, exercise	25	Open label	3 mo	Improved	Improved (S,F)*
Keeffe,[149] 1987	Diet, exercise	1	Case series	4 mo	Improved	Improved (S,I)*
Palmer,[150] 1990	Diet, exercise	39	Case series	2-111 mo	Improved	Not performed
Franzese,[235] 1997	Diet, exercise	58 (ped)	Open label	6 mo	Improved	Not performed
Vajro,[154] 1994	Diet, exercise	9 (ped)	Case series	30 mo	Improved	Improved (S,I)*
Saksena,[140] 1999	Diet, exercise					
Weight loss agents						
Harrison,[164] 2002	Orlistat	10	Open label	6 mo	Improved	Improved (S,I,F)*
Surgical						
Luyckx,[166] 1998	Gastroplasty	505	Open label	24 mo	Improved	Improved (S,I)*
Silverman,[167] 1995	Gastric bypass	91	Case series	2-61 mo	Improved	Improved (S,F)*
Cytoprotective agents						
Obinata,[236] 1996	Taurine (diet)	10 (ped)	Open label	6-17 mo	Improved	Not performed
Laurin,[170] 1996	UDCA	24	Open label	12 mo	Improved	Improved (S)*
Guma,[171] 1997	UDCA (diet)	24	Randomized, open	6 mo	Improved	Not performed
Ceriani,[172] 1998	UDCA	31	Open label	6 mo	Improved	Not performed
Antioxidants						
Fu,[184] 1998	LAB	4	Open label	12 wk	Improved	Variable
Lavine,[176] 2000	Vitamin E	11 (ped)	Open label	4-10 mo	Improved	Not performed
Gulbahar,[181] 2000	NAC	11	Open label	3 mo	Improved	Not performed
Abdelmalek,[180] 2001	Betaine	8	Open label	12 mo	Improved	Improved (S,F,I)*
Antihyperlipidemics						
Laurin,[170] 1996	Clofibrate	16	Open label	12 mo	No change	No change
Basaranoglu,[210] 1999	Gemfibrozil	46	Randomized, open label	1 mo	Improved	Not performed
Saibara,[209] 1999	Bezafibrate	2	Open label	Not reported	Not reported	Improved (S)*
Horlander,[211] 2001	Atorvastatin	7	Open label	21 mo	Improved	Improved (S,F,I)*
Nair,[212] 2002	HMG-CoA RI	13	Case control	≥6 mo	Not reported	No change
Antidiabetics						
Coyle,[202] 1999	Metformin	2	Open label	4-11 mo	Improved	Improved*
Caldwell,[189] 2001	Troglitazone	10	Open label	4-6 mo	Improved	Improved (I)*
Marchesini,[204] 2001	Metformin		Open label		Improved	Not performed
Acosta,[190] 2001	Pioglitazone	8	Case series	2-12 mo	Improved	Improved*
Neuschwander-Tetri,[191] 2002	Rosiglitazone	30	Open label	48 wk	Improved	Improved(I,F)*
Azuma,[193] 2002	Pioglitazone	7	Open label	3 mo	Improved	Not performed
Combination						
Mendez-Sanchez,[173] 2002	Diet vs. diet + ucda	23	Blind, randomized, controlled	6 wk	Improved equally in both groups	Not reported
Sanyal,[194] 2002	Vitamin E vs. vitamin E + pioglitazone	21	Randomized, controlled	6 mo	Not reported	Pioglitazone group improved (S,I,B,M)

*The primary histologic parameter showing improvement is indicated as I, inflammation; S, steatosis; F, fibrosis; B, ballooning; M, Mallory bodies. Most have shown only limited improvement in 1 or 2 parameters and some have shown worsening of some parameters despite improvement in others.

to evaluate specific dietary interventions and antioxidants in a mouse model of steatohepatitis.

Management

Published studies, summarized in Table 6, are limited by small numbers of patients, variations in the definition of NASH, and the study end points.[135] Resolution of histologic abnormalities as determined by liver biopsy remains the gold standard for treatment outcomes. Common surrogate markers include normalization of aminotransferase loss of fat as detected by noninvasive imaging. Other reported end points have included serum markers of lipid peroxidation, measures of apoptosis, indices of insulin resistance, body mass index, body fat composition, anthropometric measurements (particularly waist circumference[136,137]), lipid profiles, and mitochondrial morphology. In the future, magnetic resonance spectroscopy may prove to be the optimal means of sampling different areas of the liver for abnormalities in physiologic homeostasis. Exercise tolerance, quality of life, and cost

assessments, although often difficult issues, will be helpful to assess the overall use of an intervention.

General Considerations. Whether alcohol use should be prohibited or diminished to levels less than 20 g/d is unclear. Lacking data, a pragmatic recommendation is to tailor this to the histology, with abstinence if significant fibrosis is present. The concomitant use of medications that may promote steatohepatitis (*e.g.*, amiodarone, tamoxifen) require weighing of the risks and benefits. An increasingly common but little-explored issue is workplace exposure to hydrocarbon solvents.[78,138,139]

Exercise and Diet. Exercise and diet continue to be the cornerstones of therapy.[140] Although typically recommended together, the concept of the fit fat individual (*i.e.*, relatively well conditioned but obese) is relevant and suggests a benefit of exercise even in the absence of weight loss.[141] Exercise alters substrate use in skeletal muscle and insulin sensitivity, although only about one third of patients achieve target levels of exercise[142-144] and obese individuals may be resistant to these changes.[145,146] A small number of studies of diet and exercise therapy have been reported in both adults and children. These typically reveal improved biochemical parameters but variable changes in histology.[6,147-155] Histologic exacerbation has been observed when the rate of weight loss exceeded 1.6 g/wk. Higher-intensity exercise regimens are probably more effective in producing significant metabolic changes.[156]

Specific Diets and Weight Loss Surgery. The effects of many popular diets on the fatty liver are not known. A pragmatic approach is to recommend a reduced calorie, balanced diet such as that endorsed by the American Heart Association or, as proposed by Spieth et al. in pediatric patients, the low glycemic index diet that emphasizes dietary composition.[157] Increased polyunsaturated fats (fish, flax seed oils) alter insulin sensitivity and prostaglandin metabolism, may increase UCP expression, and may promote lipid peroxidation, but the net effect in steatohepatitis is not known.[158-162] Appetite-suppressing agents have lost favor due to their side effects.[163] Recent data suggest a role for orlistat, a lipase inhibitor, as an adjunct to weight loss.[164] Several studies have reported beneficial effects of bariatric surgery,[165-167] although precipitous weight loss has the potential to exacerbate steatohepatitis. Pretreatment with cytoprotective or antioxidant agents in this setting has not been tested. Older studies of the now abandoned jejunoileal intestinal bypass procedure supported a role for antibiotics and amino acid supplementation for patients who experienced decompensation.[168,169]

Cytoprotective Agents. These agents combine an attractive safety profile, few drug interactions, and a plausible mechanism of action at the cellular or subcellular level. Ursodeoxycholic acid, which has been studied extensively in other liver diseases, has shown some promise in preliminary studies[170-173]; the results of a randomized, multicenter study are soon to be available.

Antioxidant Agents and Iron Reduction Therapy. Among the promising agents are vitamin E,[174-176] S-adenosyl-methionine,[177-179] betaine,[180] and N-acetylcysteine.[181] Betaine (a methyl donor in an alternative pathway for remethylation of homocysteine to methionine) has shown encouraging results in adults as has vitamin E in a pediatric population. Interestingly, the use of vitamin E in patients with coronary artery disease is associated with blunted efficacy of statin drugs.[182] Silymarin,[183] a popular milk thistle extract, is used commonly by patients with liver disease but we are not aware of published studies in NAFLD. Variable results were seen in one study of combination antioxidants.[184] Histamine,[185] which possesses indirect antioxidant properties, and a group of substances known as lazaroids (21-aminosteroids),[186] may warrant pilot work. One study has shown improvement in liver enzyme levels and insulin sensitivity in a group of HFE gene–negative patients treated with serial phlebotomy for iron reduction.[187]

Antidiabetic/Insulin-Sensitizing Agents. Insulin therapy, sometimes recommended early in the course of type 2 diabetes,[188] and sulfonylureas, have not been addressed adequately. The thiazolidinediones have shown promise.[189-194] These agents activate the PPARγ nuclear transcription factor,[195] alter skeletal muscle glucose uptake (through increased GLUT4 activity),[196] decrease central adiposity,[197] promote adipocyte differentiation, alter mitochondrial mass,[198] and alter thermogenesis.[199] The efficacy of troglitazone in lipodystrophy suggests a primary effect on lipid metabolism.[200] Metformin has undergone limited study in NAFLD.[201-204] It down-regulates hepatic gluconeogenesis and also appears to divert fatty acids from triglyceride production to mitochondrial beta oxidation.[205] Other candidate agents include acarbose (an α-glucosidase inhibitor),[206] acipimox (inhibits lipolysis),[207] and d-chiro-inositol.[208]

Antihyperlipidemic Agents. Fibrates alter lipoprotein metabolism through the PPARα receptor but had no benefit in early reports.[170] However, bezafibrate showed benefit in tamoxifen-associated steatohepatitis.[209] Basaranoglu et al.[210] showed improvement in liver enzyme levels but histology was not measured in a study of gemfibrozil. A pilot study has showed improvement in biochemical and histologic parameters in a small sample of patients treated with the HMG-CoA reductase inhibitor atorvastatin.[211] However, a recent report showed no significant histologic differences between controls and patients using various statin drugs.[212] Recent reports of subclinical skel-

Table 7. Controversial Areas and Future Research Goals

Epidemiology and risk factors
What is the prevalence of NAFLD among specific populations, especially among individuals with diabetes or hyperlipidemia?
What are the risk factors for progression and what is the best means of NAFLD classification that accounts for these risk factors?
Is there a non–insulin-resistant group of individuals who have primary disorders that produce a clinical picture similar to that found in the metabolic syndrome?
What is the role of occupational exposures such as hydrocarbon fumes?
What are the effects of modest alcohol consumption?
Diagnosis
Are there more sensitive serum markers for detecting NAFLD than the transaminases?
What is the value of newer imaging modalities such as magnetic resonance spectroscopy that appear capable of sampling various areas of the liver and measuring ATP homeostasis?
What is the significance of immunologic markers such as antinuclear antibodies and IgA elevations, which are common in NAFLD?
What is the extent of sampling error on liver biopsy?
What is the spectrum of liver disease associated with insulin resistance?
What is the significance of predominantly portal-based inflammation in association with steatosis?
What is the best measure of insulin sensitivity in patients with NAFLD?
Can we identify serum markers of NAFLD and hepatic fibrosis that reliably predict who has benign steatosis and who is at risk for hepatocellular injury and progressive fibrosis?
Similarly, can we identify genetic markers that would predict who might be predisposed to either insulin resistance or to progressive liver disease?
Pathophysiology
Is NAFLD a consequence of too much insulin signaling in the liver, too little, both, or neither?
In states of insulin resistance associated with NAFLD, which of the insulin signaling pathways are impaired and in which tissues are these pathways relevant?
Conversely, which of the insulin signaling pathways are overactivated by hyperinsulinemia and what are the metabolic consequences of this overzealous signaling?
What are the relevant cytokines and other peptide mediators of insulin resistance in NAFLD?
What is the role of increased free fatty acid levels in mediating cellular injury in NAFLD?
What is the role of increased free fatty acid levels in mediating insulin resistance in NAFLD?
Is oxidant stress an important process in the pathogenesis of cellular injury and fibrosis in NAFLD or is it an epiphenomenonal consequence of cellular injury?
Why are the characteristic pathologic features of NASH often lost when it progresses to cirrhosis?
Treatment
What are the most effective behavioral and pharmacologic approaches to insulin resistance in NAFLD?
Are dietary polyunsaturated fats helpful or harmful to the liver?
What are the effects on NAFLD of treating comorbid conditions such as diabetes and hyperlipidemia with sulfonylureas or statins?

etal muscle toxicity characterized by formation of ragged red fibers and mediated by mitochondrial injury[213] are justifiable cause for concern for the use of these drugs in NAFLD. Other lipid-lowering agents (such as colesevelam or other resin-binding agents) have not been investigated. The potential role of lipid-lowering agents is questioned by observations of inherent defects of apoprotein metabolism in NASH and NAFLD.[113,214]

Liver Transplantation and Disease Recurrence. Many patients with advanced disease are poor candidates for transplantation due to comorbid conditions such as obesity and complications of diabetes. Both recurrence of NASH in patients with previously established NASH[215-218] and *de novo* occurrence of NASH after transplantation for cryptogenic cirrhosis[12,16] can occur. Posttransplantation progression to cirrhosis may develop although predictive factors and treatment have not been well defined. Immunosuppression could play a role due to the promotion of fatty liver and diabetes with corticosteroid use and more direct effects such as the effect of cyclosporine on the mitochondrion.[219]

The Future: A Call to Action

Many issues remain unresolved regarding the diagnosis and treatment of NASH. Large series of well-characterized patients will need to be followed-up to better establish the natural history of NAFLD. To achieve this ambitious goal, collaborative groups such as the National Institutes of Health–supported NASH Clinical Research Network[220] will need to pool their data for collective reporting of outcomes. Shown in Table 7 is a partial list of key areas identified during the conference that need immediate clarification to move this field ahead toward effective diagnosis, prevention, and treatment of NASH.

Appendix

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