Nonalcoholic fatty liver disease (NAFLD) is among the most common causes of chronic liver disease in the western world. It is now recognized that these patients have myriad of important co-morbidities (e.g., diabetes, hypothyroidism and metabolic syndrome). The workup of patients with suspected NAFLD should consist of excluding competing etiologies and systemic evaluation of metabolic comorbidities. NAFLD is histologically categorized into steatosis and steatohepatitis, two states with fairly dichotomous natural history. While significant progress has been made in terms of noninvasively predicting advanced fibrosis, insufficient progress has been made in predicting steatohepatitis. Currently, liver biopsy remains the gold standard for the histological stratification of NAFLD. While sustained weight loss can be effective to treat NASH, it is often difficult to achieve. Foregut bariatric surgery can be quite effective in improving hepatic histology in selected patients without liver failure or significant portal hypertension. Thiazolidinediones have shown promise and the results from the ongoing, large multicenter study should become available soon. Large multicenter studies of CB, receptor antagonists are also underway but their results will not be available for several years. Several recent studies have highlighted that cardiovascular disease is the single most important cause of morbidity and mortality in this patient population.

**Conclusion:** Health care providers should not only focus on liver disease but also concentrate on aggressively modifying and treating their cardiovascular risk factors. (HEPATOLOGY 2009;49:306-317.)

Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of elevated liver enzymes and chronic liver disease in Western countries. Its incidence in adults and children is rising rapidly because of ongoing epidemics of obesity and type 2 diabetes. It is a multifaceted metabolic disorder and is encountered in clinical practice by a variety of health care specialists ranging from primary care physicians and gastroenterologists to cardiologists, radiologists, and gynecologists. It is comprised of a spectrum of liver disease ranging from simple steatosis to full-blown steatohepatitis that is characterized by steatosis, lobular inflammation, ballooning, and fibrosis. Over the last several years, much progress has been made in terms of our understanding of its risk factors, pathogenesis, natural history, noninvasive markers, and treatment. This review is tailored toward clinicians caring for patients with NAFLD and discusses practical issues related to selected aspects of its evaluation and management.

**Evaluation of Newly Suspected NAFLD**

Suspected NAFLD represents one of the most common reasons why patients visit gastroenterologists and hepatologists in an ambulatory setting. Although some patients may have attributable abdominal symptoms and tender hepatomegaly, most are asymptomatic, and their liver disease is identified incidentally on routine blood tests or abdominal imaging. Its initial assessment consists of excluding competing and co-existing causes and identifying clinically important comorbidities.
Competing Causes

The diagnosis of NAFLD requires that there is no history of previous or ongoing significant alcohol consumption. There is no consistent agreement regarding the definition of significant alcohol consumption; however, it is generally believed that average alcohol consumption of more than two drinks per day in women and more than three drinks per day in men is necessary to develop alcoholic fatty liver.7,8 However, in individuals with metabolic risk factors such as obesity and diabetes, it is possible that alcohol consumed at lower quantities may promote hepatic steatosis. This notion is supported by a population-based study in which Ruhl and Everhart9 have shown that one or more alcohol drinks per day cause elevated alanine aminotransferase (ALT) in obese but not in normal weight individuals.9 This study is at odds with recent epidemiological data suggesting that modest wine consumption may reduce the prevalence of NAFLD.10

When the alcohol consumption history is insufficient, it becomes difficult to distinguish alcoholic and non-alcoholic forms of fatty liver, especially in those with obesity and associated metabolic risk factors. Conventional markers such as gamma glutamyl transpeptidase, mean corpuscular volume, and aspartate aminotransferase (AST)/ALT ratio are not useful, and specific serum markers for chronic alcohol abuse are of limited utility.11 The carbohydrate-deficient transferrin is the most widely used and perhaps the most specific serum marker for detecting chronic alcohol abuse.12 This test evaluates the ratio of desialylated transferrin to total transferrin, and it has 81% sensitivity with 98% specificity in detecting chronic alcohol abuse. Unfortunately, this and other tests such as the ratio of mitochondrial AST to total AST13 are not readily available in clinical practice.

More recently, investigators from the Mayo clinic have developed the “alcoholic liver disease/NAFLD index,” which consisted of five easily available variables: mean corpuscular volume, AST, and ALT values, height and weight, and sex (http://www.mayoclinic.org/gi-rst/mayomodel10.html).14 This weighted multivariate model uses logistic regression analysis to generate the alcoholic liver disease/NAFLD index score, and a score greater than 0 incrementally favors alcoholic liver disease, whereas a value less than 0 favors NAFLD in decrement. For example, an alcoholic liver disease/NAFLD index score of 8.95 would correspond to greater than 99% probability of alcoholic liver disease, whereas a value of -5.04 would correspond to greater than 99% probability of NAFLD. Because most subjects with alcoholic liver disease in this study had high model for end-stage liver disease scores, this tool is more likely applicable to patients with decompensated cirrhosis presenting for liver transplant evaluation rather than outpatients with fatty liver.

Elevated serum auto-antibodies are common in patients with NAFLD. Although low titer antinuclear antibody positivity can be seen in up to 33% of patients with NAFLD, antinuclear antibody positivity in titers greater than 1:320 is generally rare.15-17 Low titers of anti-smooth muscle and antimitochondrial antibodies also may be noted in patients with NAFLD.18 The presence of these auto-antibodies is generally thought to be an epiphenomenon, although in one study their presence correlated with more severe histological damage.19,20 In patients with suspected NAFLD and antinuclear antibody positivity at titers greater than 1:160 or anti-smooth muscle actin positivity at titers greater 1:40, a liver biopsy may be considered to exclude the presence of autoimmune hepatitis.

Mildly increased serum ferritin is not uncommon in patients with NAFLD, and it does not necessarily indicate co-existing iron overload.21 Metabolic syndrome and hyperinsulinemia are known to be associated with increased serum ferritin, and this association may be mediated by the presence of NAFLD.22 Nonetheless, elevated serum ferritin in a patient with suspect NAFLD may prompt testing for hereditary hemochromatosis (HFE gene) mutations. The prevalence of HFE gene mutations in NAFLD patients has been variable, depending on the population studied, and their relevance remains largely unknown.23,24 Although the prevalence of HFE gene mutations and their clinical relevance remain unclear,22-24 homozygote or heterozygote C282Y mutations along with elevated serum ferritin may justify a liver biopsy in patients with suspected NAFLD.

It is important to verify that chronic hepatitis B and hepatitis C have been convincingly excluded, and, depending on the clinical scenario, rare disorders such as alpha-1 antitrypsin deficiency and Wilson’s disease should also be excluded. Thorough medication history is important because several commonly prescribed medications (such as tamoxifen, methotrexate, and amiodarone) are noted for their ability to cause hepatic steatosis.1

Evaluation for Co-morbidities

The patients with NAFLD frequently have many clinically significant co-morbidities (Table 1). Obesity, type 2 diabetes, and hyperlipidemia are well known to co-exist in patients with NAFLD, and it is important to systematically characterize them. The body mass index (BMI) and waist circumference should be measured to better characterize the degree (mild, moderate, and severe) and the nature (central versus peripheral) of obesity. Type 2 diabetes and glucose intolerance are very frequent in patients
with NAFLD, and they have prognostic significance. In patients without preexisting type 2 diabetes, the presence of glucose intolerance and insulin resistance should be evaluated by obtaining fasting blood glucose, insulin levels and hemoglobin A1c. In patients without diabetes, insulin resistance should be assessed by calculating HOMA-IR (homeostasis model assessment—insulin resistance) or QUICKI (quantitative insulin-sensitivity check index). Both HOMA-IR and QUICKI are mathematical transformations of fasting blood glucose and insulin levels. In our practice, we calculate HOMA-IR ([fasting insulin [\( \mu U/mL \]) \times \text{fasting glucose [mmol/L]}]/22.5), and as a general guideline, we consider an HOMA-IR value greater than 3 to be clinically significant. If not done recently, fasting lipid profile should be obtained, because dyslipidemia is nearly universal. Typically, fasting serum triglyceride levels are high, along with low high-density lipoprotein values. Recent data suggest that high-sensitivity c-reactive protein is frequently elevated in patients with NAFLD and should be measured along with fasting lipid profile to assess cardiovascular risk (vide infra).

In addition, patients should be systematically explored for the presence of other comorbidities such as obstructive sleep apnea, hypothyroidism, and polycystic ovary syndrome. Obstructive sleep apnea is commonly present in patients with NAFLD and may contribute to the fatigue that these patients sometimes experience. Some studies have suggested a causal relationship between obstructive sleep apnea and the pathogenesis of NAFLD, but these are far from convincing. Previous studies have shown that patients with hypothyroidism and hypopituitarism have increased prevalence of NAFLD. Several studies have shown high prevalence of NAFLD in premenopausal women with polycystic ovary syndrome, and one study suggested that such individuals are at higher risk for disease progression.

### Identification of Nonalcoholic Steatohepatitis in Patients with NAFLD

The NAFLD can be categorized into simple steatosis and steatohepatitis (NASH). A well-defined case of NASH histologically exhibits macrovesicular steatosis, lobular inflammation, balloon degeneration of hepatocytes, and zone 3 pericellular fibrosis. Undoubtedly, NASH is histologically progressive and can lead to cirrhosis and associated liver dysfunction. Simple steatosis has a relatively benign course but is not totally without histological consequences. For example, investigators from the Cleveland Clinic reported in their initial paper that 4% of patients with simple steatosis developed cirrhosis and 2% had liver-related mortality over a median follow-up of approximately 8 years. The extended follow-up of the same cohort was reported in an abstract form recently, and it confirmed that simple steatosis is not entirely benign.

Liver biopsy is the current gold standard to identify steatohepatitis, but it is not without controversies and practical difficulties. Several recent studies have highlighted its sampling variability and interobserver discordance. The precise histological definition of NASH is not entirely known, and the experts believe that it should be based on pattern recognition rather than a composite score of individual components such as steatosis, ballooning, and fibrosis. It remains controversial whether ballooning or pericellular fibrosis should be considered as critical for the histological diagnosis of NASH. The recently described NAFLD Activity Score is valuable for quantifying histological changes, especially in clinical trials, but its generalizability and diagnostic utility are unknown.

Over the past several years, there has been significant interest in noninvasively predicting liver histology in patients with NAFLD. However, it remains somewhat controversial which histological finding(s) should be targeted for noninvasive assessment. Most studies have attempted to predict advanced fibrosis (bridging fibrosis/cirrhosis), but it has been argued that one should attempt to predict steatohepatitis rather than advanced fibrosis. It is our view that future studies should attempt to predict three histological states (simple steatosis, NASH without significant fibrosis, and NASH with advanced fibrosis), rather than dichotomous descriptive states.

Aminotransferase values and common imaging tests such as liver ultrasound, computed tomography, and magnetic resonance are of limited value in predicting liver histology. Numerous circulating biomarkers and prediction models have been investigated to noninvasively predict hepatic histology in patients with NAFLD, and their full discussion is beyond the scope of this review. However, cytokeratin 18 (CK-18) fragments and serum dehydroepiandrosterone, two hypothesis-driven circulating biomarkers, deserve further discussion. Based on experimental data that hepatocyte apoptosis may play an important role in the pathogenesis of NAFLD, Wick-
owska et al.\textsuperscript{51} measured plasma CK-18 fragment levels in 44 consecutive individuals with suspected NAFLD undergoing liver biopsy.\textsuperscript{51} CK-18 is a major intermediate filament protein in hepatocytes, and it is cleaved by the effector caspases (mainly caspase 3) on activation of the apoptosis cascade.\textsuperscript{52,53} Compared with individuals with normal histology and those without NASH, patients with definite NASH had significantly higher CK-18 fragment levels.\textsuperscript{54} It was suggested that CK-18 fragment levels greater than 380.2 U/L can predict definite NASH in a very precise fashion.\textsuperscript{51} A puzzling and unexplained aspect of this small cross-sectional study is that nearly 25% of patients with suspected NAFLD supposedly had normal hepatic histology on liver biopsy. More recently, Charlton et al.\textsuperscript{55} have shown that serum dehydroepiandrosterone levels had a consistent and stepwise inverse relationship with the degree of hepatic fibrosis that persisted after adjusting for age.\textsuperscript{55} This observation, which was initially made on a derivation cohort consisting of 122 patients, was subsequently reproduced on a validation cohort consisting of 361 NAFLD patients recruited from two separate academic centers.\textsuperscript{55} These provocative preliminary data deserve further study, but it may be too optimistic to assume that a single biomarker can reliably predict histology in NAFLD, a condition with relatively complex phenotype and multiple comorbidities.

There are numerous papers published in the literature describing noninvasive prediction models, but most of them consisted of small sample size and lacked rigorous external validation. Three recently described models with relatively large sample size and some level of validation have shown encouraging results.\textsuperscript{56-58} In a multicenter study consisting of 480 patients in the derivation and 253 in the validation cohorts, Angulo et al.\textsuperscript{56} have shown that a NAFLD fibrosis score consisting of six variables (age, BMI, AST/ALT ratio, hyperglycemia, platelet count, and albumin) can reliably predict advanced fibrosis. The formula for the fibrosis score was $-1.675 + 0.037 \times \text{age (years)} + 0.094 \times \text{BMI (kg/m}^2\text{)} + 1.13 \times \text{impaired glucose tolerance/diabetes (yes = 1, no = 0)} + 0.99 \times \text{AST/ALT ratio} - 0.013 \times \text{platelets (} \times 10^9\text{/L}) - 0.66 \times \text{albumin (g/dL)},$ and a low cutoff point (score $< -1.455$) signified the absence of advanced fibrosis, whereas a high cutoff point (score $> 0.676$) identified advanced fibrosis. It was concluded that NAFLD fibrosis score can be used as a triaging tool for optimizing liver biopsy yield in terms of identifying or excluding advanced fibrosis. More recently, Guha et al.\textsuperscript{57} have compared the performance of “enhanced liver fibrosis” (ELF) score with that of the “NAFLD fibrosis score” in predicting advanced fibrosis. The enhanced liver fibrosis is essentially the “original European liver fibrosis” test without “age” included in the algorithm. The original European liver fibrosis test, a model consisting of age and three serum markers of matrix turnover (tissue inhibitor of metalloproteinases, hyaluronic acid, and type III procollagen), predicted advanced fibrosis in a variety of liver disorders.\textsuperscript{59} In this report, the ELF score had excellent ability to predict different levels of fibrosis (any fibrosis, moderate fibrosis, or advanced fibrosis), and when it was combined with the “NAFLD fibrosis score,” its ability to predict different levels of fibrosis improved further.\textsuperscript{57} Harrison et al.\textsuperscript{58} have recently developed the BARD score to predict advanced fibrosis in patients with NAFLD.\textsuperscript{57} The BARD score is a weighted sum of three easily available variables (BMI $> 28$ kg/m\textsuperscript{2} [1 point], AST/ALT $\geq 0.8$ [2 points], and diabetes [1 point]), and the authors have shown that a score of 2 to 4 was associated with an odds ratio of 17 (95% confidence interval [CI]: 9.2-31.9) for predicting advanced fibrosis.\textsuperscript{57} These promising models will need to be validated by external investigators before they are recommended for wide clinical use.

| Table 2. Noninvasive Biomarkers Previously Studied or Currently Under Evaluation |
|--------------------------------------|------------------|------------------|
| **Fibrosis**                         | **Imaging Studies** | **Breath Tests** |
| BARD score\textsuperscript{58}      | Transient elastography\textsuperscript{61,131} | $[^{13}\text{C}]$ methacetin\textsuperscript{136} |
| Fibrotest\textsuperscript{135}      | MR elastography\textsuperscript{132} | $[^{13}\text{C}]$ ketoisocaproate\textsuperscript{135} |
| NAFLD fibrosis score\textsuperscript{56} | MR spectroscopy\textsuperscript{133,134} | Ethanol\textsuperscript{136} |
| ELF/OELF\textsuperscript{57,59}     | Total elastography\textsuperscript{132} | Acetone\textsuperscript{136} |
| Hyaluronic acid\textsuperscript{130} | Total antioxidant response\textsuperscript{137} | |
| TBARS\textsuperscript{119}          | Total lipid peroxide levels\textsuperscript{137} | |
| Oxidized-LDL\textsuperscript{119}   | | |
| | | |
| CK-18 fragments\textsuperscript{51,53} | | |
| TNF-$\alpha$/adiponectin ratio\textsuperscript{138,139} | | |
| hsCRP\textsuperscript{69}           | | |
| IL-6\textsuperscript{54}            | | |
| CC-chemokine ligand-2\textsuperscript{141} | | |
One French study tested “Fibrotest” for predicting advanced fibrosis in NAFLD and found encouraging results. "Fibrotest" is a popular serum test for predicting advanced fibrosis in individuals with chronic hepatitis C, but more studies are needed to test its utility in NAFLD. Similarly, liver stiffness measured by transient elastography (FibroScan) may have some role in predicting the degree of fibrosis, but the data are sparse in terms of its utility in NAFLD.61,62

In summary, there has been significant research in developing biomarkers of liver histology in patients with NAFLD, and in fact one editorialist recently opined that the future is around the corner for noninvasively diagnosing progressive NASH.63 Although this may be the case for advanced fibrosis, insufficient attention has been paid to developing markers for noninvasively identifying steatohepatitis in patients with NAFLD. Until more definite data with external validation become available, we will continue with our current practice of recommending liver biopsy to selected patients with suspected NAFLD based on the presence of certain risk factors such as older age, diabetes, severe obesity, and metabolic syndrome.

Management of Patients with NAFLD

Advice Regarding Weight Loss, Exercise, and Specific Diets. The NAFLD is largely a manifestation of obesity and metabolic syndrome and is characterized by excess calorie intake and lack of optimal health-related fitness or physical activity.64 It is generally believed that weight loss is beneficial for patients with NAFLD, but data are sparse in terms of specifics such as how, how much, and how rapidly to lose weight. Furthermore, precise hepatic and extrahepatic benefits of weight loss are not well defined. In a recent review, Bellantani et al.65 pointed out that there are only four human studies consisting of fewer than 40 total patients that evaluated the effect of calorie restriction alone, and change in liver enzymes was the primary end point in all but one study. There are 10 published studies consisting of 626 total patients that evaluated the effect of calorie restriction combined with exercise, but liver histology was the primary end point in only four studies (123 patients).65 This paucity of data makes it difficult to make evidence-based recommendations about dietary modification and exercise to treat NAFLD and NASH.

It is generally recommended that overweight and obese patients with NAFLD lose 7% to 10% of their body weight by dietary modification and exercise over the course of 6 to 12 months. This is based on short-term studies showing that gradual weight loss of this magnitude improves insulin resistance and hepatic histology.3,66

Our recommendations to enhance patient compliance with lifestyle modifications are shown in Table 3. Scientific evidence is lacking to make precise recommendations specific to modifying macronutrient composition, but it appears sensible to recommend low glycemic food with decreased saturated and trans-fat intake but increased mono and polyunsaturated fatty acid intake.67 Evolving data suggest diets consisting of high fructose should be avoided by these patients.68 Because of the lack of safety and efficacy data, popular weight-loss diets such as Atkins, Ornish, and South Beach diets should not be recommended.69 In our clinical practice, we recommend diminished portions of balanced diet (consisting of a low-glycemic and low-fat diet and increased portions of fruits and vegetables) and five to seven sessions per week of moderate aerobic exercise, with each session lasting for 30 to 45 minutes. However, such prescriptive recommendations made in a clinic setting are rarely effective both short-term and long-term.65

Orlistat, a reversible inhibitor of gastric and pancreatic lipase, may be effective in promoting limited weight loss in selected patients, but side effects such as diarrhea and bloating make it less desirable. In a randomized study, Harrison et al.69 have shown that orlistat does not cause weight loss or histological improvement above and beyond that accomplished with calorie restriction alone.69 Rimonabant (endocannabinoid receptor 1 antagonist) is approved in Europe for promoting weight loss, and it may have favorable anti-steatotic and anti-fibrotic properties.70-74 Large clinical trials are underway with this and other similar compounds in NASH, but their results will not become available for at least several years.

Advice Regarding Bariatric Surgery. Because many patients with NAFLD have severe obesity, it often comes up in clinical practice whether bariatric surgery is suitable in this patient population. Jejunoileal bypass was widely popular in mid-1950s to mid-1970s for the surgical treatment of obesity, but because of disastrous hepatic and extrahepatic consequences it is now totally abandoned.75-77 Over the last decade, an increasing number of

Table 3. Strategies to Enhance Patient Compliance in Lifestyle Modification

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Communicate with empathy</td>
<td>● Be sensitive to general stigma against obesity</td>
</tr>
<tr>
<td>Discuss pros and cons of proposed changes to lifestyle</td>
<td>● Explore reasons for perpetual poor dietary and exercise choices</td>
</tr>
<tr>
<td>Encourage self-efficacy</td>
<td>● Encourage self-efficacy</td>
</tr>
<tr>
<td>Offer specific choices of food and exercise</td>
<td>● Offer specific choices of food and exercise</td>
</tr>
<tr>
<td>Design individualized program of eating and physical activity</td>
<td>● Design individualized program of eating and physical activity</td>
</tr>
<tr>
<td>Explain treatment and its benefits</td>
<td>● Explain treatment and its benefits</td>
</tr>
</tbody>
</table>

Reproduced, with modification, from Bellantani et al., Hepatology 2008;47:746-754.
foregut bariatric surgery procedures are being performed to treat obesity and its complications, and its short-term and long-term benefits are becoming well established.78-80 The commonly performed foregut bariatric surgery procedures include roux-en-Y gastric bypass (most common), adjustable gastric banding, gastroplasty, and sleeve gastrectomy.81,82 There have been no studies that evaluated foregut bariatric surgery to specifically treat NAFLD, but many published papers described its favorable effect of hepatic histology when performed for other indications, thus introducing selection bias.78-80 In general, liver histology improves significantly after foregut bariatric surgery with very minimal risk of worsening.83,84 In a recent meta-analysis consisting of 15 studies and 766 paired liver biopsies, Mummadi et al.84 have shown that all components of NAFLD show significant improvement after foregut bariatric surgery. Pooled proportion of patients with improvement or resolution in steatosis was 93% (95% CI: 84%-98%), and improvement or resolution of steatohepatitis was 82% (95% CI: 64%-95%); improvement in fibrosis when assessed using needle biopsies was 73% (95% CI: 65%-81%). We speculate that liver disease is unlikely to worsen in association with rapid and profound weight loss unless there are additional risks such as bacterial overgrowth (for example, jejunoileal bypass) or nutrient depletion (such as kwashiorkor). This forms the basis for our view that very long roux limb (in other words, >150 cm) should be avoided in patients with advanced fibrosis. Compensated cirrhosis is not a contraindication for foregut bariatric surgery provided it is performed by an experienced surgeon and clinically evident portal hypertension is absent (no esophageal or abdominal varices). There are reports that cirrhosis may reverse after bariatric surgery.85,86

In our practice, we recommend foregut bariatric surgery as a therapeutic possibility for the severely obese NAFLD patients with advanced fibrosis who failed to lose weight despite repeated nutritional counseling. In those with cirrhosis, we exclude clinical portal hypertension by performing abdominal imaging and upper endoscopy. Bariatric surgery may be particularly attractive for carefully selected patients with Child’s A cirrhosis not only because it may stabilize or improve the liver disease but it also may enhance their future suitability for liver transplantation.

**Role of Insulin Sensitizers.** Because insulin resistance is nearly universal in patients with NASH, it is not surprising that many studies tested insulin sensitizers as its treatment. However, a large number of them are proof-of-concept studies with small numbers of patients without rigorous study design, making it difficult to make definite recommendations. Biguanides (metformin) and thiazolidinediones (pioglitazone and rosiglitazone) are the two classes of insulin sensitizers studied in humans.

**Metformin.** Although its exact mechanism of action is not entirely clear, metformin’s therapeutic benefit as an antidiabetic agent and insulin sensitizer is well recognized. Its anti-diabetic action is likely related to decreased hepatic glucoseogenesis, decreased glucose absorption, and increased insulin sensitivity by facilitating glucose uptake and utilization.86,87 In addition, its stimulatory effect on adenosine monophosphate–activated protein kinase or modulation of hepatic tumor necrosis factor alpha (TNF-α) expression may result in benefits.88,89 A summary of studies evaluating metformin to treat NASH is shown in Table 4. A recent meta-analysis published in Cochrane database showed that metformin leads to normalization of serum aminotransferases in a significantly greater proportion of patients compared with dietary modification (odds ratio: 2.83; 95% CI: 1.27-6.31) and improved steatosis by imaging (odds ratio: 5.25, 95% CI: 1.09-25.21).90 The total number of patients treated with

### Table 4. Selected Studies of Metformin in Patients with NAFLD

<table>
<thead>
<tr>
<th>Author (reference)</th>
<th>N</th>
<th>Design</th>
<th>Comparator</th>
<th>Population</th>
<th>Duration</th>
<th>Liver Enzymes</th>
<th>Histology</th>
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</thead>
<tbody>
<tr>
<td>Marchesini et al.</td>
<td>14</td>
<td>Open label</td>
<td>None</td>
<td>Adults</td>
<td>4 mo</td>
<td>Improved</td>
<td>Not evaluated</td>
</tr>
<tr>
<td>Nair et al.</td>
<td>15</td>
<td>Open label</td>
<td>None</td>
<td>Mostly nondiabetic</td>
<td>12 mo</td>
<td>Improved</td>
<td>Improved inflammation</td>
</tr>
<tr>
<td>Uygun et al.</td>
<td>36</td>
<td>Open label</td>
<td>Calorie restricted</td>
<td>Nondiabetics</td>
<td>6 mo</td>
<td>Improved</td>
<td>Improved inflammation</td>
</tr>
<tr>
<td>Bugianesi et al.</td>
<td>55</td>
<td>Randomized clinical</td>
<td>Calorie restricted</td>
<td>Non-diabetics</td>
<td>12 mo</td>
<td>Improved</td>
<td>Improved steatosis, inflammation</td>
</tr>
<tr>
<td>Schwimmer et al.</td>
<td>10</td>
<td>Open label</td>
<td>None</td>
<td>Nondiabetics</td>
<td>6 mo</td>
<td>Improved</td>
<td>Not evaluated</td>
</tr>
<tr>
<td>Loomba et al.</td>
<td>14</td>
<td>Open label</td>
<td>None</td>
<td>Nondiabetics</td>
<td>48 wks</td>
<td>Improved</td>
<td>Improved steatosis and inflammation</td>
</tr>
<tr>
<td>Nobili et al.</td>
<td>57</td>
<td>Open label</td>
<td>Antioxidant</td>
<td>Nondiabetics</td>
<td>24 months</td>
<td>No difference</td>
<td>No difference</td>
</tr>
</tbody>
</table>
metformin in controlled studies is admittedly small, and its favorable effect on hepatic histology may not be robust, but we favor its use in patients without diabetes with NASH because of its safety profile. Because most patients without diabetes with NASH have glucose intolerance, it has the added benefit of lowering the risk of developing frank diabetes.91 Because metformin has not been studied to treat NASH in individuals with diabetes, its role in the diabetic population is not known. An ongoing, multicenter study comparing metformin with vitamin E or placebo in pediatric patients with NASH (TONIC; NCT00063635) should provide more insight into metformin’s role in treating NASH.

**Thiazolidinediones.** Thiazolidinediones (TZDs) are a novel class of oral antidiabetic medications that improve insulin resistance by acting as selective peroxisome proliferator-activated receptor gamma agonists.92,93 Troglitazone, the first generation TZD, has been withdrawn from the market because of its hepatotoxicity,94 whereas rosiglitazone and pioglitazone are the second-generation TZDs that are currently available for clinical use.93,95 They redistribute fat from muscle and liver to adipose tissue and thereby improving peripheral (skeletal muscle) and hepatic insulin sensitivity.93 In addition, they increase circulating levels of adiponectin, which is produced exclusively by the adipose tissue and has insulin-sensitizing properties.96

There has been significant interest in evaluating TZDs to treat NASH, and to our knowledge, eight studies have been published either as full-length papers or solely as an abstract.97-104 Troglitazone was tested in one study,97 rosiglitazone in two,98,103 and pioglitazone in five studies.99-102,104 Four were randomized controlled studies with 213 total enrolled patients with histologically proven NASH.101-104 Selected characteristics and outcomes of three studies that randomized at least 50 patients are shown in Table 5. In general, TZDs improve hepatic histology in patients with NASH, although their favorable effect on steatosis is more striking than on other histological variables such as inflammation, ballooning, or fibrosis. Their favorable effect on liver histology and liver biochemistries disappears on their discontinuation, suggesting that long-term treatment is needed to maintain their therapeutic benefits.105 This is potentially a significant issue; recent studies have questioned the long-term

<table>
<thead>
<tr>
<th>First Author (Reference)</th>
<th>N</th>
<th>Study Design</th>
<th>Duration</th>
<th>Histological Improvement, Pre- and Post-Duration</th>
<th>Mean Weight Gain</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belfort102</td>
<td>55</td>
<td>Hypocaloric diet + Pioglitazone versus hypocaloric diet + placebo</td>
<td>6 months</td>
<td>Steatosis: Yes, No; Inflammation: Yes, Yes; Fibrosis: Yes, No; NAS: Yes, No</td>
<td>2.5 kg</td>
<td>TZD group had significant increase in serum adiponectin levels, and this inversely correlated with improved hepatic steatosis.</td>
</tr>
<tr>
<td>Ratziu103</td>
<td>63</td>
<td>Rosiglitazone versus placebo</td>
<td>12 months</td>
<td>Steatosis: Yes, No; Inflammation: No, No; Fibrosis: No, No; NAS: No, No</td>
<td>1.5 kg</td>
<td>- Primary end point was &gt;30% reduction in hepatic steatosis and predictors of response included rosiglitazone treatment, lack of diabetes at entry, and greater steatosis at baseline. - No active lifestyle modification in either groups. - Ranked assessment of pretreatment and posttreatment biopsies showed improved steatosis, ballooning, and fibrosis in the rosiglitazone group. - Relatively lower NAS at entry may at least in part explain why no change in NAS was seen in this study.</td>
</tr>
<tr>
<td>Guruprasad104</td>
<td>74</td>
<td>Diet + exercise + Pioglitazone versus Diet + exercise + Pioglitazone</td>
<td>12 months</td>
<td>Steatosis: Yes, Yes; Inflammation: Yes, No; Fibrosis: Yes, No; NAS: N/A</td>
<td>2.6 kg</td>
<td>NAS score reported. - Placebo group had mean weight loss of 3.5 kg but TZD group had mean weight gain of 2.6 kg.</td>
</tr>
</tbody>
</table>

Table 5. Randomized Controlled Trials of TZDs Consisting of at Least 50 Randomized Patients
safety of TZDs (especially rosiglitazone).106 Because most of the participants in these studies did not have diabetes, it is not clear whether TZDs are equally effective in patients with diabetes with NASH. In fact, the presence of diabetes was a negative predictor of response to rosiglitazone in one study.103 Furthermore, Ratziu et al.103 recently raised the possibility that TZDs alone without lifestyle modification may not be as effective.103 Overall, there are more questions than answers about the role of TZDs in patients with NASH, and the ongoing large U.S. multicenter study (PIVENS; NCT00063622) may provide some additional insight.

**Promising Agents**

There is intense research into developing suitable treatment for NASH, and a list of compounds that are being tested to treat NASH in humans is shown in Table 6. Oral endocannabinoid receptor antagonists are of potential benefit because of multiple potential favorable effects (on body weight, fibrogenesis, and de novo lipogenesis), and large multicenter studies are underway. Neuropsychiatric side effects are of concern, but if proven effective, they may have a role at least in a select group of patients without underlying neuropsychiatric co-morbidities. The CB-1 receptor antagonist studies in humans have just begun and their results will not be available for few years. Based on promising animal data107 and its ability to promote weight loss,108 we have initiated an open-label study of exenatide in 2006, but its recruitment has been hampered because of its injectable route of administration and potential gastrointestinal system side effects.

**Cardiovascular Disease in NAFLD**

The cardiovascular morbidity and mortality is perhaps one of the most important aspects of NAFLD and NASH, and our knowledge of their association is evolving rapidly.109-112 The patients with NAFLD have very high prevalence of cardiovascular risk factors and atherosclerosis and high incidence of cardiovascular morbidity and mortality.113,114 Over the last decade, numerous studies have demonstrated that patients with NAFLD are enriched with classic cardiovascular risk factors such as obesity, insulin resistance, type 2 diabetes, dyslipidemia, and the metabolic syndrome.115-117 Cross-sectional studies consisting of control groups have shown increased prevalence of endothelial dysfunction,118 elevated levels of oxidized low-density lipoprotein,119 and Framingham coronary risk scores109,119 in NAFLD patients. Cross-sectional studies have also shown increased prevalence of premature atheroma formation,120 carotid artery intima media thickness (surrogate for atherosclerosis),121,122 vulnerable coronary plaques,123 increased mediastinal fat, and abnormal left ventricular energy metabolism.124 Recently it has been suggested that NAFLD poses cardiovascular risk above and beyond that conferred by the presence of the metabolic syndrome.33,113,114,125 In a nested case-control, prospective study, Targher et al. have shown that NAFLD in individuals with diabetes is associated with moderately increased risk of incident cardiovascular disease even after adjusting for classic risk factors, glycemic control and the metabolic syndrome.125 Most importantly, several longitudinal studies have shown that cardiovascular disease is much more common than liver disease as a cause of death in patients with NAFLD.43,113,114 In a longitudinal study consisting of 420 Olmsted county residents with NAFLD, Adams et al.113 have shown that ischemic heart disease accounts for 25% of deaths, compared with liver disease accounting for 13% of deaths. By linking the Third National Health and Nutrition Examination Survey to linked mortality files, Ong et al.114 have shown that cardiovascular disease is the most common cause of death, exceeding liver disease among 817 individuals with suspected NAFLD in comparison with 10,468 persons without liver disease. More recently, Rafiq et al.43 have reported extended follow-up of an expanded NAFLD cohort that has been described previously. The mortality rate over an 11.1-year median follow-up (longest follow-up, 28.5 years) was
45%, and the most common cause of death was coronary artery disease. All these data provide unequivocal evidence that coronary artery disease is a serious threat to patients with NAFLD. Therefore, it has become our practice to emphasize the significance of cardiovascular disease to patients with NAFLD and their primary care providers.

Statins remain a cornerstone for managing dyslipidemia and coronary artery disease. Despite initial concerns, several recent studies have shown that statins diminish cardiovascular morbidity and mortality in patients with NAFLD; however, there are no suspected reasons why they would be any less effective. Minor fluctuations in aminotransferases on initiating statin therapy are not uncommon, but serious hepatotoxicity is quite rare, and even when it happens, it is almost universally reversible on prompt recognition and withdrawal of the offending agent.

In summary, when a patient with suspected NAFLD is seen in the clinic, it is important to carefully evaluate for competing causes and clinically important comorbidities. Many advances have been made in terms of noninvasive biomarkers for predicting advanced fibrosis, but insufficient attention has been paid to predicting steatohepatitis. Sustained weight loss can be effective to treat NASH but it is difficult to achieve. Foregut bariatric surgery can be quite effective in improving hepatic histology in selected patients without liver failure or significant portal hypertension. TZDs have shown promise; however, recent studies raised doubts about their long-term safety. Large multicenter studies of endocannabinoid receptor antagonists are underway, but their results will not be available for several years. Several recommendations made in this review are not entirely evidence-based and thus should be cautiously accepted while their results will not be available for several years. Several recommendations made in this review are not entirely evidence-based and thus should be cautiously accepted while

Note Added in Proofs
In the fall of 2008, Sanofi-Aventis has terminated its multinational clinical trials of rimonabant (CB1 receptor antagonist) to treat NASH due to safety concerns. Similarly, Pfizer also stopped its CB1 receptor antagonist development program.

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