SUMMARY

Background
Non-alcoholic fatty liver disease (NAFLD) is an increasingly prevalent condition affecting adults and children, leading to significant morbidity. It is often associated with the metabolic syndrome, although multiple pathogenetic mechanisms have been suggested. In the coming decades, it promises to be the leading cause of liver disease in industrial countries.

Aim
To provide a comprehensive, updated review of diagnosis and management of NAFLD and to appraise the evolution of new modalities in these areas.

Methods
An Ovid MEDLINE search was performed to identify pertinent original research and review articles. Selected references in these articles were also evaluated.

Results
The diagnosis of hepatic steatosis and steatohepatitis or non-alcoholic steatohepatitis (NASH) is not yet possible without liver biopsy. This is impractical given the large numbers affected by the condition. Current therapy has focused on improving insulin resistance and mediators of inflammation, factors probably associated with disease progression.

Conclusions
There are no proven non-invasive diagnostic modalities to distinguish NAFLD and NASH, but new biomarker panels are approximating the liver biopsy in accuracy. Therapeutic targets of drug development are in early stages, but a multifaceted approach will probably yield several treatment options in the years to come.
INTRODUCTION

Fatty liver disease is not a new condition, and indeed, alcohol-induced liver injury dates back thousands of years. The entity of non-alcoholic fatty liver disease (NAFLD) is also not a new condition, but was not appreciated in early reports. In the 1950s, livers consistent with non-alcoholic steatohepatitis (NASH) were described in obese individuals, but surreptitious alcohol use was suspected.1 In the now famous report of Ludwig et al. in 1980, the term NASH was coined.2 It was not until the 1990s, however, that the prevalence and increasing incidence of the condition brought it into the limelight. It was not coincidence that the recognition of NAFLD paralleled the alarming increase in body mass index (BMI) in the American population3 (Figure 1). The umbrella term of NAFLD embodies simple steatosis, NASH and advanced fibrosis or cirrhosis related to this pathological entity.

The role of insulin resistance and free fatty acids

The predominant feature of NAFLD is insulin resistance (IR), which is an under-diagnosed condition, given there is no approved pharmacological therapy for it.

Insulin resistance is the primary liaison between NAFLD and the metabolic syndrome, which is defined as visceral obesity, hypertension, IR or diabetes, and dyslipidemia.4 The metabolic syndrome becomes more prevalent with age and varies by ethnicity and gender5 (Figure 2).

Insulin, an anabolic hormone, leads to an increased synthesis of glycogen, lipids and proteins. The exact mechanisms of such actions are not fully understood. An important mediator of insulin action is the intermediary phospho-inositide-3-kinase (PI-3-kinase), the activation of which generates 3'phosphoinositides PIP2 and PIP3 (phosphatidyl-inositol-3,4-biphosphate and phosphatidyl-inositol-3,4,5-triphosphate, respectively).6 The downstream effect of insulin binding is upregulation of glucose transporter 4 (GLUT 4), which leads to increased glucose entry into cells, leading to storage as glycogen or immediate use for energy production.

The role of obesity in IR appears to be mediated in part by free fatty acids (FFAs) and adipocytokines, including tumour necrosis factor-alpha (TNF-α). Intracellular accumulation of FFAs leads to impairments in signalling through the IRS/PI-3-kinase mediated GLUT 4 activity.7 Additionally, TNF-α downregulates GLUT 4 expression via increased serine phosphorylation of IRS-1.8

Within the liver, the action of insulin leads to increased glycogen synthesis via inactivation of glycogen synthase kinase-3, GSK3, which inhibits glycogen synthase.9 Triglyceride production is regulated by insulin effects on fatty acids10 and FFAs have been implicated in upregulating Fas (CD95) death receptors leading to apoptosis.11 Other potential FFA mechanisms of hepatocyte injury are highlighted in Table 1.11–17
Multiple theories exist regarding the pathophysiology of steatosis to NASH, which is beyond the scope of this review. Figure 3 describes some of the putative factors implicated in the development of NASH.

Epidemiology and natural history

The true prevalence of NAFLD and NASH is unknown given that the disease definition and modalities used for diagnosis are not standardized. Methodology of epidemiology studies of NAFLD vary widely as well, with only limited data from large population-based studies. Based on imaging studies, the prevalence of NAFLD in the adult population ranges 14–31%. Incidence of steatosis clearly increases with obesity. A large population-based study found that 91% of obese individuals (BMI > 30 kg/m²) had evidence of steatosis on ultrasound. The prevalence of NASH is even more difficult to determine as large population-based studies are not possible given that a liver biopsy is required for diagnosis. Nonetheless, most authors cite a prevalence of 2–3%, but the prevalence of NASH can be as high as 25–36% in morbidly obese populations.

Fatty liver is pervasive in all age, gender, and ethnic groups. Ethnic background does seem to play a role in the prevalence of NAFLD in America. Analysis of surveys of National Health and Nutrition Examination Survey III revealed a significantly higher prevalence of presumed NAFLD in Mexican-Americans compared with non-Hispanic whites, even after controlling for obesity and body fat distribution. Another study found NAFLD affecting 45% of Hispanics, 33% of Caucasians, and 24% of African-Americans.

A proportion of those with simple steatosis will progress to NASH. Of those with NASH, approximately 20% develop cirrhosis. Of those with cirrhosis, 30–40% decompensate and succumb to liver-related death over a 10-year period.

DIAGNOSIS

The diagnosis of NAFLD is suspected in patients with elevated aminotransferases and, in many cases, evidence of the metabolic syndrome. The distinction between NASH and simple steatosis cannot reliably be made without liver biopsy. However, it is clear that biopsy as a screening tool to distinguish these two conditions is impractical as a population-based approach. Additionally, definitive diagnosis of NAFLD and NASH requires exclusion of the multiple other causes of hepatic steatosis (Table 2).

Biochemical features/laboratory studies

Although 80% of patients with steatosis may have aminotransferases in the normal range, in some patients with NAFLD, the diagnosis is suspected in the presence...
of mildly elevated aminotransferases. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) fluctuate, with two-thirds of patients with NASH having normal levels at any point in time. Alkaline phosphatase may also be mildly elevated. Aminotransferases greater than two times normal are predictive of septal and bridging fibrosis across different populations. Hyperbilirubinemia and a low albumin, however, indicate a state of advanced liver disease and are not otherwise found in NAFLD.

The ratio between AST and ALT has also been found to have predictive value. The ratio increases in chronic liver disease by decreased clearance of AST as sinusoidal fibrosis increases. Several other studies have found an association between advanced fibrosis on biopsy and an AST/ALT ratio of >1.

Iron studies can be difficult to interpret as increased ferritin is seen in 20–50% and elevated transferrin saturation in 5–10% of patients with NAFLD. A complete laboratory evaluation to exclude other causes of liver disease should also be performed. Clinical history to exclude significant alcohol ingestion is required for the diagnosis. A daily consumption of 20 g/day of alcohol for women and 30 g/day or more for men are commonly used as exclusionary criteria in studies; however, the validity of these cut-offs is unknown.

Histological features

The spectrum of NAFLD consists of simple steatosis, NASH, fibrosis and cirrhosis. Simple macrovesicular...
steatosis results from accumulation of lipids (triglycerides) in large and small droplets within hepatocytes. The distinction between simple steatosis and steatohepatitis can be made only histologically. There is no single pathognomonic histological lesion in NASH, but it is a constellation of pathological findings. The evolution of histopathological diagnosis and scoring systems for NASH has been well documented in a recent review.39

The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)-Sponsored NASH Clinical Research Network (CRN) developed a histological scoring system, which is used for clinical trials in NASH.40 Three histological lesions are necessary for the diagnosis of NASH: zone 3 macrosteatosis, hepatocyte ballooning and mixed lobular inflammation (Figure 4). Other findings that are common, but not necessary include mild-moderate portal inflammation, acidophil bodies, glycogenated nuclei, periodic acid stain after diastase Kupffer cells, lipogranulomas and perisinusoidal zone 3 fibrosis (Figure 5). In addition, the following may be present, but are not necessary for the diagnosis: Mallory’s hyaline in ballooned hepatocytes, megamitochondria and mild siderosis.41 The histological severity of NAFLD is determined by the Non-alcoholic fatty liver disease Activity Score (NAS) and Fibrosis Score, developed and validated by the CRN. The activity score ranges from 0 to 8 and Fibrosis Score from 0 to 4. A NAS of 0–2 is not NASH and a score of ≥5 is usually NASH.40 While this scoring

<table>
<thead>
<tr>
<th>Aetiology</th>
<th>Examples</th>
</tr>
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<tbody>
<tr>
<td>Metals</td>
<td>Antimony, Barium salts, Borates, Carbon disulphide, Chromates, Phosphorus, Thallium and uranium compounds</td>
</tr>
<tr>
<td>Cytotoxic drugs</td>
<td>l-Asparaginase, Azacitidine, Azauridine, Methotrexate</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>Azaserine, Bleomycin, Puromycin, Tetracycline</td>
</tr>
<tr>
<td>Other drugs</td>
<td>Highly active antiretrovirals, Amiodarone, Tamoxifen, Oestrogens, Glucocorticoids, Coumadin, Dichloroethylene, Ethionine, Ethyl bromide, Hydrazine, Perhexilene maleate</td>
</tr>
<tr>
<td>Other metabolic disorders and infections</td>
<td>Inflammatory bowel disease, Small bowel bypass, Kwashiorkor and marasmus, Starvation and cachexia, Total parenteral nutrition, HIV, HCV</td>
</tr>
</tbody>
</table>
system is very useful for assessing change in clinical trials, it is not meant to replace a full interpretation of histological findings by a pathologist.

There are limitations to scoring systems based on liver biopsy. As a single percutaneous liver biopsy yields only a minute percentage (1/50 000 or 0.002%) of the total hepatic tissue, paired biopsies have been evaluated in several published studies.42–45 These studies, in aggregate, concluded that there is a significant sampling variability and that the histological lesions of NASH are unevenly distributed throughout the liver parenchyma and can lead to substantial misdiagnosis and staging inaccuracies.42, 44, 45 For example, Ratziu et al. reported on 51 patients with NAFLD who underwent paired biopsies. The discordance rate for ballooning was 18%; steatosis would have been missed in 24% of cases if only one biopsy had been done. A difference of one stage of fibrosis or more was seen in 41% of paired biopsies.45 In contrast, Larson et al. found a minimal variability between the right and left lobes of the liver for steatosis, fibrosis, and NAS > 5.43

Imaging

Although pathological diagnosis is the gold standard for making the diagnosis of fatty liver disease, some physicians and patients are reluctant to proceed with liver biopsy. In addition to the sampling variability noted above, variability in pathologists’ interpretation, cost and morbidity contribute to the search for additional modalities for diagnosis and staging of disease. Radiological features of NAFLD in combination with abnormal aminotransferases are often used to make the presumed diagnosis. After a diagnosis of NAFLD has been made pathologically, quantitative assessment of hepatic fat using computed tomography (CT), magnetic resonance imaging (MRI) or magnetic resonance spectroscopy may be an alternative to serial biopsies in the monitoring of patients with fatty liver disease, either clinically or in the context of clinical trials.46

Ultrasonography showing hyperechogenic liver tissue with fine echoes (bright liver) in contrast to the lower echogenicity of the spleen or kidney is characteristic of steatosis.48 Both liver fibrosis and steatosis may have similar appearance on ultrasound; however, a coarser echo pattern is generally seen with fibrosis.46

The sensitivity of ultrasonography is highly variable from 60% to 94%, with lower sensitivity in patients with mild steatosis.48 The sensitivity of ultrasound is 80% with ≥30% steatosis, but only 55% with steatosis of 10–19%.48 The specificity for detecting steatosis with ultrasound is 88–95%.48 However, in morbid obesity, sensitivity decreases to 49% and specificity to 75%.49 There is also significant interobserver and intraobserver variability as well as operator dependence in the assessment of fatty liver by ultrasound, which can limit usefulness.50 Furthermore, ultrasound cannot quantify the amount of fat present or stage fibrosis. Doppler ultrasound is another modality that may be helpful for the diagnosis of fatty infiltration. The compression on hepatic veins caused by fat deposition in hepatocytes can cause an abnormal hepatic vein Doppler waveform pattern.46, 51–53 This modality is not currently performed in clinical practice.

Unenhanced CT shows a low attenuation of the hepatic parenchyma in steatosis with the liver appearing darker than the spleen. The severity of the steatosis correlates with the density of the liver and the liver-to-spleen attenuation ratio.54, 55 The sensitivity and positive predictive value of unenhanced CT for detecting >33% steatosis is 93% and 76% respectively in a study by Saadeh et al.56

Contrast enhanced CT using a qualitative evaluation of the differential liver-spleen attenuation demonstrates a sensitivity of 54% and specificity of 95%.46 Iron increases the attenuation of the hepatic parenchyma, therefore, limiting the usefulness of CT for evaluating steatosis in the presence of iron overload states.57

Magnetic resonance imaging has been evaluated in detecting steatosis with different methods:
conventional pulse sequence, chemical shift imaging and fat saturation techniques. The conventional pulse sequence MRI is not useful because of limited sensitivity. MRI using the gradient-echo chemical shift technique and the fat saturation technique utilize the differences in resonant frequencies between water and fat. Both these techniques have been shown to detect steatosis accurately. Chave et al. demonstrated a sensitivity and specificity of 80% and 71% respectively for a threshold of 10% steatosis using the chemical shift technique. Qayyum et al. found that liver fat quantification with fat-saturated fast spin-echo MRI was significantly better than the out of phase gradient-echo MR (chemical shift technique) particularly in patients with cirrhosis. Hepatic iron content distorts the magnetic field, which interferes with accuracy in this technique in patients with primary or secondary iron overload.

Other modalities being evaluated include magnetic resonance spectroscopy, contrast enhanced ultrasound, and dual energy X-ray absorptiometry. None of the imaging technologies to date can distinguish between steatosis and NASH.

### Biomarkers in NAFLD

A growing number of potential biomarkers are being studied in the search for non-invasive indicators of the extent of disease, response to therapeutic modalities, and prognosis in NAFLD. Progressive fibrosis is related to inflammation, oxidative stress and apoptosis. These are some of the mechanisms that lead to liver injury and disease progression in NAFLD. Markers of these processes are potential targets for study.

Inflammation is thought to be a key component in the development of NAFLD; however, there is no clear marker identified as a predictor of disease. The adipocyte derived cytokines TNF-α and adiponectin are being evaluated for their role in NAFLD. TNF-α is increased by oxidative stress and is pro-inflammatory, whereas adiponectin may have an antifibrotic effect. Multiple studies have demonstrated that an imbalance of these two cytokines is correlated with NASH.

C-reactive protein (CRP), another inflammatory marker, has mixed results in NAFLD. Several studies showed no predictive value. However, recently, Yone-da et al. showed a significant elevation of high sensitivity assays for CRP in cases of NASH compared with cases of simple steatosis. High sensitivity CRP was also significantly elevated in those with advanced fibrosis compared with mild fibrosis. Reverse transcriptase-polymerase chain reaction also showed intrahepatic mRNA expression of CRP was increased in patients with NASH compared to simple steatosis ($P = 0.0228$).

Oxidative stress is felt to be a significant element in the progression from simple steatosis to steatohepatitis. Multiple by-products of oxidative stress have been measured including: lipid peroxidation products (lipid peroxides, thiobarbituric acid-reacting substances, oxidized low density lipoprotein (LDL), vitamin E levels, glutathione peroxidase (GSH-Px) activity, erythrocyte GSH-Px activity, Cu to Zn superoxide dismutase activity and breath ethane. While some of these markers may be found to be useful after further study, others have not been found to correlate with NAFLD.

Apoptosis, or programmed cell death, is increased in NASH. Cytokeratin 18 (CK-18), a marker of apoptosis, independently predicted NASH on multivariate analysis with an area under the curve (AUC) of 0.93 and a specificity for NASH of 94% and sensitivity of 90.5%. A large multicentre validation study is underway. CK-18 fragment levels were also measured in children with NAFLD and were correlated with NAS to predict severity of liver disease. Median CK-18 levels in patients with NAS ≥ 4 (477 U/L; range 157–2614) were significantly higher than in patients with NAS of ≤3 (median 209 U/L; range 148–494; $P = 0.049$). The CK-18 test may indeed prove to be a clinically useful biomarker in NAFLD.

### Diagnostic panels to assess steatosis and NASH

Bedogni et al. developed the Fatty Liver Index (FLI) by evaluating a cohort from the Dionysos Nutrition & Liver Study. A total of 224 subjects with suspected liver disease (excluding hepatitis B and C) were selected and matched with 287 subjects without suspected liver disease. After serial analysis, four predictors [triglycerides, BMI, gamma-glutamyl transpeptidase (GGT) and waist circumference] were entered into a model to generate the FLI. The authors reported that an FLI < 30 could be used to rule out and >60 to rule in hepatic steatosis. A limitation of this study is that the diagnosis of fatty liver was based on ultrasoundography.
these are the SteatoTest for quantitative steatosis and the NashTest for the categorical diagnosis of NASH. The SteatoTest includes the six components of the ActiTest plus BMI, glucose, triglycerides and cholesterol adjusted for age and gender. The AUC in the training group was 0.79 and in the three validation groups the AUC ranged from 0.72 to 0.86 for advanced steatosis (>5%). The NashTest is a slight variation of the SteatoTest and ActiTest to try to distinguish steatosis from NASH. AUC for the diagnosis of NASH in the training and validation groups were both 0.79. With the two groups pooled together, the sensitivity and specificity were 33% and 94% respectively. For borderline NASH, the sensitivity improved to 88%, but specificity dropped to 50%.

Shimada et al. evaluated the combination of adiponectin level, homeostasis model assessment insulin resistance (HOMA-IR), and serum type IV collagen level to separate simple steatosis from early stage NASH (Brunt stage 1–2). The individual components and well as the combined panel were evaluated, with the combination panel demonstrating a superior sensitivity and specificity (94% and 74% respectively). They concluded that approximately 90% of patients with early stage NASH could be predicted by this combination.

Non-invasive assessment of fibrosis

Of particular clinical interest is the determination of the extent of fibrosis using non-invasive markers, although this is still hampered by the comparison with the ‘gold’ standard, liver biopsy, with its previously noted limitations. Fibrosis is a dynamic process that involves remodelling of the extracellular matrix. Evaluation of the turnover of matrix components in relation to fibrosis has been studied singly and in a variety of combinations to determine the extent of fibrosis. Some of the components evaluated include transforming growth factor-beta, hyaluronic acid (HA), aminoterminal peptide of pro-collagen III (P3NP), type IV collagen propeptide, laminin, matrix metalloproteinases and tissue inhibitors of metalloproteinases (TIMPs).

Suzuki et al. evaluated HA in 79 patients with NAFLD. HA predicted severe fibrosis (stage 3–4) with an AUC of 0.90, but was poor for detecting mild fibrosis. A subsequent study by Palekar et al. showed that HA levels differentiated between simple steatosis and NASH in 80 patients with NAFLD (Receiver operating characteristic curve (ROC) 0.64). HA also predicted severe fibrosis with an ROC of 0.885, sensitivity 85.7%, and specificity 80.3%, with a cut-off level of 45.3 µg/L.

Endothelin-1 (ET-1) is another mediator in hepatic fibrosis. Degertekin et al. evaluated the role of ET-1 in determining the extent of fibrosis in three groups of patients with NAFLD. Group I (n = 40) had NASH, Group II (n = 12) had simple steatosis with elevated aminotransferases, and Group III had steatosis by ultrasound with normal aminotransferases. They found that mean ET-1 levels were significantly higher in NASH patients compared with those with simple steatosis. There was also a correlation between the severity of fibrosis and the serum ET-1 levels in patients with NASH.

Transient elastography uses pulse-echo ultrasound to measure wave velocity as a measure of liver stiffness. Its advantages include that it is non-invasive, it can assess a large sample area of the liver and allows multiple readings, which may avoid sampling error. Yoneda et al. showed that in 97 patients with NAFLD, there was a significant correlation between liver stiffness measurement and fibrosis stage. However, in a study by Fraquelli et al., they found a reduced interobserver agreement in assessing fibrosis in patients with high BMI (≥25) and steatosis; however, this study included only 13 patients with NASH. Although Foucher et al. demonstrated that in a large group of patients, liver stiffness correlated with fibrosis regardless of the aetiology of liver disease, only 4% of the patients had NASH. Furthermore, it should be noted that 47 of the 758 patients (6.2%) were excluded from the analysis because of unsuccessful liver stiffness measurement mostly because of the patients being overweight. It is possible that the presence of steatosis may interfere with the wave velocity such that the stiffness of a fibrotic liver is counterbalanced by the coexisting steatosis. Kim et al. demonstrated that transient elastography could not determine the amount of steatosis present on liver biopsy.

Diagnostic panels to assess fibrosis

The NAFLD Fibrosis score is a panel consisting of age, BMI, platelet count, albumin, AST/ALT ratio, and hyperglycaemia. This model was constructed using 480 patients and validated in 253. By using high and low cut-off values, advanced fibrosis could be ascertained with a negative predictive value (NPV)
of 93% in the construct group and 88% in the validation group. The positive predictive values for the groups were 90% and 82%, with AUC equal to 0.88 and 0.82 respectively. A limitation of the model was that 25% of the cases were in the indeterminate range. Nonetheless, liver biopsy could have been avoided in 75%.

The Original European Liver Fibrosis test is a panel of markers consisting of age, tissue inhibitor of metalloproteinase 1 (TIMP 1), HA, and P3NP.\textsuperscript{84} Guha et al. studied this panel and modifications to it in a cohort of 196 patients with NAFLD in a validation study. By removing age from the panel, the Enhanced Liver Fibrosis (ELF) panel had an AUC of 0.90 for severe fibrosis, 0.82 for moderate fibrosis, and 0.76 for no fibrosis. Removing age from the panel did not change the performance. The diagnostic accuracy was improved by adding ‘simple markers’ (the NAFLD Fibrosis Score components of Angulo et al.\textsuperscript{83}) to the panel, with AUC of 0.98 for severe fibrosis, 0.93 for moderate fibrosis, and 0.84 for no fibrosis. Based on a utility model, 82% (ELF) and 88% (combined panel) of liver biopsies could potentially be avoided for the diagnosis of severe fibrosis.\textsuperscript{85}

The BMI, Age, ALT, Triglyceride (BAAT) score (0–4), consisting of BMI $\geq$ 28 kg/m\(^2\), age $\geq$ 50 years, ALT greater than or equal to two times normal and serum triglycerides $\geq$ 1.7 mmol/L was evaluated in 93 consecutive patients by Ratziu et al.\textsuperscript{30} The NPV for fibrosis was 100% for a total score of 0–1. For detection of septal fibrosis, a score of 4 had 100% specificity but only 14% sensitivity.

FibroTest-FibroSURE is a proprietary algorithm combining five biochemical markers consisting of $\alpha_2$-macroglobulin, apolipoprotein A1, haptoglobin, total bilirubin, and GGT corrected for age and gender. The ActiTest (necroinflammatory index) adds ALT to the same markers.\textsuperscript{86} Both these were developed for patients with viral hepatitis. In a validation study for patients with NAFLD, the AUC of the FibroTest for advanced fibrosis is 0.81 (95% CI: 0.74–0.86). Limitations include patients with Gilbert’s syndrome, cholestasis and acute inflammation, which raise haptoglobin. In addition, in 33% of the cases, the presence or absence of advanced fibrosis could not be predicted.\textsuperscript{86}

These studies and several others\textsuperscript{87} suggest that single markers or a combination of markers may be used for non-invasive diagnosis and staging of NAFLD. However, none of these markers has been prospectively validated in different populations. In addition, they have not been evaluated for their utility in monitoring disease activity or progression. New approaches and advancing technology may also help identify novel biomarkers.\textsuperscript{88}

**TREATMENT**

Hepatic steatosis without evidence of NASH is not considered associated with increased liver-related morbidity or mortality.\textsuperscript{89} However, the presence of hepatic cell necrosis and inflammation warrants more intensive monitoring because NASH can progress to cirrhosis, end-stage liver disease and hepatocellular carcinoma. Currently, there are no approved therapies for NAFLD. There are many proposed agents being evaluated currently, each targeting a different step in the pathogenesis of development of hepatic steatosis or progression to steatohepatitis (Figure 6). The proper dosing, duration of treatment, safety, and tolerability of these treatments remain under investigation.

**Insulin resistance**

As described earlier, IR is believed to be a central mechanism involved in the development of hepatic steatosis. IR can be targeted through a multifaceted approach involving weight loss, surgical intervention, or pharmacological therapy.

Weight loss. Obesity stimulates a downregulation of GLUT-4 expression in adipose tissue.\textsuperscript{90} Muscle contraction from exercise induces a transient increase in intracellular calcium content and AMP/adenosine triphosphate ratios, which cause activation of downstream protein kinases. This triggers phosphorylation of substrates responsible for GLUT-4 translocation.\textsuperscript{90} Weight loss via diet and exercise together can significantly impact glucose homeostasis by increasing insulin sensitivity. Multiple studies have demonstrated decreased hepatic steatosis and serum transaminases,\textsuperscript{91–96} however, durability of weight loss as well as its effect on steatohepatitis is uncertain (Table 3).

A target loss of 10% of baseline weight as initial goal is recommended if BMI is $>25$ kg/m\(^2\).\textsuperscript{97} Weight loss should be gradual (1–2 pounds/week), as rapid weight loss $>1.6$ kg has been associated with exacerbation of steatohepatitis.\textsuperscript{98} In general, dietary recommendations have focused on a reduced daily caloric
Metabolic syndrome

Insulin resistance
Dyslipidemia

Statins
Clofibrate
Gemfibrozil
Probucol
Omega-3-FAs
Aquaporin
Rimonabant

Inflammation
Oxidative stress
Vitamin E
Betaine
Ursodeoxycholic acid

Steatosis

TZD, thiazolidinedione; FA, fatty acid; AT Rec Blocker, angiotensin II receptor blocker

Figure 6. Therapeutic agents under investigation for non-alcoholic steatohepatitis.

<table>
<thead>
<tr>
<th>Biopsy-proven NASH</th>
<th>Lifestyle intervention</th>
<th>Anthropometric change</th>
<th>Histology</th>
<th>ALT</th>
<th>Insulin resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y⁹¹ 50% CHO</td>
<td>BMI +</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>30% fat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>20% protein</td>
<td></td>
<td></td>
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<tr>
<td>(Total kcal/day = 25 kcal/kg)</td>
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<tr>
<td>Y⁹² Low CHO (&lt;20 g/day), ketogenic diet plus exercise</td>
<td>Weight loss +</td>
<td>+</td>
<td>=</td>
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<tr>
<td>Y⁹³ 40–45% CHO</td>
<td>Weight loss =</td>
<td>=</td>
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<td>+</td>
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<td>35–40% fat</td>
<td>BMI =</td>
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<tr>
<td>15–20% protein</td>
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<td></td>
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<tr>
<td>plus exercise</td>
<td></td>
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</tr>
<tr>
<td>N⁹⁴ 500 kcal daily dietary reduction plus exercise</td>
<td>Weight loss +</td>
<td>n/a</td>
<td>+</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>BMI +</td>
<td></td>
<td></td>
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<tr>
<td>N⁹⁵ Aerobic exercise 30 min/day plus moderately-restricted diet (25 kcal/kg)</td>
<td>BMI +</td>
<td>n/a</td>
<td>+</td>
<td>=</td>
<td></td>
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<tr>
<td>N⁹⁶ 54% CHO</td>
<td>BMI +</td>
<td>n/a</td>
<td>+</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>25% fat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>21% protein</td>
<td></td>
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</tbody>
</table>

+, statistically significant improvement; =, unchanged or improvement that did not reach statistical significance; n/a, not applicable; ALT, alanine aminotransferase; CHO, carbohydrate; BMI, body mass index; kcal, kilocalorie; NASH, non-alcoholic steatohepatitis.

Table 3. Lifestyle modification for treatment of NASH
intake as well as moderate daily exercise to promote weight loss and improve insulin sensitivity.

The role of pharmacological agents that induce weight loss is an area of interest that is currently being evaluated. Orlistat, an enteric lipase inhibitor, and sibutramine, a serotonin and norepinephrine reuptake inhibitor that increases satiety, have been associated with improved aminotransferases, as well as possible decreased hepatic inflammation and fibrosis.\textsuperscript{99, 100} Orlistat use has been limited by gastrointestinal side effects, and neither of these compounds is commonly prescribed.

The weight loss approach with the most dramatic and durable effect on obesity and NASH is bariatric surgery. In comparison with traditional diet and exercise, bariatric surgery more reliably effects substantial, long-term weight reduction for those with morbid obesity (BMI > 35 kg/m\textsuperscript{2}). With advances in technique, bariatric surgery results in significantly less malabsorption and nutritional deficiency.\textsuperscript{101} Laparoscopic assisted gastric banding is a minimally invasive procedure with very low perioperative morbidity and mortality.\textsuperscript{102} In addition to weight loss, multiple studies have shown a significant improvement in obesity-related comorbidities such as type-2 diabetes mellitus,\textsuperscript{103} hypertension, dyslipidemia, obstructive sleep apnoea, infertility, depression and quality of life.\textsuperscript{104}

With regard to its effect on the liver, bariatric surgery also produces a significant improvement in liver histology and biochemistry, probably because of the decrease in cytokines and chronic inflammation associated with obesity. Histologically, surgical weight loss has been associated with significantly improved steatosis, necroinflammation and fibrosis. Changes in anthropometrics, histology, biochemistries, and IR of studies investigating the effect of bariatric surgery on biopsy-proven NASH are shown in Table 4.\textsuperscript{105–112} In one study, biopsies performed 2 years postsurgery showed complete resolution of steatosis and fibrosis in 84\% and 75\% of patients respectively.\textsuperscript{109} Moreover, other studies have demonstrated complete reversal of cirrhosis.\textsuperscript{108}

**Insulin sensitizers.** Agents that improve insulin sensitivity are currently undergoing extensive evaluation with regard to safety and ability to improve histological features of fibrosis and inflammation. Thiazolidinediones are high-affinity ligands of peroxisome

### Table 4. Bariatric surgery for treatment of non-alcoholic steatohepatitis

<table>
<thead>
<tr>
<th>Type of surgery</th>
<th>Anthropometrics</th>
<th>Histology</th>
<th>Insulin resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wt loss</td>
<td>% Wt loss</td>
<td>BMI</td>
</tr>
<tr>
<td>LAGB\textsuperscript{105}</td>
<td>34.0 kg</td>
<td>51.6%</td>
<td>13</td>
</tr>
<tr>
<td>LRYGB\textsuperscript{106}</td>
<td>50.2 kg</td>
<td>n/a</td>
<td>18.2</td>
</tr>
<tr>
<td>LRYGB, LAGB, or LSG\textsuperscript{107}</td>
<td>103 kg</td>
<td>30.4%</td>
<td>17</td>
</tr>
<tr>
<td>RYGB\textsuperscript{108}</td>
<td>n/a</td>
<td>n/a</td>
<td>22.3</td>
</tr>
<tr>
<td>BPD or BPD-DS\textsuperscript{109}</td>
<td>38 kg</td>
<td>30.6%</td>
<td>16</td>
</tr>
<tr>
<td>RYGB\textsuperscript{108}</td>
<td>n/a</td>
<td>60%</td>
<td>19.3</td>
</tr>
<tr>
<td>VRG or LABG\textsuperscript{111}</td>
<td>32 kg</td>
<td>n/a</td>
<td>12.2</td>
</tr>
<tr>
<td>RYGB\textsuperscript{112}</td>
<td>36.4 kg</td>
<td>28.9%</td>
<td>13.5</td>
</tr>
</tbody>
</table>

+, statistically significant improvement; =, unchanged or improvement that did not reach statistical significance; −, Worsening; n/a, not applicable; Wt, weight; BMI, body mass index; Stea, steatosis; Infl, inflammation; Fibr, fibrosis; ALT, alanine aminotransferase; LAGB, laparoscopic adjustable gastric banding; LRYGB, laparoscopic roux–en–Y gastric bypass; LSG, laparoscopic sleeve gastrectomy; RYGB, roux–en–Y gastric bypass; BPD, bilio-pancreatic diversion; BPD-DS, bilio-pancreatic diversion with duodenal switch; VRG, vertical banded gastroplasty; FBS, fasting blood sugar; HOMA %S, homeostatic model assessment %insulin sensitivity; HOMA-IR, homeostatic model assessment insulin resistance.

*Aspartate aminotransferase used.
proliferator-activated receptor-γ, stimulating the storage of FFAs in subcutaneous adipocytes as opposed to liver and omental fat, thereby improving insulin sensitivity. This class may also have anti-inflammatory properties, as demonstrated by a decrease in nuclear factor kappa-B levels and an increase in inhibitor kappa-B and adiponectin levels. In multiple small pilot studies, both rosiglitazone and pioglitazone, have significantly improved insulin sensitivity, transaminases and liver histology. Table 5 summarizes thiazolidinediones studies in patients with biopsy-proven NASH. However, treatment with these agents may need to be life-long, as evidenced by one study that found an increase in serum ALT, IR, and steatosis after drug cessation. Additionally, recent data have suggested possible cardiovascular risks, congestive heart failure, osteoporosis, and weight gain associated with this class of drugs. Larger, multi-year clinical trials are necessary to determine the efficacy and safety of thiazolidinediones in this population.

Metformin improves IR by decreasing hepatic glucose production and increasing skeletal muscle glucose uptake. Specifically, it reduces hepatic expression of TNF-α, a cytokine that interferes with insulin-receptor mediated signalling pathways in hepatocytes. Metformin can also increase fatty acid oxidation and suppress lipogenesis. Small studies have shown that metformin results in a significant improvement in aminotransferases as well as histology in patients with NASH (Table 6). Metformin appears to be well tolerated in the NAFLD population, with most common side effect being GI intolerance. Unlike the thiazolidinediones, metformin does not cause weight gain, and in fact, may be associated with mild weight loss. Although metformin has been associated with elevated lactate levels, there have been no cases of lactic acidosis. Nonetheless, metformin should be used with caution in patients with liver disease as it can exacerbate hepatic encephalopathy and hepatic failure.

### Table 5. Thiazolidinediones for treatment of NASH

<table>
<thead>
<tr>
<th>Biopsy-proven NASH</th>
<th>Drug</th>
<th>Histology</th>
<th>ALT Effects</th>
<th>Study design</th>
<th>N</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>Pioglitazone</td>
<td>+ + = +</td>
<td>↓ fasting glucose, ↓ fasting insulin, ↓ TNF-α, ↓ TGF-β, ↑ adiponectin</td>
<td>Randomized (1:1), placebo control</td>
<td>55</td>
<td>24 weeks</td>
</tr>
<tr>
<td>Y</td>
<td>Rosiglitazone</td>
<td>+ + + +</td>
<td>↑ serum insulin, ↑ QUICKI, ↓ HOMA</td>
<td>Randomized (1:1), double-blind, placebo controlled</td>
<td>63</td>
<td>48 weeks</td>
</tr>
<tr>
<td>Y</td>
<td>Pioglitazone</td>
<td>+ + + +</td>
<td>↓ fasting insulin, ↓ fasting FFA</td>
<td>Randomized (1:1), control vs. vitamin E 400 IU/day alone</td>
<td>20</td>
<td>24 weeks</td>
</tr>
<tr>
<td>Y</td>
<td>Rosiglitazone</td>
<td>= = = +</td>
<td>↓ HOMA-IR</td>
<td>Nonrandomized, 3 arms, open label vs. metformin vs. diet</td>
<td>47</td>
<td>48 weeks</td>
</tr>
<tr>
<td>Y</td>
<td>Pioglitazone</td>
<td>+ + + +</td>
<td>↓ HOMA-IR, ↑ QUICKI, ↓ fasting insulin, ↓ fasting FFA</td>
<td>Single arm, open label</td>
<td>18</td>
<td>48 weeks</td>
</tr>
<tr>
<td>Y</td>
<td>Rosiglitazone</td>
<td>= + + +</td>
<td>↓ HOMA-IR, ↑ QUICKI</td>
<td>Single arm, open label</td>
<td>30</td>
<td>48 weeks</td>
</tr>
</tbody>
</table>

+, statistically significant improvement; =, unchanged or improvement that did not reach statistical significance; n/a, not applicable; Stea, steatosis; Infl, inflammation; Fibr, fibrosis; ALT, alanine aminotransferase; FFA, free fatty acid; TNF-α, tumour necrosis factor-alpha; TGF-β, transforming growth factor-beta; QUICKI, quantitative insulin-sensitivity check index; HOMA-IR, homeostasis model assessment insulin resistance; NASH, non-alcoholic steatohepatitis.

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caution in patients with renal insufficiency or congestive heart failure. Metformin has shown encouraging results in improving IR as well as biochemical and histological markers of NASH. However, large randomized, controlled trials are necessary to determine long-term safety and effect of metformin on steatohepatitis. Exedin-4 (nateglinide) improves insulin sensitivity by stimulating the release of insulin from the pancreas and stimulating growth of pancreatic beta cells. Studies on ob/ob mice have demonstrated a significant improvement in hepatic steatosis and IR. A small pilot study, which investigated the use of nateglinide in five diabetic patients with NASH, found a significant improvement in biochemical and histological markers of disease.

### Oxidative stress

The mechanism of progression from the steatosis of NAFLD to the necroinflammatory state of NASH is poorly understood; however increased production of pro-inflammatory mediators probably plays a role. Adipose tissue is a metabolically active tissue, which produces adipocytokines, which are involved in the pathogenesis of inflammation associated with NASH. Adipocytokine pathways offer several targets for potential drug development for the treatment of NASH.

The antioxidant properties of vitamin E results from its ease in donating hydrogen from its hydroxyl group to neutralize free radicals and thereby to prevent lipid peroxidation. Multiple randomized clinical trials have failed to find a significant effect of vitamin E on transaminases, hepatic inflammation, and fibrosis. Moreover, long-term high dose vitamin E supplementation (>400 IU/day) has been associated with increased all-cause mortality. It is currently being evaluated in a large, multi-centre trial.

Betaine is a metabolite of choline that assists with the synthesis of S-adenosylmethionine. Increased levels of this powerful antioxidant may serve a protective role against development of hepatic steatosis. A small pilot study of 10 patients with NASH showed biochemical and histological improvement after 1 year treatment with betaine. Given the low cost and tolerability, these agents may serve a role in treatment of NASH; however, additional studies are needed.

### Altered lipid metabolism

In addition to IR, alterations in lipid metabolism may be an antecedent for development of steatosis and NASH. Targeting the mechanisms of lipid production...
and accumulation in the liver has generated several potential therapies for NASH. Hydroxymethylglutaryl-CoA reductase inhibitors (also known as ‘statins’) are a class of highly prescribed medications used to treat hyperlipidemia. Given the risk of asymptomatic liver enzyme elevation and hepatotoxicity related to statins, there has been concern regarding their use in patient with fatty liver disease. However, this patient population has not been shown to be at a greater risk. A study that investigated the safety of statins found no increase in liver biochemistries after 6 months of therapy.\textsuperscript{142} Though statins may have the potential to increase hepatic lipogenesis,\textsuperscript{143} a recent study by Ekstedt showed no progression of liver fibrosis after 10.3–16.3 years of statin therapy.\textsuperscript{144} In fact, these follow-up biopsies found a significant reduction in fatty infiltration.

Several statins have been investigated in small pilot studies\textsuperscript{145–149} (Table 7). However, because these studies have small sample sizes and lack placebo control, more extensive studies must be performed before statins can be recommended for the treatment of NASH.

Other dyslipidemia agents have been investigated. Clofibrate is a lipid-lowering agent that has been shown to lower hepatic triglycerides in animal models, although the exact mechanism of action remains unknown.\textsuperscript{150} However, an open label, 1-year pilot study failed to show an improvement in lipid profile, aminotransferases and histological grade of steatosis.\textsuperscript{151} In a randomized controlled trial, gemfibrozil resulted in decreased aminotransferases and GGT compared to placebo.\textsuperscript{152} Probucol, a lipophilic bisphenol that lowers total and LDL cholesterol, also has potent antioxidant properties. Ten patients with biopsy-proven NASH who underwent 1 year of probucol showed normalization of aminotransferases and reduced histological evidence of steatohepatitis.\textsuperscript{153} Finally, omega-3 fatty acid supplementation has also been shown to decrease significantly serum aminotransferases, GGT, triglycerides and fasting glucose as well as to improve steatosis on ultrasound.\textsuperscript{154}

Conclusions about lipid altering agents in the treatment of NAFLD are limited because of small sample sizes, open label study design, and poor methodological quality. However, given that those with fatty liver disease often have metabolic syndrome, a lipid-lowering agent may play a beneficial role in management of comorbidities associated with metabolic syndrome and dyslipidemia.

### Evolving therapies

Multiple novel agents are currently under investigation. Rimonabant selectively antagonizes cannabinoid type 1 (CB1) receptors in the central nervous system, resulting in decreased food consumption and caloric intake.\textsuperscript{155} Activation of hepatic CB1 receptors stimulates fatty acid synthesis in hepatocytes and increases lipogenesis.\textsuperscript{156} Consequently, inhibition of these receptors by rimonabant may result in decreased hepatic steatosis and fibrosis.\textsuperscript{157}

<table>
<thead>
<tr>
<th>Biopsy-proven NASH</th>
<th>Drug</th>
<th>Histology</th>
<th>ALT</th>
<th>Study design</th>
<th>N</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>Atorvastatin 10 mg/day\textsuperscript{145}</td>
<td>n/a</td>
<td>+</td>
<td>Nonrandomized vs. ursodeoxycholic acid</td>
<td>44</td>
<td>6 months</td>
</tr>
<tr>
<td>Y</td>
<td>Pravastatin 20 mg/day\textsuperscript{146}</td>
<td>Improved*</td>
<td>+</td>
<td>Single arm, open label</td>
<td>5</td>
<td>6 months</td>
</tr>
<tr>
<td>N</td>
<td>Atorvastatin 10–80 mg/day\textsuperscript{147}</td>
<td>n/a</td>
<td>+</td>
<td>Single arm, open label</td>
<td>22</td>
<td>6–12 months</td>
</tr>
<tr>
<td>N</td>
<td>Rosuvastatin 10 mg/day\textsuperscript{148}</td>
<td>n/a</td>
<td>+</td>
<td>Single arm, open label</td>
<td>23</td>
<td>8 months</td>
</tr>
<tr>
<td>N</td>
<td>Atorvastatin 20 mg/day\textsuperscript{149}</td>
<td>Improved†</td>
<td>+</td>
<td>Nonrandomized, 3 arms, open label vs. omega-3 fatty acid vs. orlistat</td>
<td>88</td>
<td>24 weeks</td>
</tr>
</tbody>
</table>

+, statistically significant improvement; n/a, not applicable; omega-3 FA, omega-3 fatty acids; NASH, non-alcoholic steatohepatitis.

* Three of five patients with improved inflammation; one with improved steatosis; no improvement in fibrosis.

† Repeat liver biopsies in only eight of 88 subjects: six of eight with improved inflammation; no improvement in fibrosis.
The renin-angiotensin system (RAS), via angiotensin II (ATII), activates hepatic stellate cells, the main matrix-producing cells in the process of liver fibrosis.\textsuperscript{158} Several animal studies have demonstrated a marked decrease in hepatic fibrosis and stellate cell activation with inhibition of RAS, specifically at the ATII type 1a receptor.\textsuperscript{159–163} A pilot study using losartan in seven patients with NASH and hypertension found a significant decrease in aminotransferases as well as serum markers of fibrosis.\textsuperscript{164}

Aquaporins (AQPs) are integral membrane channel proteins that facilitate the movement of water through cell membranes.\textsuperscript{165} A sub-family of AQPs, called aquaglyceroporins, coordinates movement of both water and glycerol across cell membranes. Two aquaglyceroporins, adipose-specific AQP7 and liver-specific AQP9, may be involved in the pathogenesis of IR and may be reasonable targets of drug development.\textsuperscript{166} AQP7 facilitates the movement of glycerol from adipocytes into the bloodstream, while AQP9 allows uptake of glycerol into the hepatocyte, which stimulates hepatic gluconeogenesis. In the normal feeding state, insulin inhibits expression of adipose-specific AQP7 and liver-specific AQP9, thereby reducing glycerol export out of adipose tissue and increasing fat accumulation. However, as evidenced by mouse models,\textsuperscript{167} IR actually causes an increase in these AQP. As a result, increased glycerol is exported from adipocytes to hepatocytes, resulting in increased gluconeogenesis and worsening hyperglycaemia.

Other studies have proposed a possible role of intestinal bacterial overgrowth in the development of NASH. Bacterial overgrowth results in endogenous ethanol production, which increases intestinal permeability to gut bacterial products, such as lipopolysaccharide.\textsuperscript{168, 169} Both ethanol and lipopolysaccharide are hepatotoxic, stimulating release of cytokines, in particular TNF-\textgreek{z}. In small pilot studies, probiotic VSL\#3 has improved aminotransferases in patients with NAFLD.\textsuperscript{170} Similarly, anti-TNF antibodies have improved hepatic inflammation and transaminases in obese, insulin resistant mouse models.\textsuperscript{169} Although results may seem promising in animal studies, there are no large randomized trials in humans.

Caveolins are plasma membrane proteins that are involved in skeletal muscle insulin signalling and energy metabolism.\textsuperscript{171} Caveolin-1 deficient mice develop marked lipid profile abnormalities\textsuperscript{172} as well as disturbances in the insulin signalling pathway.\textsuperscript{173} Likewise, caveolin-3, found predominantly in skeletal muscle, may also participate in insulin signalling and energy metabolism. Caveolin-3 knockout mice displayed increased whole body adiposity and hyperinsulinaemia, proposed to be secondary to diminished insulin receptor stability, resulting in defective insulin signalling, and subsequent IR.\textsuperscript{171} Caveolins may serve as potential new therapeutic targets for improving lipid trafficking within hepatocytes.

One final novel therapy under investigation is oligofructose, a nondigestible oligomer of \textgreek{b}-D-fructose found in chicory root, onion, asparagus, artichoke and garlic.\textsuperscript{174} In rat studies, fructose supplementation was found to reduce serum and hepatic triglyceride by decreasing hepatic de novo lipogenesis.\textsuperscript{175, 176} Daubioul et al. performed a small, placebo-controlled pilot study using 8 weeks of oligofructose on seven patients with biopsy-proven NASH.\textsuperscript{177} Oligofructose resulted in decreased aminotransferase and insulin levels.

\section*{Conclusions}

Non-alcoholic fatty liver disease is and will continue to be a major liver health issue in Western countries in the coming decades. The exact pathogenesis and natural history are still being defined. Simple steatosis is very prevalent, but fortunately does not appear to progress to advanced liver disease in majority of individuals. Those with the NASH variant have a poorer prognosis, with a significant proportion progressing to advanced fibrosis. This is a slow process, but will begin to appear in younger individuals as the obesity prevalence in children continues to rise. A major challenge is finding a diagnostic scheme to distinguish this aggressive histological variant without resorting to liver biopsy. To date, no acceptable replacement for histological assessment has been found, but diagnostic panels are improving and will soon have reasonable sensitivity and specificity to allow at least an adjuvant modality to diagnose or suggest NASH.

The therapeutic arena for NAFLD continues to develop and focuses on improving IR and decreasing inflammatory microenvironments to prevent or slow the development of NASH. There are no leading drug candidates at this point, although there are several promising concepts in drug development. Currently, therapy should address the features of the metabolic syndrome, including diabetes, obesity and dyslipidemia. Bariatric surgery is becoming a more mainstream option for morbid obesity, but maintenance of weight control will require dieting and exercise.
A multidisciplinary approach utilizing liver specialists, surgeons, endocrinologists, dietitians and psychologists is likely to be of greatest benefit to the patient with NAFLD.

ACKNOWLEDGEMENT
Declaration of personal and funding interests: None.

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