# Nonalcoholic fatty liver disease

**Leon A. Adams, Paul Angulo, Keith D. Lindor**

**Abstract**

NONALCOHOLIC FATTY LIVER DISEASE is emerging as the most common chronic liver condition in the Western world. It is associated with insulin resistance and frequently occurs with features of the metabolic syndrome. Disease presentation ranges from asymptomatic elevated liver enzyme levels to cirrhosis with complications of liver failure and hepatocellular carcinoma. Current treatment recommendations are limited to weight loss and exercise, although several promising medications are on the horizon. In this article we discuss the etiology, pathogenesis and diagnosis of nonalcoholic fatty liver disease as well as approaches to its management.

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The diagnosis of nonalcoholic fatty liver disease (NAFLD) requires evidence of fatty changes in the liver in the absence of a history of excessive alcohol consumption. The histologic spectrum of NAFLD spans from generally benign, bland steatosis to steatosis with evidence of hepatocellular inflammation and damage (nonalcoholic steatohepatitis, or NASH), which may be complicated by progressive fibrosis and cirrhosis. NAFLD can be primary or secondary depending on the cause (Table 1). Secondary causes require exclusion, as these conditions have different prognoses and treatment.[1] In this review we focus on primary NAFLD and discuss the current knowledge of the pathophysiology and natural history of NAFLD, appropriate management strategies and emerging treatment options.

## Epidemiology

Hepatic steatosis detected by magnetic resonance spectroscopy is found in 31% of adults in the United States[2] and in 33% of potential live liver donors undergoing liver biopsy.[3] Ultrasonography detects fatty changes in the liver in 12.9%–16.4% of individuals.[4–6] The prevalence of steatosis tends to be higher among males[6–8] and in certain ethnic groups (e.g., up to 45% of Hispanic people).[2] Prevalence increases with age, from 2.6% among children to 26% among people 40–59 years old.[5,9]

NAFLD is more frequent among people with diabetes (50%) and obesity (76%), and it is almost universal among diabetic people who are morbidly obese.[4,10,11] Obesity, diabetes and the metabolic syndrome are also risk factors for NASH and for advanced fibrosis on liver biopsy.[12–14] NASH is present in 18.5% of obese subjects (compared with 2.7% of lean individuals) and in 50% of severely obese people with diabetes.[15,16] The metabolic syndrome confers an odds ratio (OR) for NASH of 3.2 (95% confidence interval [CI] 1.2–8.9) and an OR for advanced fibrosis of 3.5 (95% CI 1.1–11.2).[17] Among people who are not obese and do not have diabetes, risk factors for NAFLD are impaired fasting glycemia (OR 2.8, 95% CI 1.5–5.20), hypertriglyceridemia (OR 2.8, 95% CI 2.0–4.0), hyperuricemia (OR 2.6, 95% CI 1.6–4.1), central obesity (OR 2.4, 95% CI 1.7–3.4), hypertension (OR 1.7, 95% CI 1.2–2.4) and low levels of high-density lipoprotein (HDL) cholesterol (OR 1.4, 95% CI 11.0–2.0).[18]

## Pathogenesis

The liver plays a central role in lipid metabolism, importing serum free fatty acids and manufacturing, storing and exporting lipids and lipoproteins. However, the pathophysiology that leads to NAFLD is not well understood; in particular, the factors that lead to progressive hepatocellular damage after triglyceride accumulation are not well elucidated. It appears that alteration of local and systemic factors (particularly insulin resistance) that control the balance between the influx or synthesis of hepatic lipids and their export or oxidation leads to hepatic triglyceride accumula-

**Table 1: Causes of nonalcoholic fatty liver disease**

| Cause | Associations | Steatosis type |
|---|---|---|
| **Primary** | Features of the metabolic syndrome | Macrovesicular |
| **Secondary** | | |
| Nutritional | Total parental nutrition, rapid weight loss, starvation, intestinal bypass surgery | Macrovesicular |
| Drugs | Glucocorticoids, estrogens, tamoxifen, methotrexate, zidovudine | Macrovesicular |
| | Amiodarone, ASA, intravenous tetracycline, didanosine, cocaine, perhexilene, hypervitaminosis A, diltiazem | Microvesicular |
| Toxins | Toxic mushrooms (*Amanita phalloides, Lepiota*) | Macrovesicular |
| | Petrochemicals, phosphorus, *Bacillus cereus* toxin | Microvesicular |
| Metabolic | Lipodystrophy, dysbetalipoproteinemia, Weber–Christian disease, Wolman's disease | Macrovesicular |
| | Acute fatty liver of pregnancy, Reye's syndrome | Microvesicular |
| Other | Inflammatory bowel disease, HIV infection, small-bowel diverticulosis with bacterial overgrowth | Macrovesicular |

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tion. The steatotic liver is then thought to be vulnerable to secondary insults, which lead to hepatocellular inflammation and fibrosis. A variety of factors have been implicated to produce a second "hit," including hormones derived from adipose tissue (adipocytokines), oxidative stress and gut-derived bacterial endotoxin.[19]

### Insulin resistance

The pathogenesis of insulin resistance is complex and is likely to involve many genetic polymorphisms that influence insulin secretion and action as well as environmental factors that promote obesity and immobility.[20] Hyperinsulinemia increases serum free fatty acid levels, which are taken up by the liver and drive triglyceride production and hepatic steatosis (Fig. 1).[21] In addition, chronic hyperinsulinemia promotes *de novo* hepatic lipogenesis through upregulation of lipogenic transcription factors[21–23] and may activate profibrotic cytokines such as connective tissue growth factor.[24]

### Hepatic lipid metabolism

Lipids are normally exported from the liver in very-low-density lipoproteins (VLDL), which are formed by microsomal triglyceride transfer protein (MTP) incorporating triglyceride into apolipoprotein B (apo B). A reduction in MTP activity and apo B synthesis and secretion may impair hepatic lipid export and favour hepatic triglyceride accumulation.[25–28]

### Inflammatory and fibrotic mediators in NAFLD

Adipocytokines (tumour necrosis factor-α [TNF-α], leptin and adiponectin), free fatty acids, mitochondrial dysfunction, bacterial endotoxin and vascular disturbance have all been implicated in the development of hepatic inflammation and fibrosis in patients with NAFLD.[29–33] These factors may be directly hepatotoxic or generate oxygen radicals with subsequent lipid peroxidation, cytokine induction and liver damage.[19]

TNF-α promotes insulin resistance and liver inflammation. Levels are increased in patients with NAFLD, perhaps secondary to gut-derived endotoxin or TNF-α polymorphisms.[28,31,34–36] Leptin and adiponectin are important regulators of adiposity and insulin resistance and have been found to promote liver fibrogenesis in animal models.[30,37–39] Leptin and adiponectin levels are altered in patients with NASH; however, their exact pathogenic role has yet to be elucidated.[31,40]

As a result of insulin resistance, serum free fatty acid levels are increased in NASH patients and may be directly hepatotoxic or produce damaging reactive oxygen species.[23,32,33] Oxidative stress may be exacerbated further by ultrastructural mitochondrial lesions, which impair respiratory chain function.[23,33]

As liver injury progresses, fat-laden hepatocytes and perisinusoidal fibrosis may impair microvascular hepatic blood flow. This effect may decrease oxygen and nutrient exchange and thus stimulate a microvascular inflammatory

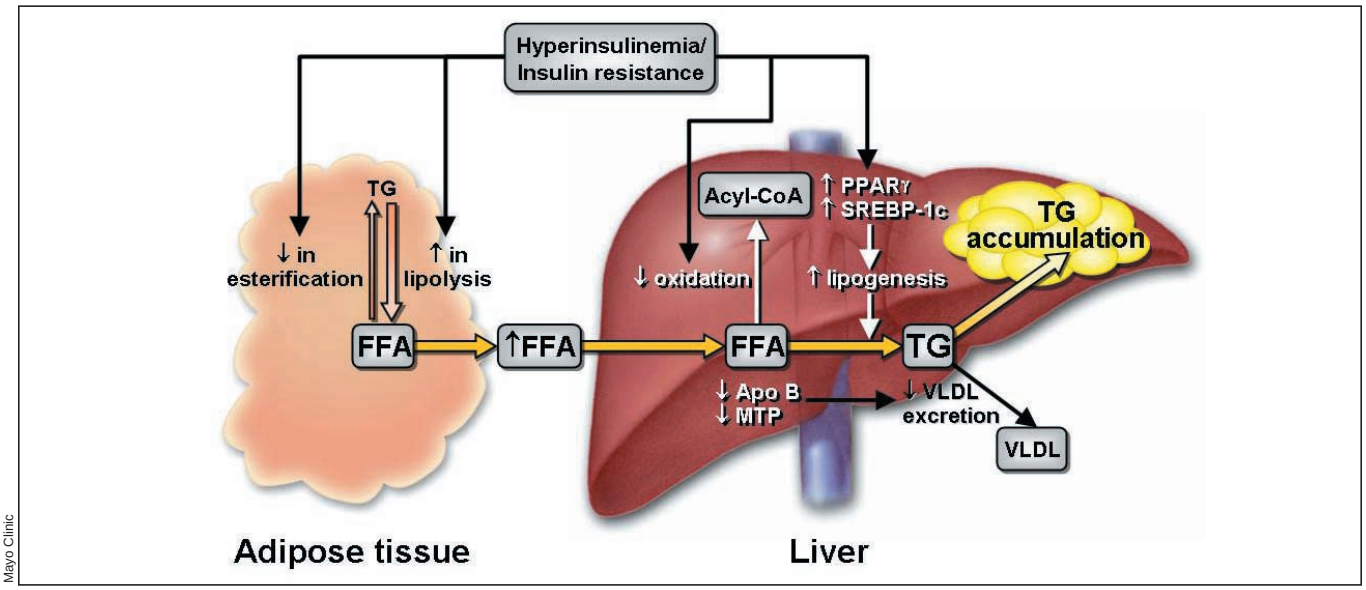


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**Fig. 1: Development of nonalcoholic hepatic steatosis.** Insulin resistance enhances triglyceride (TG) lipolysis and inhibits esterification of free fatty acids (FFA) within adipose tissue. The result is increased serum FFA levels, which are taken up by the liver. Hepatic TG synthesis is driven by the increased influx of FFA and favoured by insulin upregulated lipogenic transcription factors, such as peroxisome proliferator-activated receptor gamma (PPARγ) and sterol regulatory element binding protein (SREBP)-1c. Alternative metabolism of FFA by oxidation is inhibited by insulin. TG export via very-low-density lipoproteins (VLDL) may be inhibited by decreased synthesis of apolipoprotein B (apo B) or reduced incorporation of TG with apo B by microsomal triglyceride transfer protein (MTP). See the animated figure at www.cmaj.ca/cgi/content/full/172/7/899/DC1.

response and an escalating cycle of liver damage and vascular insufficiency.[41,42]

## Natural history

The natural history of NAFLD has been examined in relatively small numbers of select patients, and thus the evolution of the disease in the general population is not well defined. However, patients who have NAFLD appear to have a higher mortality than people in the general population.[43] Patients with pure steatosis have a benign prognosis: follow-up of 198 patients for up to 21 years revealed progression to cirrhosis in 3 patients and liver-related death in only 1.[44–46] In contrast, up to 11% of NASH patients may die of liver-related causes.[45] Diabetes is a risk factor for fibrosis progression and for overall and liver-related death among NAFLD patients.[47,48]

Many patients with cryptogenic cirrhosis have metabolic risk factors for NAFLD and are likely to represent cases of previously unrecognized NAFLD,[49,50] particularly because hepatic steatosis may disappear with the development of cirrhosis.[48,51] NAFLD may also present as cirrhosis complicated by hepatocellular carcinoma: at least 13% of cases of hepatocellular carcinoma were attributable to NASH in one study.[52] Overall, fibrosis progression in patients with NAFLD appears to be slow: in previous studies, it took several decades for cirrhosis, hepatocellular carcinoma and liver decompensation to develop in a small proportion of patients with NASH.[48,51,53]

## Clinical features

NAFLD is usually asymptomatic, although fatigue and discomfort in the right upper quadrant of the abdomen may be reported.[51] The majority (56%–79%) of patients are overweight (body mass index [BMI] > 25 kg/m$^2$), and one-third have the metabolic syndrome.[17,54,55] Lean patients (BMI < 25 kg/m$^2$) usually have at least one metabolic risk factor.[17] Hepatomegaly may be present, although signs of chronic liver disease are uncommon.[51,56]

Liver enzyme levels in NAFLD patients fluctuate, normal values being present in up to 78% of patients at any one time.[57,58] When levels are elevated, the increase is mild and often restricted to one or both of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The AST:ALT ratio is usually less than 1, although it may reverse in the presence of cirrhosis.[59] Liver enzyme levels do not reliably correlate with liver histology, and the full range of disease may be seen in patients with NAFLD who have normal transaminase levels.[12,51]

Ferritin levels are increased in 20%–50% of patients, and elevated transferrin saturation (> 55%) is present in 5%–10%.[13,55,60] Autoantibodies are identified in 23%–36% of NAFLD patients and are associated with more advanced fibrosis.[61,62]

## Diagnosis

### *Imaging*

Ultrasonography and CT and MRI scanning are reliable for detecting moderate to severe fatty changes in the liver. Hepatic fat causes increased echogenicity on ultrasound, compared with the lower echogenicity of the spleen or renal cortex. In noncontrast CT scans, the fatty liver is hypodense and appears darker than the spleen. Hepatic vessels

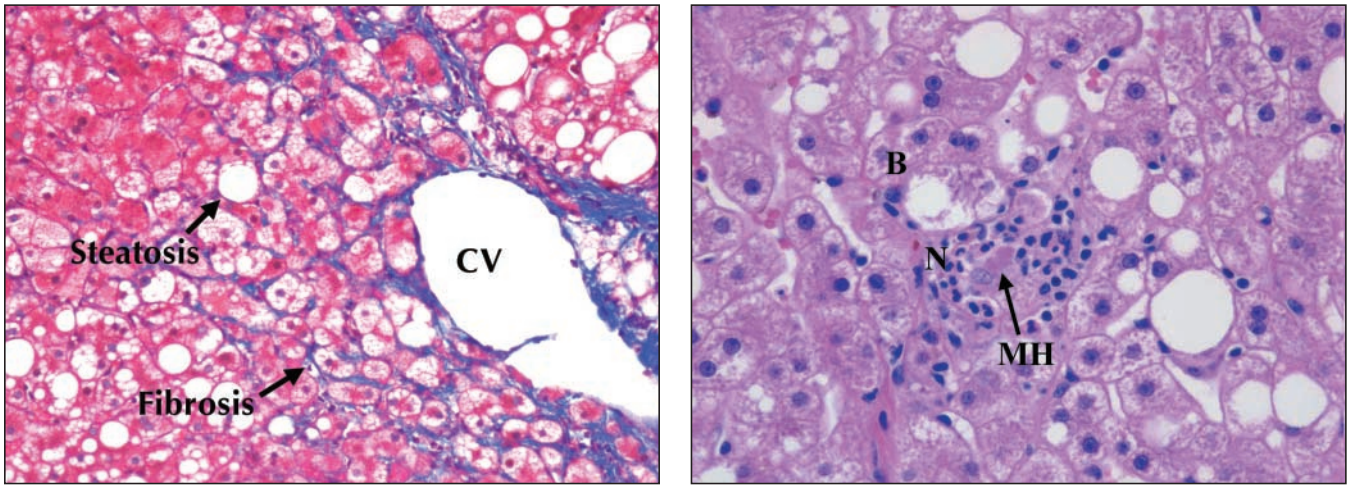


**Fig. 2: Left:** Histologic features of fibrosis in nonalcoholic steatohepatitis (NASH). Trichrome stain of liver (magnification × 200) demonstrating macrovesicular steatosis and fibrosis, most prominent in zone 3 near the central vein (CV) of the hepatic lobule. Typically early fibrosis is pericellular and perisinusoidal, giving the appearance of "chickenwire." **Right:** Histologic features of necrotic inflammation in NASH. Hematoxylin and eosin stain of liver (magnification × 400) with injured ballooned hepatocytes (B) and a mild neutrophilic infiltrate (N). Aggregations of Mallory's hyaline (MH) comprised of eosinophilic cytoskeleton filaments are also observed.

give the appearance of being relatively brighter and can be mistaken for contrast injection. No imaging method is able to distinguish between simple steatosis and NASH or indicate the stage of fibrosis.[63] The sensitivity and specificity of ultrasonography for detecting fatty infiltration decreases as BMI increases and thus varies from 49% to 100% and from 75% to 95% respectively.[63–65] The sensitivity of each imaging method increases with the degree of fatty infiltration, with at least 33% steatosis being optimal for detection.[63]

### *Liver biopsy*

The "gold standard" for diagnosing NAFLD is clinicopathological correlation, with confirmation of steatosis by liver biopsy and exclusion of other causes (e.g., alcohol) clinically. However, because alcoholic liver disease and NAFLD have similar histologic features (Fig. 2), they cannot be distinguished by means of liver biopsy. The cutoff limit of alcohol intake that distinguishes between alcoholic and nonalcoholic fatty liver disease is not known, although 20 g/d for women and 30 g/d for men is commonly used.[66] One standard drink typically contains 10–20 g of alcohol.

A minimum of 5%–10% steatosis by weight is considered a requirement for the diagnosis of NAFLD.[66] Steatosis is generally macrovesicular, although it may be mixed with microvesicular droplets, which are seen with defective mitochondrial oxidation of free fatty acids.

Histologic features used to distinguish NASH from simple steatosis are controversial and vary in the literature. A recent conference defined NASH as zone 3 predominate macrovesicular steatosis in combination with hepatocyte ballooning and a mixed inflammatory infiltrate,[66,67] often with characteristic perisinusoidal and pericellular fibrosis.

Liver biopsy performed on patients who have persistently elevated liver enzyme levels and no viral serologic markers of chronic liver disease will reveal NAFLD 66%–90% of the time.[68–70] The positive predictive value of fatty changes on ultrasound in this setting is estimated to be 92%–96%, whereas the negative predictive value of a normal scan is estimated to be 55%–87%.[64] Despite this, a clinical diagnosis of NAFLD before biopsy based on serologic test results and findings from imaging studies is correct in only 53%–83% of cases.[68,71] However, in primary care settings where NAFLD is common, a positive ultrasound result in association with metabolic risk factors in the absence of viral serologic evidence of chronic liver disease is likely to be adequate for diagnosis.

In addition to confirming the clinical diagnosis, liver biopsy is valuable for excluding other liver disease and for monitoring disease progression.[71] It provides prognostic information by distinguishing steatosis from NASH and by determining the stage of fibrosis. However, sampling error may lead to variation of one fibrosis stage in 24%–37% of biopsies.[72] Liver biopsy may also be useful when the diagnosis is uncertain (e.g., in the presence of autoantibodies[62] or elevated iron indices), before participation in a clinical trial or when there is concern of advanced fibrosis, which may alter patient screening and surveillance.[73] The decision to perform a biopsy should be made on an individual basis and discussed with the patient, with the benefits weighed against the small but definite risks of harm.

### *Serologic testing*

Other causes of liver disease can usually be excluded through history taking, examination and serologic testing. In the presence of elevated liver aminotransaminase levels, it may be possible to exclude viral hepatitis, hemochromatosis, autoimmune hepatitis, chronic cholestatic disease, $\alpha_1$-antitrypsin deficiency and Wilson's disease through appropriate serologic testing.

## Management

### *Weight loss and pharmacotherapy*

The aim of treatment is to slow the progression of NAFLD and to prevent liver-related illness and death. However, because disease progression is slow and the magnitude of disease-related morbidity and mortality is uncertain, it is unclear which patients will benefit most from treatment. In addition, most therapeutic trials to date have been uncontrolled, of short duration and lacking histologic end points, which has led to limited treatment recommendations.[74] Studies involving more than 10 participants, lasting longer than 6 months and having histologic end points are reported in Table 2.[14,15,75–86]

Both weight loss and exercise improve insulin resistance[87] and are recommended in conjunction with the treatment of associated metabolic abnormalities. In one study, patients following a restricted diet (25 calories [105 kJ] per kilogram ideal body weight) and exercise regimen over 3 months had reduced liver enzyme levels and hepatic steatosis compared with control subjects, although it is unknown whether hepatic inflammation and fibrosis improved in the longer term.[88] Rapid weight loss due to a very low energy diet (< 500 kcal [< 2090 kJ] daily) or jejunoileal bypass should be avoided because of the risk of worsening inflammation and fibrosis.[75,89] A reasonable target is the loss of 10% of body weight over 6 months.[74]

Of the only 3 randomized placebo-controlled trials reported to date, none showed convincing histologic evidence of improvement with any of the investigated medications (antioxidants [vitamins E and C], hepatoprotective agents [ursodeoxycholic acid] or metformin) compared with placebo.[76,77,90] Thiazolidinediones improve insulin sensitivity by activating the peroxisome proliferator-activated receptor gamma (PPARγ) and have shown promise in pilot trials involving patients with NASH, although weight gain has been a troublesome side effect.[78,79] In addition, the initial PPARγ agonist troglitazone was withdrawn because of its hepatotoxic effects.[91] The second-generation thioglita-

zones rosiglitazone and pioglitazone appear to be safer, although their use is currently contraindicated in the presence of active liver disease. Both pentoxifylline (a TNF-α inhibitor) and atorvastatin (used in NASH patients who have hyperlipidemia) improved aminotransaminase levels in uncontrolled trials, although histologic evidence of their benefit is unknown.[92–95] The risk of statin-induced hepatotoxic effects does not appear to be increased among patients with unexplained elevation of liver enzyme levels presumably due to NAFLD.[96] In a randomized controlled trial of probucol, an antioxidant and lipid-lowering agent, aminotransferase levels were significantly improved in the treatment group compared with the placebo group.[97] Pilot studies of betaine and losartan revealed histologic and biochemical evidence of improvement, although the samples were small (fewer than 10 participants).[80,98] Metformin has shown promise in small trials, and larger, randomized trials are in progress.[81] However, because of the lack of evidence at this time, pharmacotherapy for NAFLD cannot be recommended outside of clinical trials.

### *Follow-up*

Monitoring patients with NAFLD is difficult because liver enzyme levels tend to improve regardless of whether liver fibrosis worsens or improves.[48] In addition, it may take several decades of monitoring before the development of complications is observed. Therefore, follow-up should be focused on patients who have risk factors for advanced disease.

### *Liver transplantation*

Liver transplantation may be required if cirrhosis develops and is complicated by liver failure or hepatocellular carcinoma. Currently, about 3% of all transplants in North America are performed because of end-stage NAFLD,[53] although this figure does not include patients with cryptogenic cirrhosis or those precluded from undergoing liver transplantation because of comorbidities related to insulin resistance. Recurrence of steatosis after transplantation is common (in 60%–100% of transplant recipients),[53,99] with progression to steatohepatitis reported in one-third of cases.[53]

## Conclusions

NAFLD is common, and its prevalence is likely to increase with the rising incidence of obesity and diabetes. A minority of patients with NAFLD, particularly those with NASH and diabetes, are at risk of liver-related complications such as cirrhosis and hepatocellular carcinoma. In ad-

**Table 2: Trials of treatment of nonalcoholic fatty liver disease[66]**

| Trial | Treatment | No. of patients | Study type | Duration of study, mo | Outcome* | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Liver enzyme levels | Steatosis | Inflammation | Fibrosis |
| Andersen et al[75] | Very low calorie diet | 41 | Open label | 4–23 | Improved | Improved | Variable | Variable |
| Dixon et al[14] | Bariatric surgery | 36 | Case series | 26 | Improved | Improved | Improved | Improved |
| Luyckx et al[82] | Bariatric surgery | 69 | Case series | 27 | Improved | Improved | Worsened | No change |
| Silverman et al[15] | Bariatric surgery | 91 | Case series | 2–61 | No change | Improved | No change | Improved |
| Kral et al[83] | Bariatric surgery | 104 | Case series | 6–111 | Improved | Improved | No change | Worsened |
| Harrison et al[84] | Orlistat | 10 | Open label | 6 | Improved | Variable | No change | Variable |
| Hasegawa et al[85] | Vitamin E | 10 | Open label | 12 | Improved | Variable | Variable | Variable |
| Harrison et al[76] | Vitamins E and C | 45 | RCT | 6 | No change | No change | No change | Improved† |
| Abdelmalek et al[80] | Betaine | 10 | Open label | 12 | Improved | Improved | Variable | Improved |
| Laurin et al[86] | UCDA | 24 | Open label | 12 | Improved | Improved | No change | No change |
| Lindor et al[90] | UCDA | 126 | RCT | 24 | No change | No change | No change | No change† |
| Laurin et al[86] | Clofibrate | 16 | Open label | 12 | No change | No change | No change | No change |
| Nair et al[81] | Metformin | 15 | Open label | 12 | No change | Variable | Variable | Variable |
| Uygun et al[77] | Metformin | 34 | RCT | 6 | Improved | Improved | No change | No change |
| Promrat et al[79] | Pioglitazone | 18 | Open label | 12 | Improved | Improved | Improved | Improved |
| Neuschwander-Tetri et al[78] | Rosiglitazone | 30 | Open label | 12 | Improved | Improved | Improved | Improved‡ |

Notes: RCT = randomized controlled trial, UCDA = ursodeoxycholic acid.
*Variable result indicates improvement in some subjects and worsened condition in others, with no trend detected; often associated with a small sample.
†No difference when compared with placebo arm.
‡Improvement seen in zone 3 fibrosis but not overall fibrosis stage.

dition, the overall mortality appears to be higher among NAFLD patients than among people in the general population, probably because of underlying pathogenic factors such as insulin resistance. Currently, treatment is limited to weight loss, exercise and the control of metabolic risk factors. Effective pharmacotherapies are awaited, and several promising agents are on the horizon.

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From the Division of Gastroenterology and Hepatology, Mayo Clinic and College of Medicine, Rochester, Minn.

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***Correspondence to:*** *Keith D. Lindor, Division of Gastroenterology and Hepatology, Mayo Clinic and College of Medicine, 200 First St. SW, Rochester MN 55905, USA*