Treatment options for nonalcoholic fatty liver disease

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Abstract: Nonalcoholic fatty liver disease comprises a range of disorders from steatosis and steatohepatitis through to cirrhosis. Nonalcoholic steatohepatitis can progress to cirrhosis and liver-related death. Therefore, managing this common disorder is becoming an important public health issue. Lifestyle measures are commonly suggested but robust data are lacking. Trials with antioxidants (vitamin E, betaine) as well as cytoprotectants (ursodeoxycholic acid) have been disappointing. While data for insulin sensitizers such as metformin are less conclusive, thiazolidinediones appear promising. However, not all patients respond to thiazolidinediones. Moreover, issues related to weight gain, cardiovascular risk need to be addressed. The use of endocannabinoid antagonists and insulin secretagogues are novel strategies to combat this disorder.

Keywords: fatty liver, insulin sensitizers, nonalcoholic steatohepatitis, nonalcoholic fatty liver disease, endocannabinoids, thiazolidinediones, ursodeoxycholic acid, vitamin E, lifestyle intervention, physical activity

Introduction

Nonalcoholic fatty liver disease (NAFLD) comprises a spectrum of liver disorders from simple steatosis through to steatohepatitis and cirrhosis [Farrell and Larter, 2006]. The prevalence of NAFLD in the general population is up to 30% [Browning and Horton 2004]. This figure is even higher among persons with type 2 diabetes (50%), obesity (76%) and the morbidly obese (nearly 100%) [Adams *et al.* 2005]. A similar epidemic of NAFLD looms large in the developing world [Chitturi *et al.* 2007, 2004], with a reported prevalence of 10–29% in some countries within the Asia-Pacific region.

The natural history of this disorder varies by histologic subtype [Matteoni *et al.* 1999]. The benign nonprogressive course of simple steatosis is well documented [Teli *et al.* 1995]. By contrast, 10–15% with nonalcoholic steatohepatitis (NASH) can develop progressive hepatic fibrosis and cirrhosis [Farrell and Larter, 2006; Adams *et al.* 2005]. Once hepatic decompensation sets in, the outlook is dismal [Sanyal *et al.* 2006;

Hui *et al.* 2003]. The survival curves for these patients are similar to those observed for patients with end-stage chronic viral hepatitis [Hui *et al.* 2003]. In addition to patients with known NASH-related cirrhosis, the pool of patients with advanced hepatic fibrosis is further expanded by cases of cryptogenic cirrhosis [Caldwell and Crespo, 2004]. These patients have a high prevalence of obesity and diabetes as compared with controls with alcoholic or viral hepatitis-associated cirrhosis. Therefore, it is generally accepted that these patients are likely to represent instances of end-stage NAFLD [Caldwell and Crespo, 2004]. Finally, the growing prevalence of hepatocellular carcinoma is, in part, attributable to the rise in obesity and diabetes, both of which are common substrates for NAFLD [Bugianesi *et al.* 2002, Nair, 2002]. Therefore, the management of NAFLD is more than of passing interest.

In the three decades since Ludwig's seminal paper [Ludwig *et al.* 1980], there is better insight into the key pathways underlying hepatic steatosis and the initiation/perpetuation of progressive liver injury in NAFLD/NASH. Insulin resistance

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plays a pivotal role in the development of NAFLD [Marchesini *et al.* 2003; Chitturi *et al.* 2002]. NAFLD is now regarded as the hepatic manifestation of the metabolic syndrome. Early identification of fatty liver is becoming important as it portends a significantly increased risk of future type 2 diabetes, hypertension and other metabolic disorders [Chitturi and Farrell, 2007a; Fan *et al.* 2007]. In addition to insulin resistance, other processes and pathways of relevance to NAFLD/NASH include oxidative stress, adiponectin and other adipocyte-derived hormones (adipocytokines), proinflammatory cytokines such as tumour necrosis factor- α (TNF- α), activated peroxisome proliferator-activated receptors (PPAR) and genetic factors [reviewed by Day, 2006; Farrell and Larter, 2006; Browning and Horton, 2004; and Chitturi and Farrell, 2001]. More recently, the contributions of the endocannabinoid system to NAFLD have gained prominence [Mallat and Lotersztajn, 2008]. In view of the diverse mechanistic processes involved, it is hardly surprising that a bewildering array of management options have been evaluated.

Limitations of current approaches

Much of the published data fall well short of current requirements of evidence-based practice. Methodological issues include the use of an open-labelled/nonrandomized design, small sample size (often less than 20) and the lack of baseline and/or follow-up histology (a prerequisite for the diagnosis of NASH). There are other inherent difficulties in interpreting NAFLD trials. Serum transaminases often used as an endpoint, are unreliable in assessing histologic improvement. Normal transaminase levels do not preclude significant liver injury [Mofrad *et al.* 2003]. Even the definition of what should be the 'normal range' of serum transaminases, has been questioned [Prati *et al.* 2002]; an upper limit of 30 U/l (for men) and 19 U/l (for women) have been suggested.

Although liver biopsy is widely relied upon as the gold standard and as the surrogate endpoint in clinical trials, this raises methodologic issues. First, significant histologic sampling error can occur [Ratziu *et al.* 2005]. Second, histologic appraisal is no substitute for 'hard' endpoints such as the impact on preventing or delaying liver-related complications or death (not demonstrated in any study to date). Finally, the

'unseen hand' of voluntary lifestyle changes should not be underestimated because these can potentially account for the biochemical and histologic improvement observed in placebo-treated trial recipients.

Management of NAFLD

Current strategies in management of NAFLD include lifestyle measures (diet and exercise), the use of weight-reducing drugs, antioxidants and cytoprotective agents, insulin sensitizers and a miscellaneous group of agents including angiotensin receptor blockers, lipid-lowering agents, endocannabinoid receptor antagonists among others. The rationale for their use is outlined in Table 1.

Lifestyle modification/weight-reducing drugs

Diet Weight reduction through changes in lifestyle is generally considered to be the cornerstone of management of NAFLD/NASH. However, robust data are lacking to support this recommendation. A meta-analysis of 517 studies of weight reduction in NAFLD revealed only a few studies (15) without methodologic problems [Wang *et al.* 2003]. Small studies have shown a reduction in the degree of steatosis, sometimes inflammation but not usually fibrosis [Huang *et al.* 2005; Hickman *et al.* 2004; Ueno *et al.* 1997]. The latter may relate to the short duration of follow up. Gradual weight loss is usually recommended (less than 1.6 kg/week) because rapid weight reduction can lead to worsening of steatohepatitis. By contrast, the consequences of rapid weight loss with current bariatric procedures (as opposed to crash diets) appear to favour improvement in metabolic control.

As a group, persons with NAFLD consume significantly higher amounts of meat, soft drinks and saturated fat but lower levels of dietary fibre, antioxidants and omega-3 fatty acids [Zelber-Sagi *et al.* 2007; Musso *et al.* 2003] than controls. A recent review of diets suitable for NAFLD patients recommended a monounsaturated fat/n-3 fatty acid/high fibre enriched diet along with foods with a low glycaemic index while placing restrictions on simple carbohydrates and sweet drinks [Zivkovic *et al.* 2007]. However, these recommendations need prospective validation.

Three small studies have examined the role of n-3 polyunsaturated fatty acid (n-3 PUFA) supplements in NAFLD. The most recent has

Table 1. The rationale of current management approaches to the treatment of nonalcoholic fatty liver disease.

Intervention/drug class	Example	Rationale
Weight reduction/ nutritional supplementation	Diet/exercise Orlistat, sibutramine Bariatric surgery n-3 polyunsaturated fatty acids	Weight loss improves insulin sensitivity; improves the metabolic profile Decreased free fatty acid lipotoxicity in hepatocytes; Hepatic PPAR- α activation; suppresses hepatic de novo lipogenesis; antioxidant and anti-inflammatory effects
Antioxidants	Vitamin E N-acetylcysteine	Oxidative stress, directly or through cytokine release, contributes to inflammatory cell recruitment, perpetuation of liver injury and hepatic fibrogenesis; N-acetylcysteine is a glutathione prodrug
Cytoprotective agents	Ursodeoxycholic acid	Membrane stabilizing properties, antiapoptosis
Anticytokine treatment	Pentoxifylline	TNF- α levels are increased in NASH; TNF- α impairs insulin sensitivity and is involved in hepatic inflammatory cell recruitment in NASH. Pentoxifylline reduces TNF- α production and secretion.
Insulin sensitizers	Metformin Thiazolidinediones Glucagon-like protein-1-receptor agonist Nateglinide	Promotes weight loss; improves hepatic insulin sensitivity; activates AMP-activated kinase and consequent hepatic fatty acid β -oxidation PPAR- γ agonists improve peripheral and hepatic insulin sensitivity; increase adiponectin levels; have anti-inflammatory and antifibrotic effects Insulin secretagogue; reduces appetite; upregulates hepatic PPAR- α mRNA and downregulates SREBP-1 expression; reduces free fatty acid flux to the liver Insulin secretagogue; upregulates hepatic PPAR- α and adiponectin receptor R2 mRNA expression
Lipid-lowering drugs	Gemfibrozil Fenofibrate, bezafibrate atorvastatin, pravastatin	Hyperlipidaemia is a component of the metabolic syndrome Some have anti-inflammatory, antifibrogenic and antioxidant effects. Fibrates promote hepatic fatty acid β -oxidation through activation of hepatic PPAR- α
Angiotensin converting enzyme inhibitors	Losartan	Renin-angiotensin blockade reduces experimental hepatic fibrosis; Telmisartan has partial PPAR- γ agonist properties
Endocannabinoid antagonists	Rimonabant	Reduce hepatic lipogenesis; antifibrotic properties; lowers TNF- α ; increases serum adiponectin

PPAR: peroxisome proliferators activated receptor; TNF: tumour necrosis factor; SREBP-1: sterol regulatory element binding protein 1.

well-documented effects on reducing hepatic lipogenesis and also anti-inflammatory and antioxidant properties [Eritsland, 2000]. Twenty-three Japanese patients with NASH were administered highly purified eicosapentaenoic acid (EPA), one of the main constituents of n-3 PUFA. Improvement in transaminases and reduced steatosis (ultrasound assessed) was observed. Follow up histology ($n=7$) showed improved nonalcoholic steatosis scores (NAS) as well as reduction of hepatic fibrosis [Tanaka *et al.* 2008]. The other two studies included one randomized and one non-randomized controlled trial. In the first, 40 patients were prescribed the American Heart Association diet along with or without n-3 PUFA (2 g daily)

for 6 months [Spadaro *et al.* 2008]. In the second report, 42 patients with NAFLD were treated with n-3 PUFA capsules (1 g daily) for 12 months. Fourteen subjects who refused treatment served as controls [Capanni *et al.* 2006]. In both studies, reductions in triglyceride, ALT and hepatic steatosis (by ultrasound) were documented in the PUFA group [Spadaro *et al.* 2008; Capanni *et al.* 2006]. Reduction in serum TNF- α and insulin resistance were also observed in the PUFA group [Spadaro *et al.* 2008]. A bleeding tendency through inhibition of platelet function is a known side effect [Eritsland, 2000]. This was not observed in these three studies but may be important in patients with advanced hepatic fibrosis [Tanaka *et al.* 2008].

Exercise Patients with NAFLD have lower levels of physical activity and of cardiovascular fitness as compared with controls [Krasnoff *et al.* 2008; Church *et al.* 2006]. Therefore, it appears reasonable to recommend increasing levels of physical activity along with dietary modification, a more effective combination than either alone [Day and Harrison, 2007]. For example, in the Diabetes Prevention Program (DPP) study, lifestyle modification was superior to even drug therapy (metformin) in the prevention of diabetes [Knowler *et al.* 2002]. Given the common theme of insulin resistance in NAFLD and type 2 diabetes, it is appropriate to actively promote lifestyle measures as a primary treatment strategy. Dietary approaches have been discussed in the previous section. With respect to exercise, it is not clear whether this should involve aerobic exercise or resistive training or both. A plan of 30–45 minutes of moderate-intensity aerobic exercise three to five times per week is recommended by many cardiac societies and seems reasonable in this patient group.

Weight-reducing drugs Lifestyle change is not easy to achieve or sustain for some individuals. For the latter, drug treatment or surgery (in the morbidly obese) may be considered. Of the drugs available, only *orlistat* and *sibutramine* have been evaluated in NAFLD.

Orlistat is a lipase inhibitor that reduces dietary fat absorption by 30%. In clinical trials, subjects receiving orlistat achieved a 3% greater reduction in weight as compared with controls. Reduction in waist circumference, total cholesterol and improved insulin sensitivity were also documented [Drew *et al.* 2007].

Three small studies have evaluated orlistat in NAFLD [Hussein, 2007; Zelber-Sagi, 2006; Harrison *et al.* 2004]. Only one of these was a double-blind placebo-controlled randomized clinical trial [Zelber-Sagi *et al.* 2006]. Forty-four patients were randomized to receiving orlistat or placebo for 6 months. All patients underwent nutritional counselling and were encouraged to exercise. Follow-up liver biopsies were obtained in about half of the participants. Weight reduction (mean 7.7 kg) and reversal of hepatic steatosis (by ultrasound in 24%) was observed in the orlistat group. More patients in the orlistat group had a reduction in transaminases as compared with

controls (48% *vs* 26%, respectively). No significant changes in the grade of inflammation or fibrosis stage were observed. However, subjects in the placebo arm also lost weight (6%) and had reversal of steatosis (17%), illustrating the positive impact of lifestyle changes and reiterating the importance of a placebo arm in NAFLD trials. Other open-labelled studies with orlistat have also documented some histologic improvement. However, orlistat is associated with side effects, such as bloating, oily spotting and occasionally faecal incontinence, which may limit its widespread use [Drew *et al.* 2007].

Sibutramine is a serotonin-norepinephrine reuptake inhibitor that induces satiety through its central actions and induces weight reduction comparable to that achieved with orlistat [Eckel, 2008]. Sibutramine has adrenergic side effects such as tachycardia and elevation of blood pressure (relevant to this obese, hypertensive population).

A Turkish study evaluated sibutramine ($n=13$) and orlistat ($n=12$) in obese patients with NAFLD receiving an hypocaloric diet [Sabuncu *et al.* 2003]. Significant weight loss was achieved in both groups (10.2% and 8.4%, respectively). Improved insulin sensitivity, reduced transaminases and regression of steatosis (ultrasound assessed) were observed in both groups but no histology was available.

Bariatric surgery Surgical methods of weight reduction are recommended for treating morbid obesity (body mass index ≥ 40 kg/m²) and for patients with body mass index ≥ 35 kg/m² in the presence of co-morbid conditions (type 2 diabetes, hypertension). Jejuno-ileal bypass is now obsolete due to its association with liver injury and metabolic complications. Reduction in visceral as opposed to subcutaneous fat is critical for optimization of the metabolic milieu; insulin sensitivity is correlated with visceral rather than subcutaneous fat mass. Techniques such as abdominal liposuction are effective in reducing subcutaneous (but not visceral) fat. As expected, liposuction does not lead to improvement in insulin sensitivity nor metabolic profile and is therefore unsuitable for application in patients with NAFLD [Klein *et al.* 2004].

Contemporary surgical approaches include roux-en-Y gastric bypass, laparoscopic gastric banding

and biliopancreatic diversion [de Freitas *et al.* 2008]. These operations achieve a reduction of at least a 50% loss of the excessive weight (which includes visceral fat reduction) and patients benefit from an improvement in glycaemic control, lowered blood pressure and better metabolic profiles. More invasive procedures such as biliopancreatic diversion are associated with greater degrees of weight loss but have a higher complication rate.

Formal trials of bariatric surgery in NAFLD are lacking. Liver histologic improvement has been documented with all techniques [Klein *et al.* 2006; Dixon *et al.* 2004]. Hepatic steatosis nearly always improves. The effects on steatohepatitis and fibrosis have been more varied. In general, steatohepatitis is much less frequently observed in follow-up liver biopsies. Downstaging of hepatic fibrosis stage has also been documented. One study also demonstrated reduction in serum markers of fibrogenesis (such as transforming growth factor-beta 1, TGF- β 1) as well as a reduction in expression of genes involved in pathways of inflammation [Klein *et al.* 2004]. However, there are a few instances where the follow-up biopsies have shown worsening of steatohepatitis and fibrosis stage [de Ridder *et al.* 2007]. Possible factors influencing the outcome include the rapidity of weight loss and interval between the operation and second liver biopsy [Angulo, 2006]. Although the degree of inflammation may appear worse in 'early' biopsies (at 1 year), this does not preclude improvement later. Improvement in hepatic fibrosis may not also be discerned in 'early' biopsies. Bariatric surgery has been performed successfully in patients with cirrhosis and also after liver transplantation. Cirrhosis does carry a higher operative risk [Brolin *et al.* 1998]. However, most surgeons would consider gastric restrictive techniques to be relatively safe but would not undertake malabsorptive procedures for these patients [Brolin *et al.* 1998]. A number of factors such as the overall nutritional status, severity of liver impairment (Child-Pugh, MELD scores) need to be considered, operative decisions individualized and these procedures are best undertaken in multidisciplinary obesity centres.

Oxidative stress and antioxidants

Reactive oxygen species (ROS) generated by microsomal and peroxisomal free fatty acid

oxidation play an important part in progression of simple steatosis to steatohepatitis and progressive hepatic fibrosis [Farrell and Larter, 2006; Chitturi and Farrell, 2001]. They bind with intracellular organelles, disrupt cell signalling, contribute to membrane peroxidation, elicit cytokine release and can activate hepatic stellate cells [Farrell and Larter, 2006; Chitturi and Farrell, 2001].

Antioxidants Vitamin E, betaine and N-acetylcysteine have been evaluated in NAFLD. Published studies with vitamin E include three randomized controlled (including 16 to 45 cases) [Vajro *et al.* 2004; Harrison *et al.* 2003; Kugelmas *et al.* 2003] and several open-label trials [Hasegawa *et al.* 2001; Lavine *et al.* 2000]. Vitamin E was used in doses of 300–1200 mg daily for 3–12 months. Most but not all [Kugelmas *et al.* 2003] documented improved liver tests as well as reduced levels of markers of oxidative stress and profibrogenic cytokines (TGF- β 1) [Hasegawa *et al.* 2001]. However, histologic data are scanty. In one study where subjects were treated with a combination of vitamin E and C, histologic improvement (including fibrosis) was observed in post-treatment liver biopsies. However, these changes were not significantly different from those obtained from placebo-treated controls [Harrison *et al.* 2003]. Vitamin E has also been used in combination with ursodeoxycholic acid (UDCA) ($n=15$) in a three-arm trial involving placebo and UDCA/placebo as the other arms [Dufour *et al.* 2006]. Improvement in liver tests were observed in the UDCA/placebo and vitamin E/UDCA groups but histologic improvement (mainly hepatic steatosis) was observed only in the UDCA/vitamin E arm.

The enthusiasm for vitamin E therapy has been dampened by safety concerns. A meta-analysis published in 2005 found that high-dose vitamin E supplementation (>400 iu/day) was associated with an increase in all-cause mortality [Miller *et al.* 2005]. An excess of cardiovascular deaths among vitamin E recipients (as compared with placebo) was also reported in the Cambridge heart antioxidant study (CHAOS) [Stephens *et al.* 1996]. These have not been documented in NAFLD studies but the lack of robust evidence of efficacy for vitamin E has diminished its importance in this setting. Another antioxidant, N-acetylcysteine (a glutathione prodrug), was evaluated in 11 patients with NASH.

Improvement in transaminases was observed but histology was lacking [Gulbahar *et al.* 2000].

Betaine This naturally occurring metabolite of choline raises s-adenosylmethionine levels (SAME). SAME participates as a methyl donor in several reactions including production of glutathione and phosphatidylcholine (lecithin); the latter is a component of very low density lipoprotein (VLDL) and is involved in exporting triglyceride out of the liver [Chang *et al.* 2006]. SAME has been previously shown to attenuate experimental hepatic steatosis [Oz *et al.* 2006]. Betaine increased activation of hepatic AMP-activated protein kinase (which promotes fatty acid β -oxidation) along with reduction in hepatic lipogenic enzyme activity in a mouse model of sucrose-induced hepatic steatosis [Song *et al.* 2007].

One hundred and ninety one patients with NAFLD were randomized to placebo or treated with a combination of betaine glucuronate, diethanolamine glucuronate and nicotinamide ascorbate; improvement in liver enzymes and hepatic steatosis (by ultrasound) was observed in subjects receiving the betaine combination [Miglio *et al.* 2000]. A pilot study of ten patients treated with betaine anhydrous for 1 year at the Mayo clinic also showed histologic improvement in all three components (steatosis, inflammation and fibrosis) [Abdelmalek *et al.* 2001]. Unfortunately, despite its earlier promise, the experience with betaine in NASH has been disappointing. A subsequent study from the Mayo group (available only in abstract form) did not confirm these findings [Abdelmalek *et al.* 2006].

Cytoprotective agents: ursodeoxycholic acid (UDCA)

UDCA has cytoprotective properties including inhibition of apoptosis and stabilization of plasma membranes, and is effective in preventing experimental hepatic steatosis. Small open-labelled and controlled studies in NAFLD were encouraging [Kiyici *et al.* 2003; Laurin *et al.* 1996]. However, UDCA was not found to be effective in a multicentre randomized controlled trial [Lindor *et al.* 2004]. One hundred and sixty six patients with NASH were randomized to UDCA or placebo for 2 years. Liver biopsies were obtained at baseline and at 2 years. Improvement in transaminases and steatosis were observed in both arms of the study but

differences between the groups were not significant. Although this was a well-conducted study, one-third of the subjects did not undergo follow-up biopsies. Voluntary changes in lifestyle were also not controlled for. UDCA as a monotherapy for NAFLD cannot be recommended but it is being evaluated as part of drug combination trials in progress.

Anticytokine treatment: pentoxifylline

Tumor necrosis factor (TNF)- α is implicated in hepatic inflammatory cell recruitment in NASH, a key step in initiation and perpetuation of liver injury [Chitturi and Farrell, 2007b]. Further, TNF- α impairs reduce insulin sensitivity through interference with insulin receptor signalling. Pentoxifylline attenuates the production of TNF- α and other proinflammatory cytokines and has been shown to attenuate hepatic steatosis in a methionine-choline mouse model of NASH [Koppe *et al.* 2004].

Four studies have evaluated pentoxifylline in NASH [Satapathy *et al.* 2007; Lee *et al.* 2006; Adams *et al.* 2004; Satapathy *et al.* 2004]. Improvement in liver enzymes and liver histology (one study) has been documented. However, these studies involved small samples (9–20 patients) and were not placebo-controlled trials. Further, more than 40% of patients withdrew from gastrointestinal adverse effects in one study [Adams *et al.* 2004].

Insulin sensitizers

Metformin This drug, the cornerstone of type 2 diabetes management, has a number of important effects which are relevant to patients with NAFLD. These include weight reduction, improve insulin sensitivity through decreasing hepatic glucose output and increasing peripheral glucose uptake, reduced hepatic lipogenesis and increased hepatic fatty acid β -oxidation (by activating AMP-activated protein kinase) and suppression of the lipogenic transcription factor (sterol regulatory element binding protein-1, SREBP-1) [Zhou *et al.* 2001; Hundal *et al.* 2000; Bailey and Turner, 1996; Stumvoll *et al.* 1995].

The use of metformin in NAFLD has been evaluated in six studies (Table 2). Controls comprised subjects managed by diet alone, vitamin E or placebo. Improvement in serum transaminases were observed in all studies but histologic follow up was obtained in only three studies. In one study, there was only transient improvement in transaminases

Table 2. Metformin in the treatment of nonalcoholic fatty liver disease.

Author	<i>n</i>	Duration of treatment (months)	Transaminase reduction	Histologic improvement
Marchesini <i>et al.</i> (2001)	14	4	Yes	No data
Nair <i>et al.</i> (2004)	15	12	Yes but transient only	Yes; reduction in steatosis (3 of 10 cases) inflammation (2/10) and fibrosis (1/10)
Uygun (2004) (randomized controlled trial)	17	6	Yes	No
Bugianesi <i>et al.</i> (2005) (randomized controlled trial)	55	6	Yes	Yes; reduction in hepatic steatosis, inflammation and fibrosis; not all had follow histology
Schwimmer <i>et al.</i> (2005)	10	6	Yes	No data
Duseja <i>et al.</i> (2007)	7	6	Yes	No data

[Nair *et al.* 2004]. Although histologic improvement has been documented, only a small proportion of cases have had follow-up biopsies. In one study, there was improvement in hepatic necro-inflammatory activity in metformin-treated cases but this was not different from the response in the placebo arm [Uygun *et al.* 2004]. Minor gastrointestinal side effects can occur with metformin. Raised serum lactate was observed in some cases but no cases of lactic acidosis have been reported. Although the theoretical basis for the use of metformin is sound, hard clinical evidence is yet to accrue. Metformin is being studied in multicentre clinical trials.

Thiazolidinediones The thiazolidinediones (TZD) are peroxisome proliferator-activated receptor- γ (PPAR- γ) agonists. They improve insulin sensitivity by reducing insulin resistance in adipose tissue, liver and skeletal muscles [Yki-Jarvinen, 2004; Shulman, 2000]. They increase plasma adiponectin levels and activate AMP-activated protein kinase [Bajaj, 2004]. As mentioned above, the consequence of these effects are enhanced hepatic lipid β -oxidation and inhibition of hepatic lipogenesis. Adiponectin has also anti-inflammatory and antifibrotic properties. Thus, many of the key pathways operational in NASH (insulin resistance, low adiponectin levels, activation of pro-inflammatory cytokines) are targeted by TZDs.

Troglitazone was the first TZD to be studied in NAFLD. Improvement in steatohepatitis was observed but it was subsequently withdrawn due to hepatotoxicity [Caldwell *et al.* 2001]. However, it paved the way for the use of second-generation TZDs, rosiglitazone and pioglitazone which are

not associated with significant liver injury. There are seven studies (four randomized trials) that have evaluated these agents in NAFLD (Table 3). All have documented reduction in transaminases, hepatic fat content and necro-inflammatory activity. In one of these studies [Ratzu, 2008], no statistically significant differences in hepatic necro-inflammatory or fibrosis were observed with rosiglitazone. However, the median nonalcoholic steatosis severity scores were low in both groups (4). This makes it quite difficult to adequately discern significant histologic differences between these groups. In fact, the overall proportion of patients showing histologic progression of necro-inflammation or fibrosis was lower than those receiving placebo, suggesting *some* benefit. Four studies have shown reduction in fibrotic severity. Therefore, there is wide expectation that the TZDs will occupy a prime position in the management of NAFLD.

However, there are important caveats that need to be considered. First, recurrence of the disease is invariable when the drugs are stopped [Lutchman *et al.* 2007]. Long-term treatment seems necessary. Second, weight gain is a concern. This is mainly due to redistribution of fat from the visceral to subcutaneous compartment. However, this could lead to compliance issues in this generally obese patient population. Third, the importance of nonhepatic side effects such as increased cardiovascular mortality (for rosiglitazone), risk of heart failure and fractures has to be fully clarified [Grey *et al.* 2007; Nissen *et al.* 2007]. Finally, not all patients respond to TZDs [Ratzu *et al.* 2008]. In one study, responders to rosiglitazone treatment had lower levels of gamma glutamyl transpeptidase, were less likely to have diabetes and

Table 3. Thiazolidinediones in the treatment of nonalcoholic steatohepatitis.

First author/ date of publication	n	Drug	Thiazolidinedione dose/study duration	Transaminase reduction	Histology
Caldwell <i>et al.</i> (2001)	10	Troglitazone	400 mg for 3–6 months	Yes	Steatosis, inflammation reduced
Neuschwander- Tetri <i>et al.</i> (2003)	30	Rosiglitazone	4 mg bid for 48 weeks	Yes	Steatosis, inflammation reduced; zone 3 perisinusoidal fibrosis reduced
Promrat <i>et al.</i> (2004)	18	Pioglitazone	30 mg daily for 48 weeks	Yes	Steatosis, inflammation and fibrosis reduced
Sanyal <i>et al.</i> (2004)	10	Pioglitazone + vitamin E vs vitamin E alone**	30 mg daily for 6 months	Yes	Steatosis, inflammation, ballooning and pericellular fibrosis significantly reduced in pioglitazone-vitamin E combination arm
Tiikkainen <i>et al.</i> (2004)	9*	Rosiglitazone vs metformin	8 mg daily for 16 weeks	Yes	Not done; hepatic fat reduced (assessed by magnetic resonance imaging)
Belfort <i>et al.</i> (2006)	26*	Pioglitazone + hypocaloric diet vs placebo + hypocaloric diet **	45 mg daily for 6 months	Yes	Steatosis, inflammation reduced in pioglitazone group; changes in fibrosis not different from placebo
Aithal <i>et al.</i> (2007)	30*	Pioglitazone + diet/ exercise vs place- bo + diet/exercise**	30 mg daily for 12 months	Yes	Steatosis and fibrosis reduced in both groups; reduction in hepatocellu- lar injury only in pioglitazone group. End of treatment biopsies showed significant differences (reduction) between the groups in steatosis, inflammation and fibrosis.
Ratziu <i>et al.</i> (2008)	32*	Rosiglitazone vs placebo**	8 mg daily for 48 weeks	Yes	Steatosis reduced

*Number of subjects enrolled in the thiazolidinedione group.
**Randomized controlled trial.

tended to have higher levels of adiponectin [Ratziu *et al.* 2008]. This suggests that additional strategies (e.g. combination with other drug classes) may be necessary in nonresponders.

Combination of metformin with rosiglitazone
One study examined the effect of combining metformin with rosiglitazone in patients with type 2 diabetes or glucose intolerance [Akyuz *et al.* 2007]. Those receiving oral hypoglycaemic agents or insulin were excluded. Subjects were randomized to metformin alone, rosiglitazone alone or a combination of the two drugs. Approximately 20 patients were included in each arm of the study. Liver biopsies were performed at entry and 12 months after treatment. Significant improvement in transaminases and the NAS score was observed only in rosiglitazone recipients.

No significant changes in the fibrosis stage were recorded. There are several limitations to this study. These include lack of blinding, absence of a placebo arm and exclusion of patients with normal glucose tolerance. Finally, follow-up liver biopsies were available only from 35 of the 64 patients enrolled.

Glucagon-like protein-1-receptor agonist (incretin analogues)
Glucagon-like peptide 1 (GLP-1) is secreted postprandially by intestinal L cells. It potentiates meal-induced insulin production and secretion, inhibits glucagon secretion, reduces gastric emptying and also reduces appetite. GLP-1 is rapidly degraded by the enzyme dipeptidyl-peptidase IV (DPPIV) and is unsuitable for clinical use. Instead, a long-acting, DPPIV-resistant GLP-1 receptor

Table 4. Lipid-lowering agents in the treatment of nonalcoholic fatty liver disease.

First author/date of study	n	Drug	Treatment duration (months)	Transaminase reduction	Histologic improvement
Laurin <i>et al.</i> [1996]	16	Clofibrate	12	No	No
Basaranoglu <i>et al.</i> [1999]	46	Gemfibrozil	1	Yes	No data
Saibara <i>et al.</i> [1999]	2	Bezafibrate	10	No data	Yes; steatosis
Horlander <i>et al.</i> [2001]	7	Atorvastatin	12	Yes	Yes; only inflammation improved
Kiyici <i>et al.</i> [2003]	27	Atorvastatin	6	Yes	Yes; steatosis
Rallidis <i>et al.</i> [2004]	5	Pravastatin	6	Yes	Yes; steatosis and inflammation better
Antonopoulos <i>et al.</i> [2006]	23	Rosuvastatin	8	Yes	No data
Merat <i>et al.</i> [2007]	10	Probucol	12	Yes	Yes; steatosis and inflammation better
Fernandez-Miranda <i>et al.</i> [2008]	16	Fenofibrate	12	Yes	Yes; reduced hepatocellular ballooning

agonist exendin-4 (exenatide) has found application in patients with type 2 diabetes. Administration of exenatide reversed hepatic steatosis in obese mice [Ding *et al.* 2006; Tushuizen *et al.* 2006]. Further, markers of oxidative stress were reduced and there was upregulation of PPAR- α mRNA (activation of hepatic PPAR- α enhances fatty acid β -oxidation), down regulation of SREBP-1, as well as reduction in free fatty acid flux to the liver (through insulin-inhibition of peripheral lipolysis). All of these actions could collectively contribute to reducing hepatic steatosis.

A 59-year-old male with poorly controlled type 2 diabetes was treated with exenatide for 44 weeks [Tushuizen *et al.* 2006]. He was also receiving metformin. Improvement in lipid profile, blood pressure and insulin sensitivity were observed along with a decrease in liver fat content from 16% to 4% (measured by magnetic resonance spectroscopy) and transaminase levels.

Nateglinide This insulin secretagogue, a derivative of D-phenylalanine, restores early-phase insulin secretion and prevents meal-time surges of glucose [Tentolouris *et al.* 2007]. Nateglinide was effective in attenuating experimental hepatic steatosis [Mine *et al.* 2008]. There was upregulation of hepatic PPAR- α and adiponectin receptor R2 mRNA expression [Mine *et al.* 2008]. Five patients with type 2 diabetes and NASH were treated with nateglinide for 20 weeks [Morita *et al.* 2005]. Improvement in

liver tests, glucose tolerance and liver histology were observed.

Lipid-lowering drugs

Fibric acid derivatives Early trials with lipid lowering agents in NAFLD have yielded conflicting results (Table 4). There is interest in the use of fibric acid derivatives because of their capacity to increase hepatic fatty acid β -oxidation through activation of PPAR- α . In the methionine-choline deficient model of NASH, bezafibrate was also shown to downregulate several inflammatory cytokines [Nagasawa *et al.* 2006], reduce oxidative stress and also suppress TGF- β 1-induced fibrogenic responses [Nakano *et al.* 2008]. A pilot study showed improvement in aminotransferases with *gemfibrozil* but not clofibrate [Basaranoglu *et al.* 1999; Laurin *et al.* 1996]. More recently, *bezafibrate* has been evaluated in a small Japanese case series of patients with tamoxifen-induced NASH [Saibara *et al.* 1999]. Reduction in hepatic steatosis was observed. A study of 12 months treated with *fenofibrate* showed reduction in the degree of hepatocellular ballooning but not in other histologic components [Fernandez-Miranda *et al.* 2008].

3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (statins) Beyond the theoretical benefits of serum lipid lowering in reducing hepatic steatosis, the statins also have antioxidant properties [Yamamoto *et al.* 1998]. Most of the studies published are small case series ($n = 5-22$) using atorvastatin, pravastatin and rosuvastatin [Antonopoulos *et al.* 2006;

Rallidis *et al.* 2004; Kiyici *et al.* 2003]. Not all have had follow-up histology. Improvement in transaminases, inflammatory grades but not hepatic fibrosis have been documented [Rallidis *et al.* 2004].

Abnormalities of serum transaminases were documented in early statin trials. However, these tend to be infrequent (<2% of cases), often transient, modest in degree [$<1.5 \times$ upper limit of normal (ULN)] and numerically similar to subjects receiving placebo [Lewis *et al.* 2007]. Yet, in the past, there has been reluctance to use these drugs in patients with liver disease due to perceived fears of hepatotoxicity. The safety of statin use in NAFLD has been reiterated by recent studies [Lewis *et al.* 2007; Chalasani, 2005; Browning, 2006]. There is no justification for withholding these drugs in persons with NAFLD, a group at a generally higher risk of cardiovascular disease.

Ezetimibe This recently introduced cholesterol and plant sterol absorption inhibitor reduces plasma low-density lipoprotein cholesterol by binding to Niemann-Pick C1 like 1, a selective intestinal cholesterol transporter. Ezetimibe treatment for 4 weeks reduced hepatic lipid content and ALT levels in a mouse model of NAFLD [Zheng *et al.* 2008]. It has also shown to reduce oxidative stress and decrease serum TNF- α levels [Assy *et al.* 2006]. Human data are yet to accrue.

Endocannabinoid antagonists

The appetite-stimulating actions of marijuana have been recognized for centuries. Research into the central actions of this drug was boosted by the discovery of Δ^9 -tetrahydrocannabinol (Δ^9 -THC) as its psychoactive constituent. More recently, endogenous lipid derivatives, the endocannabinoids (ECs), were found to have a range of actions that mimic the activity of Δ^9 -THC [Caraceni *et al.* 2008; Mallat and Lotersztajn, 2008]. The ECs have emerged as important mediators of a diverse range of biologic processes including regulation of appetite, energy balance, immune and inflammatory responses. EC activities are mediated through specific cannabinoid receptors, CB1 and CB2. The CB1 receptor is expressed in the brain and some peripheral tissues including the liver. CB2 is predominantly expressed within the cells of the immune system. The EC system is upregulated in chronic liver diseases and has been implicated in NAFLD,

cirrhotic cardiomyopathy, hepatic reperfusion-ischaemic injury as well as portal hypertension [Mallat and Lotersztajn, 2008].

The involvement of the EC system in NAFLD has been only recently documented [Kunos and Osei-Hyiaman, 2008; Osei-Hyiaman D *et al.* 2005]. In one study, an endocannabinoid, N-arachidonoyl-ethanolamine (anandamide, AEA) was found to promote diet-induced obesity and fatty liver in mice [Osei-Hyiaman, 2005]. This was achieved through AEA-mediated overexpression of hepatic SREBP1, a lipogenic transcription factor that promotes *de novo* hepatic lipogenesis. This action was shown to be mediated through the CB1 receptor because these effects were blunted in mice pretreated with a CB1 receptor antagonist (rimonabant). CB1 (-/-) mice were also less susceptible to the obesogenic and steatogenic effect of a high-fat diet. Similar effects were observed in rimonabant-treated obese Zucker fa/fa rats. An increase in serum adiponectin and a decrease in TNF- α levels were observed with rimonabant treatment [Gary-Bobo *et al.* 2007]. The EC system also mediates pro- and anti-fibrogenic responses within the liver [Siegmund and Schwabe 2008]. Therapeutic manipulation of the EC system therefore could be useful in preventing progressive hepatic fibrosis in NAFLD and other liver disorders.

Human data are limited to a single case report of rimonabant in a 21-year-old obese woman with NASH [Banasch *et al.* 2007]. She had failed to achieve weight reduction with several diet protocols over 5 years. On this occasion, she was treated with rimonabant and was also placed on a specific diet (WeightWatchers). Significant weight loss (10%) was achieved. Improvement in aminotransferases and also the metabolic profile (improved insulin sensitivity, lipid profile and glucose tolerance as well as increased adiponectin levels) were observed. Larger trials are underway with rimonabant in human NAFLD. Depression and neuropsychiatric effects are known side effects of rimonabant that could limit its widespread use.

Angiotensin-receptor blockers

Several lines of evidence have highlighted the role of the renin-angiotensin system (RAS) in hepatic fibrogenesis. Blockade of the angiotensin II receptor has been shown to decrease hepatic stellate cell activation and thereby attenuate hepatic fibrosis in several animal models of

hepatic fibrosis [Hirose *et al.* 2007; Yokohama *et al.* 2006]. Angiotensin II has also been shown to contribute to NAFLD through induction of oxidative stress [Wei *et al.* 2008].

One small study has evaluated the effects of losartan, an angiotensin II type 1 receptor antagonist in patients with NASH. Seven Japanese patients with hypertension and NASH were treated with losartan for 48 weeks [Yokohama *et al.* 2004]. Paired liver biopsies were obtained before and after treatment. There was significant reduction in necro-inflammatory activity and fibrosis. There was also a reduction in transforming growth factor β -1, hyaluronic acid and other serum markers of hepatic fibrosis. Indirect evidence for the protective role of RAS blockade was provided by a recent retrospective study of factors associated with *de novo* NAFLD following liver transplantation. Use of angiotensin-converting enzyme inhibitors was associated with a significantly lower risk of NAFLD following liver transplantation (odds ratio 0.09, 95% CI: 0.1–0.92) [Seo *et al.* 2007].

These preliminary results need to be verified in larger studies. However, it does raise certain questions. For instance, is this a class effect applicable to all angiotensin receptor blockers (ARB)? Recent data suggest that all ARBs are not the same with respect to their metabolic effects. In one study involving a rat model of NAFLD, telmisartan but not valsartan was found to reduce steatohepatitis to a similar extent as pioglitazone. Telmisartan not only differs structurally from valsartan but also resembles pioglitazone. Further, telmisartan has partial PPAR- γ agonist properties [Benson *et al.* 2004] and may be even superior to the TZDs because it reduces both subcutaneous and visceral fat [Fujita *et al.* 2007]. This is a potential advantage over the TZDs, which are associated with weight gain due to redistribution from visceral fat.

Approach to management

The current approach to managing NAFLD remains largely empirical. A few general recommendations can be made. Optimizing metabolic control through control of type 2 diabetes and/or hyperlipidemia is important from a cardiovascular perspective. Limited data available do suggest improvement in some components of NAFLD with diet/exercise alone. Ideally, this should be undertaken with the assistance of a dietician and a personal trainer. Specific drug therapy for

NAFLD cannot be recommended outside clinical trials. Patients with morbid obesity who have failed diet/exercise/drug therapy can still benefit from bariatric surgery and should be referred to tertiary centres with multidisciplinary support for such procedures.

For patients with advanced hepatic fibrosis/cirrhosis, monitoring for liver-related complications including hepatocellular carcinoma is mandatory. Persons with signs of hepatic decompensation should be referred for liver transplant assessment. For patients with steatosis alone, specialist follow up is generally not necessary but metabolic monitoring by general practitioners should continue. For those with NASH, where the long-term outcome can be quite variable, periodic monitoring in the liver clinic is suggested. Signs of progressive liver impairment (platelet count, signs of portal hypertension, coagulopathy) should be regularly assessed. This, of course, presumes that a liver biopsy has been performed to identify NASH in the first place. If no liver histology is available, then the best possible strategy at present would be to target patients with high risk of hepatic fibrosis, particularly older patients with obesity and type 2 diabetes. A number of scoring systems are available. None are perfect but they could help identify a significant proportion of patients who may need ongoing surveillance.

Future perspectives

Besides the endocannabinoid antagonists, a number of potentially useful strategies are being evaluated in NAFLD. Some of these include novel molecules such as caspase inhibitors whereas others include combination of drugs already in clinical use. Analogous to the treatment of other chronic diseases such as type 2 diabetes or hypertension, drug combinations involving agents of more than one class may become necessary to treat patients refractory to monotherapy (see FLIRT trial above, [Ratziu *et al.* 2008]) or to reduce side effects (e.g. weight gain with the glitazones). Overall, since treatment appears to be for life, it is important to seek simple lifestyle options which are likely to be easily adopted at a community level in dealing with this ubiquitous metabolic problem.

Conflict of interest statement

None declared.

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