A growing burden: the pathogenesis, investigation and management of non-alcoholic fatty liver disease

P Riley, J O'Donohue, M Crook

J Clin Pathol 2007;**60**:1384–1391. doi: 10.1136/jcp.2006.044891

Non-alcoholic fatty liver disease (NAFLD) is the most common hepatic disorder in western countries, and its incidence is increasing. This review outlines the significant health burden posed by NAFLD and discusses what is presently known about its pathogenesis, including the roles of the metabolic syndrome, obesity, insulin resistance, hepatic steatosis, reactive oxygen species, inflammatory cytokines and adipocytokines. The way in which NAFLD is clinically diagnosed is described, and areas of uncertainty surrounding its investigation are identified, before discussing the relative merits of the limited treatment options available and looking ahead to potential therapeutic strategies for the future.

therefore presents a significant, growing health burden in the developed world.

PATHOGENESIS

The classic “two-hit” theory of NAFLD originally proposed by Day and James²⁰ describes peripheral insulin resistance as the initiating event, with an increase in adipocyte lipolysis and hyperinsulinaemia fuelling hepatic triacylglyceride (TAG) synthesis and steatosis.²¹ Reactive oxygen species (ROS) then drive peroxidation of the accumulated hepatic lipids,²² producing toxic products that damage mitochondria and stimulate further production of ROS.²³ Such a positive feedback loop results in cellular damage, activation of Kupffer cells and the generation of proinflammatory cytokines which drive hepatic inflammation,²⁴ with concurrent activation of hepatic stellate cells establishing a pattern of repair that leads to hepatic fibrosis.^{23 24}

However, the link between the development of hepatic steatosis and the generation of ROS is still poorly understood. Hepatic steatosis has been shown to promote hepatic insulin resistance,^{25 26} and may induce the up-regulation of carnitine palmityl transferase I as well as a reduced affinity for its physiological inhibitor, malonyl CoA, facilitating the mitochondrial uptake of free fatty acids (FFAs).^{23 27} With hepatic FFA pools overflowing, such an increase in β -oxidation could conceivably generate sufficient numbers of ROS to trigger lipid peroxidation.^{23 27} But can steatosis really drive fibrosis alone?

The natural history of NAFLD would suggest not, as statistically only a small proportion of patients develop advanced liver disease.^{28 29} Much of what is known about the progression from hepatic steatosis to fibrosis comes from studies of patients with chronic hepatitis C (HCV), 20–30% of whom have been estimated to develop cirrhosis within 10–30 years.³⁰ A number of such studies have demonstrated a significant association between hepatic steatosis and fibrosis.^{31–33} Applying the two-hit theory, ROS may be produced as a direct effect of the virus on hepatic mitochondria and/or the immune response to viral infection,³⁴ with steatosis providing an abundance of fuel on which such radicals can act.³⁵ In support, steatosis has been associated with markers of oxidative stress in patients with hepatitis C.³⁶ While this provides good evidence for the potential of such a pathogenetic mechanism, it does not explain how ROS may be generated in the absence of a secondary hepatic insult.

Furthermore, a causal relationship between hepatic steatosis and fibrosis has not been estab-

Non-alcoholic fatty liver disease (NAFLD) covers a progressive spectrum of liver pathology, from hepatic steatosis to inflammation and fibrosis characteristic of non-alcoholic steatohepatitis (NASH) and cirrhosis (figs 1 and 2).¹ It is associated with the development of hepatocellular carcinoma (HCC),^{2 3} a significant proportion of which develops from “cryptogenic cirrhosis”,⁴ believed to represent the end-stage of previously undetected NASH.^{5 6} Epidemiological data suggest that NAFLD is the most common hepatic disorder in western countries,⁷ and its incidence is increasing.⁸ A recent study in the United States found that 34% of the population had hepatic steatosis⁹; reports from the Asia-Pacific region suggest similarly high levels paralleling the rise in obesity and type 2 diabetes.¹⁰

NAFLD is widely regarded as a product of insulin resistance due to its strong association with insulin resistant features also seen in the metabolic syndrome.^{11–13} Similarities in prevalence, clinical features and outcome have led some to argue that NAFLD is an unrecognised component of the latter,^{14 15} while others have suggested it should be renamed as “metabolic liver disease”.¹⁶ In fact NAFLD may precede the development of the metabolic syndrome, implying its presence is an early indicator of a premorbid cardiovascular state.^{16 17} As for whether the development of advanced liver disease is a valid concern among patients with co-existing cardiovascular risk factors, El-Serag *et al*¹⁸ showed an increased incidence of non-alcoholic chronic liver disease and HCC in patients with diabetes, and de Marco *et al*¹⁹ found that the increase in liver related mortality exceeded the increase in cardiovascular related mortality in a cohort of type 2 diabetics. NAFLD

See end of article for authors' affiliations

Correspondence to: Mr P Riley, Department of Gastroenterology, University Hospital Lewisham, Lewisham High Street, London SE13 6LH, UK; paul.riley@kcl.ac.uk

Accepted 10 April 2007
Published Online First
4 May 2007

Figure 1 Hepatic steatosis of non-alcoholic fatty liver disease (courtesy of Professor Bernard Portmann, Consultant Histopathologist, Institute of Liver Studies, King's College Hospital, London, UK).

lished. It is possible that the “progression” from the former to the latter reflects instead the effects of an underlying pathology that produces steatosis and fibrosis concurrently. Insulin resistance is an obvious candidate, and a number of studies have shown it to be a reliable predictor of disease severity.^{37–38} It underlies the “first hit” of hepatic steatosis in NAFLD, and there is good evidence to suggest that it is responsible for the hepatic steatosis seen in non-genotype 3 chronic HCV.^{34–39} Several studies have demonstrated an association between insulin resistance and hepatic fibrosis in cohorts of patients with NAFLD^{37–40–41} or HCV,^{42–44} and it may promote fibrosis through the following:

- Hepatic steatosis promoting hepatic insulin resistance,^{25–26} facilitating the β -oxidation of FFAs and generation of ROS.
- An increased delivery of FFAs to the liver where they may undergo peroxisomal oxidation^{45–46} and stimulate cytochrome P450 2E1^{47–48} generating ROS, or drive the production of inflammatory cytokines in hepatocytes directly via a lysosomal pathway.⁴⁹
- Hyperinsulinaemia and hyperglycaemia stimulating the expression of the fibrogenic growth factor CTGF in hepatic stellate cells.⁵⁰

Recently it has become clear that insulin resistance can increase serum uric acid levels, and hyperuricaemia may worsen NAFLD.⁵¹ However, more studies are required to evaluate the links between insulin resistance and hyperuricaemia, and determine its significance in the context of NAFLD.

Steatosis and insulin resistance have therefore emerged as potentially significant factors in the evolution of the relatively benign fatty liver to the morbid hepatic fibrosis characteristic of advanced NAFLD. However, their complex inter-relationship and pathogenic significance remain frustratingly unclear,⁵² and in contrast to the evidence presented above some studies have failed to find an association between steatosis^{53–55} or insulin resistance⁵⁶ and the progression of liver disease.

Also unclear is the role of the proinflammatory cytokine tumour necrosis factor α (TNF- α). One study has demonstrated an increase in the hepatic production of TNF- α in response to diet-induced obesity and hepatic steatosis,⁵⁷ and it is known to promote insulin resistance, both directly via serine phosphorylation of IRS-1 residues downstream of the insulin receptor,^{39–52–58} and indirectly via the perturbation of the growth

hormone axis⁵² and disruption of β -cell function.⁵⁸ Transgenic experiments with the HCV core protein in mice have demonstrated that hepatic insulin resistance following HCV infection is driven by the up-regulation of TNF- α and can be reversed by TNF- α blockade.⁵⁹ It has been pointed out, however, that other inflammatory liver diseases such as hepatitis B are not associated with insulin resistance despite raised intrahepatic and circulating levels of the cytokine.⁶⁰ Aside from its potential effects on glucose metabolism, TNF- α has also been shown to induce mitochondrial abnormalities⁶¹ which may promote the production of ROS, and is known to play a role in the recruitment of Kupffer cells, activation of hepatic stellate cells and fibrogenesis in mouse models of NASH.⁶²

In fact the most likely source of TNF- α in NAFLD is adipocytes.⁵² Plasma levels have been shown to correlate directly with body fat mass,⁶³ and it has been suggested that the metabolic stress placed on adipose tissue in obesity stimulates macrophage infiltration and localised TNF- α production, with the cytokine acting in a paracrine fashion to suppress secretion of the adipokine mediator adiponectin.^{24–58} Adiponectin is known to enhance insulin sensitivity,⁶⁴ and is predominantly secreted by visceral fat.⁶⁴ Consequently the expansion of the visceral adipocyte compartment may paradoxically suppress its secretion,⁶⁵ and NAFLD has been associated with a reduction in plasma adiponectin,^{65–67} explaining the well described observation that abdominal waist to hip ratio predicts insulin resistance and NAFLD better than body mass index, which says nothing about fat distribution.^{17–65–68} A number of studies have found an association between low levels of adiponectin and hepatic steatosis in patients with NAFLD^{65–69–70} or HCV.⁷¹ Adiponectin is also believed to provide a hepatoprotective role against inflammation and fibrosis,^{65–66} and the extent of hypo adiponectinaemia has been shown to correlate well with the severity of necroinflammation and hepatic fibrosis in patients with NAFLD.^{69–72–73}

In contrast, levels of the adipocytokine leptin appear to be significantly raised in patients with NASH^{74–75} or HCV,^{76–77} an effect which some have ascribed to the obesity-related phenomenon of “leptin resistance”.⁷⁸ In both disorders raised levels have been found to correlate with steatosis^{74–79} and leptin is known to modulate insulin sensitivity,⁷⁸ as well as being implicated in the development of hepatic fibrosis. Both leptin and leptin receptor deficient rodents either fed choline deficient diets^{80–81} or treated with profibrotic xenobiotics,⁸² failed to develop the hepatic fibrosis seen in their normal littermate controls. Leptin has been shown to increase the expression of the profibrotic cytokine TGF- β in sinusoidal endothelial and Kupffer cells, increase PDGF-dependent proliferation of hepatic stellate cells,⁸² and up-regulate their expression of proangiogenic and proinflammatory cytokines.⁸³ Furthermore, activated hepatic stellate cells have been identified as a non-adipocyte source of leptin themselves.⁸⁴ However, some researchers have found no increase in serum leptin concentration between patients with NASH or HCV and controls,^{85–86} and no correlation between leptin level and hepatic histology in patients with NAFLD^{85–87} or HCV.^{86–88–89} One study even describes the reduction of serum leptin with progressive hepatic injury.⁹⁰

Clearly insulin resistance, hepatic steatosis, reactive oxygen species, inflammatory cytokines and adipocytokines all have a part to play in the development and/or progression of NAFLD. However their precise roles and the sequence of events remain unclear (fig 3). The disorder shares many similarities with the development of hepatic steatosis and fibrosis in HCV, and the same key players appear to be involved. Nonetheless, the complex inter-relationship between host risk factors and the cytopathic effect of the virus, especially genotype 3 HCV,⁹¹ means we should be wary of drawing comparisons between

Figure 2 Non-alcoholic steatohepatitis (courtesy of Professor Bernard Portmann, Consultant Histopathologist, Institute of Liver Studies, King's College Hospital, London, UK).

apparently similar hepatic pathologies. Critically, the trigger that initiates the conversion of benign hepatic steatosis to steatohepatitis in NAFLD remains unknown. Further large scale, prospective, well controlled studies adjusting for a large number of confounding metabolic factors are required to elicit further information about this prognostically important question.

INVESTIGATION

No specific laboratory test exists for the diagnosis of NAFLD; it is primarily a diagnosis of exclusion. Suspicion is typically aroused by the incidental finding of persistently raised serum transaminases, particularly alanine transaminase (ALT), which is known to be a more specific indicator of hepatocyte injury than aspartate transaminase (AST).⁹² However, the guidelines on what constitutes a clinically significant elevation, and the period of time that must elapse before such an elevation may be considered persistent, are far from clear.

A recent comprehensive review of the significance of elevated serum transaminases in primary care suggests that the first line of investigation should be a detailed history and physical examination to uncover risk factors for, and/or clinical features of, liver disease.⁹³ If risk factors are present, the subsequent course of action is dependent on their nature: some predispose to hepatic pathology directly (eg excessive alcohol consumption), while others predispose to hepatic pathology indirectly (eg intravenous drug use). The authors recommend that patients with a direct risk factor should undergo a six month trial of appropriate interventional therapy, with further investigation reserved for those with an indirect risk factor, those with clinical features, or those in whom such interventional therapy fails to work. These guidelines imply that patients who are obese and/or diabetic without other direct risk factors, indirect risk factors or clinical features of liver disease may be assumed to have NAFLD and treated accordingly for six months without further investigation, the pre-emptive diagnosis being confirmed by the successful reduction of serum transaminases.

Patients with NAFLD may present with raised serum transaminases that are not associated with any risk factors or clinical features of liver disease. For such patients Smellie *et al*⁹³ recommend further investigation on the basis of a single measurement of raised ALT $>3 \times$ upper limit of normal (ULN),

while two measurements taken six months apart are required before further investigation is warranted if the raised ALT is $<3 \times$ ULN, to distinguish between a transient elevation that is clinically insignificant and a persistent rise which may indicate pathological change. However, the range of transient, clinically insignificant elevations in ALT is poorly defined, and there is little evidence to suggest that a threshold of $3 \times$ ULN optimises the investigative approach. What is clear is that once innocuous elevations in ALT have been ruled out, the size of the rise gives little or no indication of the extent of liver disease. Indeed, Smellie *et al* point out the "poor correlation between level of rise and biopsy findings" and hence "do not recommend a lower threshold" for further investigation. At one end of the spectrum, normal serum ALT has been documented in significant numbers of patients with NAFLD,⁹⁴⁻⁹⁶ and at the other, raised levels have been found in patients with no evidence of hepatic pathology.⁹⁷ This suggests that NAFLD is under-diagnosed in society, and supports the need for an active diagnostic approach that recognises the independent importance of risk factors for the disease. The significance of absolute transaminase levels is unclear, and more research is required to determine the relevance of commonly quoted "threshold" limits.

When the decision to undertake further investigation into raised serum transaminases is made, Smellie *et al* suggest initially performing a full blood count to screen for excessive alcohol consumption and hypersplenism; an autoantibody count to screen for primary biliary cirrhosis and autoimmune hepatitis; ferritin levels to screen for haemochromatosis; and hepatitis serology to screen for hepatitis B or hepatitis C as the cause. If a diagnosis is not confirmed, a second set of investigations should be initiated, with anti-endomysial antibody detection to screen for coeliac disease; α_1 -antitrypsin measurement to screen for α_1 -antitrypsin deficiency; caeruloplasmin and urinary copper levels to screen for Wilson disease; and a liver ultrasound (US) to screen for biliary duct obstruction and a liver tumour. If the above treatable disorders have been ruled out, evidence from a recent large scale study by Skelly *et al*⁹⁸ suggests that the most likely cause is NAFLD. They performed liver biopsies on 354 patients with unexplained raised serum liver function enzymes (including serum ALT) $>2 \times$ ULN for >6 months, and found that 32% of the patients had hepatic steatosis and 34% of the patients had NASH. Given the previously stated poor correlation between the degree of liver enzyme abnormality and extent of liver disease, there is no reason to suggest that the results would have been any different if the cohort had included patients with an unexplained persistent elevation in serum ALT of any size.

From a diagnostic point of view, a liver US performed to exclude biliary duct obstruction or a liver tumour as the cause of an elevation in serum ALT may also be used to detect hepatic steatosis. Several trials suggest that US has a high sensitivity and specificity for the detection of fatty livers. Saverymuttu *et al*⁹⁹ selected a cohort of 85 patients that had undergone liver biopsy; they showed that US blindly identified hepatic steatosis in 94% of the 48 patients with the condition, and 100% of the patients for whom steatosis was histologically graded moderate or severe, with a specificity of 84%. Mathiesen *et al*¹⁰⁰ investigated 165 asymptomatic patients with persistently raised serum transaminases and found that US detected biopsy-proven hepatic steatosis 90% of the time, with a specificity of 82%. However, evidence suggests that US is not a reliable investigation for the diagnosis or grading of fibrosis⁹⁹⁻¹⁰⁰; a liver biopsy is widely recognised as the only definitive test for NASH and cirrhosis.¹⁰¹

Figure 4 details a suggested algorithm for the investigation of persistently raised serum transaminases.

Figure 3 Schematic diagram illustrating the pathogenesis of non-alcoholic fatty liver disease. Visceral obesity results in a paradoxical inhibition of adiponectin secretion from adipose tissue which produces peripheral insulin resistance, promoting hyperinsulinaemia and the generation of hepatic steatosis. Hepatic steatosis drives hepatic insulin resistance, which produces a surge in mitochondrial β -oxidation, creating reactive oxygen species (ROS) which stimulate peroxidation of triacylglyceride (TAG), and establishes a positive feedback loop. The resulting cellular damage stimulates the production of proinflammatory cytokines that drive the progression from hepatic steatosis to non-alcoholic steatohepatitis (NASH). Insulin resistance, tumour necrosis factor α (TNF- α), low serum adiponectin and high serum leptin may be incidental, play permissive roles or directly enhance such a transition. Ultimately the activation of hepatic stellate cells drives hepatic fibrosis (not shown). FFA, free fatty acid.

MANAGEMENT

Given the strong association between obesity, insulin resistance and NAFLD, weight loss would appear to be an appropriate management strategy. The most rational approach involves lifestyle modifications incorporating diet and exercise; a number of studies indicate that such a weight loss regime can successfully reduce raised serum transaminases^{11 102–104} and hepatic steatosis^{103 105 106} in patients with NAFLD. Kugelmas *et al*¹⁰⁴ studied the effects of weight loss in patients with biopsy-proven NASH, achieved through a step 1 American Heart Association diet¹⁰⁷ with at least 30 minutes light exercise per day. They found a significant reduction in raised serum transaminases at 6 weeks that was maintained at 12 weeks, and also report a significant reduction in plasma levels of IL-6, an established marker of the severity of the hepatic inflammatory response,¹⁰⁸ suggesting that such a regime may also reduce hepatic inflammation. Two case reports^{109 110} and a recent pilot study by Huang *et al*¹¹¹ support this claim, showing that weight loss achieved through a combination of diet and exercise can induce the regression of the histological features of NASH. Furthermore, in the Huang *et al* study there was a significantly greater reduction in mean waist circumference in the patients showing histological improvement, as well as a greater reduction in visceral fat on CT, showing the importance of reducing visceral fat stores in the treatment of NAFLD.

However, therapies based on lifestyle modification alone are notoriously hard to sustain. A number of studies have shown that weight loss following bariatric surgery can have a beneficial effect on patients with NAFLD. Gastroplasty,^{112 113} gastric banding,^{114 115} Roux-en-Y gastric bypass^{116 117} and gastric bypass via biliopancreatic diversion¹¹⁸ have all been shown to induce weight loss with a concurrent improvement in liver biochemistry and histology, including reductions in serum transaminase levels, hepatic steatosis, inflammation and

fibrosis. Recently, pilot studies have suggested that pharmacologically induced weight loss may also improve NAFLD. Orlistat, an inhibitor of gastric and pancreatic lipases typically used to manage obesity, and sibutramine, a centrally acting serotonin and noradrenaline reuptake inhibitor which has direct effects on appetite, have both been shown to be effective at inducing weight loss, reducing raised serum transaminases and regressing hepatic steatosis in patients with NAFLD.^{119 120}

Weight loss is an attractive therapy for NAFLD for a number of good reasons. Evidence suggests that it is effective; there are a number of ways in which it may be achieved; and it simultaneously tackles cardiovascular risk factors of the associated metabolic syndrome.¹²¹ However, a recent review pointed out the lack of randomised, controlled trials investigating the effects of weight loss on hepatic pathology.¹²² Some studies have questioned its efficacy; Fan *et al*^{123 124} found that weight loss following a low calorie diet reduced hepatic steatosis but had no effect on hepatic inflammatory changes in rodent models of NASH. Others have shown that weight loss can produce a significant deterioration in hepatic function. Severe dieting results in a dramatic increase in peripheral lipolysis which furthers the supply of FFAs to the liver, and has been linked with reduced levels of the antioxidant glutathione, providing the conditions for a paradoxical increase in hepatic inflammation and fibrosis.¹²⁵ Rapid, diet-induced weight loss has been shown to precipitate the development and degeneration of steatohepatitis.^{126 127} Similar concerns exist regarding weight loss achieved through surgery. Jejunoileal bypass for morbid obesity has long been recognised as a primary cause of NASH,^{128 129} and weight loss following gastroplasty has also been associated with hepatic degeneration.¹³⁰

Given the difficulty of dietary intervention, the relative risks of bariatric surgery, the scarcity of data surrounding the effects of pharmacologically induced weight loss, and the controversy

Figure 4 Algorithm for the investigation of persistently raised serum transaminases, based on Smellie *et al.*⁹³ Non-alcoholic fatty liver disease may be diagnosed via the presence of obesity and/or diabetes (direct risk factors) in the absence of other direct risk factors, indirect risk factors or clinical features of liver disease, or by exclusion. Liver ultrasound may diagnose hepatic steatosis but liver biopsy is required to diagnose non-alcoholic steatohepatitis or cirrhosis.

over the effectiveness of weight loss as a treatment for NAFLD, it is important that alternative therapeutic strategies are developed.

The central role of insulin resistance in the pathogenesis of NAFLD has stimulated considerable interest in the use of insulin sensitisers. Metformin has been shown to eliminate fatty liver disease in insulin resistant ob/ob mice,¹³¹ and studies in patients with NAFLD have shown similarly beneficial hepatic effects, including reductions in persistently raised serum transaminases,^{132–134} hepatic fat content,^{133, 134} necroinflammation, fibrosis, and a significant improvement in coexisting positive criteria for the metabolic syndrome.¹³⁴ Preliminary reports also suggest that thiazolidinediones may be effective at reducing hepatic steatosis, inflammation and fibrosis.¹³⁵ Alternative approaches to overcoming insulin resistance are to stimulate insulin secretion or target critical events in the development of NAFLD downstream of the insulin receptor. Nateglinide, which stimulates insulin secretion, has been shown to improve liver histology in a cohort of five patients with NASH,¹³⁶ and L-carnitine,¹³⁷ β-aminoisobutyric acid¹³⁸ and antisense oligonucleotide inhibitors of acetyl CoA carboxylases,¹³⁹ all of which increase mitochondrial FFA oxidation, have shown promising results in rodent models.

Antioxidant therapies aim to prevent the lipid peroxidation thought to be central to the progression from hepatic steatosis to NASH. S-nitroso-N-acetylcysteine has been shown to inhibit the development of NAFLD in association with a reduction in lipid peroxidation in rats,¹⁴⁰ while a three month course of N-acetylcysteine reportedly produced a significant reduction in raised transaminases in a cohort of patients with NASH.¹⁴¹ Pentoxifylline, an inducer of hepatic glutathione, is reported to decrease raised serum ALT and hepatic inflammation in diet-

induced steatohepatitis in mice,¹⁴² and has also been shown to be effective in reducing steatohepatitis in patients with NASH.¹⁴³ Some antioxidants may intervene earlier in the pathogenesis of NAFLD, suggesting that either our understanding of the initial events is incomplete or the drugs in question have multiple therapeutic targets. The ROS scavenger manganese tetrakis 4-benzoic acid porphyrin has been shown to rescue manganese superoxide dismutase knockout mice from hepatic steatosis¹⁴⁴ and was recently shown to improve hepatic steatosis in ob/ob mice.¹⁴⁵ Ursodeoxycholic acid, another inducer of hepatic glutathione,¹⁴⁶ has been shown to prevent the development of hepatic steatosis in rats¹⁴⁷ and reduce raised serum ALT in patients with NAFLD.¹⁴⁸ Given the apparent hepatoprotective effects of glutathione, the absence of data regarding the effects of S-adenosylmethionine, which has been shown to reverse decreased hepatic levels in patients with liver disease,¹⁴⁹ is notable.

Many of the pharmacological treatments mentioned above may also exert immunoregulatory effects, exhibiting anti-inflammatory properties through direct or indirect inhibition of pro-inflammatory cytokines to prevent and reduce hepatic inflammation. Metformin has been shown to inhibit hepatic expression of TNF-α, and reverse TNF-α inducible responses that may promote hepatic steatosis and necrosis in insulin-resistant ob/ob mice.¹³¹ L-carnitine has been shown to reduce the induction of TNF-α expression by alcohol in rats,¹⁵⁰ and pentoxifylline has also been shown to reduce TNF-α expression in rodent models of NASH.¹⁴³

Despite the pressing need for a definitive treatment for NAFLD, and the apparent potential for therapeutic intervention, no reliable pharmacological agent has been found to date.

Take-home messages

- Non-alcoholic fatty liver disease presents an increasingly significant health burden in the developed world.
- Determinants of the clinically significant progression from hepatic steatosis to non-alcoholic steatohepatitis remain unclear. Insulin resistance, hepatic steatosis, reactive oxygen species, inflammatory cytokines and adipocytokines may play important roles.
- Guidelines for managing asymptomatic patients with raised serum transaminases are ambiguous. An active diagnostic approach that recognises the importance of risk factors for non-alcoholic fatty liver disease is required.
- Gradual, controlled weight reduction through a balanced diet and regular exercise is the treatment of choice.
- More research into the progression from hepatic steatosis to non-alcoholic steatohepatitis, and pharmaceutical agents that target critical steps in the pathogenesis of non-alcoholic fatty liver disease, is required.

This is due, in part, to a lack of well controlled, large scale, randomised trials of the drugs that have shown benefit in preliminary animal or patient studies. There is also a lack of information regarding the effects of such treatments on liver histology. Consequently current treatment places an emphasis on gradual, controlled weight reduction through a balanced diet and regular exercise.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the contribution of Professor Bernard Portmann, Consultant Histopathologist, Institute of Liver Studies, King's College Hospital, London, UK, for supplying histological pictures of hepatic steatosis and non-alcoholic steatohepatitis.

Authors' affiliations

P Riley, J O'Donohue, Department of Gastroenterology, University Hospital Lewisham, London, UK

M Crook, Department of Biochemistry, University Hospital Lewisham, London, UK

Competing interests: None.

REFERENCES

- Sass DA, Chang P, Chopra KB. Nonalcoholic fatty liver disease: a clinical review. *Dig Dis Sci* 2005;**50**:171–80.
- Bugianesi E, Leone N, Vanni E, et al. Expanding the natural history of non-alcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. *Gastroenterology* 2002;**123**:134–40.
- Shimada M, Hashimoto E, Taniguchi M, et al. Hepatocellular carcinoma in patients with non-alcoholic steatohepatitis. *J Hepatol* 2002;**37**:154–60.
- Bugianesi E. Review article: steatosis, the metabolic syndrome and cancer. *Aliment Pharmacol Ther* 2005;**22**(Suppl 2):40–3.
- Cuadrado A, Orive A, Garcia-Suarez C, et al. Non-alcoholic steatohepatitis (NASH) and hepatocellular carcinoma. *Obes Surg* 2005;**15**:442–6.
- Ayata G, Gordon FD, Lewis WD, et al. Cryptogenic cirrhosis: clinicopathologic findings at and after liver transplantation. *Hum Pathol* 2002;**33**:1098–104.
- Reid AE. Nonalcoholic steatohepatitis. *Gastroenterology* 2001;**121**:711–23.
- Portincasa P, Grattagliano I, Palmieri VO, et al. The emerging problem of non-alcoholic steatohepatitis (NASH). *Rom J Gastroenterol* 2005;**14**:43–51.
- Browning JD, Szczepaniak LS, Dobbins R, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 2004;**40**:1387–95.
- Chitturi S, Farrell GC, George J. Non-alcoholic steatohepatitis in the Asia-Pacific region: future shock? *J Gastroenterol Hepatol* 2004;**19**:368–74.
- Knobler H, Schattner A, Zhornitsky T, et al. Fatty liver—an additional and treatable feature of the insulin resistance syndrome. *Q J Med* 1999;**92**:73–9.
- Bugianesi E, McCullough AJ, Marchesini G. Insulin resistance: a metabolic pathway to chronic liver disease. *Hepatology* 2005;**42**:987–1000.
- Machado M, Cortez-Pinto H. Non-alcoholic fatty liver disease and insulin resistance. *Eur J Gastroenterol Hepatol* 2005;**17**:823–6.
- Carulli N. Metabolic syndrome—cardiovascular disease risk and more. *Aliment Pharmacol Ther* 2005;**22**(Suppl 2):1–2.
- Powell EE, Jonsson JR, Clouston AD. Dangerous liaisons: the metabolic syndrome and nonalcoholic fatty liver disease. *Editorial Annals of Internal Medicine* 2005;**143**:753.
- Kim HJ, Kim HJ, Lee KE, et al. Metabolic significance of nonalcoholic fatty liver disease in nonobese. *Nondiabetic Adults Arch Intern Med* 2004;**164**:2169–75.
- Friis-Liby I, Aldenborg F, Jerlstad P, et al. High prevalence of metabolic complications in patients with non-alcoholic fatty liver disease. *Scand J Gastroenterol* 2004;**39**:864–9.
- El-Serag HB, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. *Gastroenterology* 2004;**126**:460–8.
- de Marco R, Locatelli F, Zoppini G, et al. Cause-specific mortality in type 2 diabetes. The Verona Diabetes Study. *Diabetes Care* 1999;**18**:353–8.
- Day CP, James OFW. Steatohepatitis: a tale of two 'hits'. *Gastroenterology* 1998;**114**:842–5.
- Bugianesi E, Zannoni C, Vanni E, et al. Non-alcoholic fatty liver and insulin resistance: a cause-effect relationship? *Dig Liver Dis* 2004;**36**:165–73.
- Albano E, Mottaran E, Occhino G, et al. Review article: role of oxidative stress in the progression of non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 2005;**22**(Suppl 2):71–3.
- Fromenty B, Robin MA, Igoudjil A, et al. The ins and outs of mitochondrial dysfunction in NASH. *Diabetes Metab* 2004;**30**:121–38.
- Day CP. From fat to inflammation. *Gastroenterology* 2006;**130**:207–10.
- Kim JK, Fillmore JJ, Chen Y, et al. Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. *Proc Natl Acad Sci USA* 2001;**98**:7522–7.
- Chou CJ, Haluzik M, Gregory C, et al. WY14,643, a peroxisome proliferator-activated receptor alpha (PPARalpha) agonist, improves hepatic and muscle steatosis and reverses insulin resistance in lipotrophic A-ZIP/F-1 mice. *J Biochem* 2002;**277**:24484–9.
- Pessayre D, Fromenty B. NASH: a mitochondrial disease. *J Hepatol* 2005;**42**:928–40.
- Day CP. Natural history of NAFLD: remarkably benign in the absence of cirrhosis. *Gastroenterology* 2005;**129**:375–8.
- Teli MR, James OF, Burt AD, et al. The natural history of nonalcoholic fatty liver: a follow-up study. *Hepatology* 1995;**22**:1714–19.
- Alberti A, Vario A, Ferrari A, et al. Review article: chronic hepatitis C—natural history and cofactors. *Aliment Pharmacol Ther* 2005;**22**(Suppl 2):74–8.
- Bocato S, Pistis R, Noventa F, et al. Fibrosis progression in initially mild chronic hepatitis C. *J Viral Hepat* 2006;**13**:297–302.
- Fartoux L, Chazouilleres O, Wendum D, et al. Impact of steatosis on progression of fibrosis in patients with mild hepatitis C. *Hepatology* 2005;**41**:82–7.
- Leandro G, Mangia A, Hui J, et al. Relationship between steatosis, inflammation and fibrosis in chronic hepatitis C: a meta-analysis of individual patient data. *Gastroenterology* 2006;**130**:1636–42.
- Lonardo A, Adinolfi LE, Loria P, et al. Steatosis and hepatitis C virus: mechanisms and significance for hepatic and extrahepatic disease. *Gastroenterology* 2004;**126**:586–97.
- Koike K. Steatosis in chronic hepatitis C: fuel for overproduction of oxidative stress? *J Gastroenterol* 2005;**40**:664–5.
- Kitase A, Hino K, Furutani T, et al. In situ detection of oxidised n-3 polyunsaturated fatty acids in chronic hepatitis C: correlation with hepatic steatosis. *J Gastroenterol* 2005;**40**:617–24.
- Bugianesi E, Manzini P, D'Antico S, et al. Relative contribution of iron burden, HFE mutations, and insulin resistance to fibrosis in non-alcoholic fatty liver. *Hepatology* 2004;**39**:179–87.
- Haukeland JW, Konopski Z, Linnestad P, et al. Abnormal glucose tolerance is a predictor of steatohepatitis and fibrosis in patients with non-alcoholic fatty liver disease. *Scand J Gastroenterol* 2005;**40**:1469–77.
- Zekry A, McHutchison JG, Diehl AM. Insulin resistance and steatosis in hepatitis C virus infection. *Gut* 2005;**54**:903–6.
- Harrison SA, Torgerson S, Hayashi PH. The natural history of non-alcoholic fatty liver disease: a clinical histopathological study. *Am J Gastroenterol* 2003;**98**:2042–7.
- Adams LA, Sanderson S, Lindor KD, et al. The histological course of nonalcoholic fatty liver disease: a longitudinal study of 103 patients with sequential liver biopsies. *J Hepatol* 2005;**42**:132–8.
- Bugianesi E, Marchesini G, Gentilecore E, et al. Fibrosis in genotype 3 chronic hepatitis C and non-alcoholic fatty liver disease: role of insulin resistance and hepatic steatosis. *Hepatology* 2006;**44**:1648–55.
- Hickman JI, Powell EE, Prins JB, et al. In overweight patients with chronic hepatitis C, circulating insulin is associated with hepatic fibrosis: implications for therapy. *J Hepatol* 2003;**39**:1042–8.
- D'Souza R, Sabin CA, Foster GR. Insulin resistance plays a significant role in liver fibrosis in chronic hepatitis C and in the response to antiviral therapy. *Am J Gastroenterol* 2005;**100**:1509–15.
- DeCraemer D, Pauwels M, Van den Branden C. Alterations of peroxisomes in steatosis of the human liver: a quantitative study. *Hepatology* 1995;**22**:744–52.
- Rao MS, Reddy JK. Peroxisomal beta-oxidation and steatohepatitis. *Semin Liver Dis* 2001;**21**:43–55.
- Chalasani N, Gorski JC, Asghar MS, et al. Hepatic cytochrome P450 2E1 activity in nondiabetic patients with non-alcoholic steatohepatitis. *Hepatology* 2003;**37**:544–50.

- 48 **Robertson G**, Leclercq I, Farrell GC. Nonalcoholic steatosis and steatohepatitis. II. Cytochrome P-450 enzymes and oxidative stress. *Am J Physiol Gastrointest Liver Physiol* 2001;**281**:G1135-9.
- 49 **Feldstein AE**, Werneburg NW, Canbay A, et al. Free fatty acids promote hepatic lipotoxicity by stimulating TNF- α expression via a lysosomal pathway. *Hepatology* 2004;**40**:185-94.
- 50 **Paradis V**, Perlemuter G, Bonvoust F, et al. High glucose and hyperinsulinaemia stimulate connective tissue growth factor expression: a potential mechanism involved in progression to fibrosis in non-alcoholic steatohepatitis. *Hepatology* 2001;**34**:738-44.
- 51 **Tsouli SG**, Liberopoulos EN, Mikhailidis DP, et al. Elevated serum uric acid levels in metabolic syndrome: an active component or an innocent bystander? *Metabolism* 2006;**55**:1293-301.
- 52 **Picardi A**, D'Avola D, Gentilucci UV, et al. Diabetes in chronic liver disease: from old concepts to new evidence. *Diabetes Metab Res Rev* 2006;**22**:274-83.
- 53 **Sanyal AJ**. Review article: non-alcoholic fatty liver disease and hepatitis C—risk factors and clinical implications. *Aliment Pharmacol Ther* 2005;**22**(Suppl 2):48-51.
- 54 **Ryder SD**. Progression of hepatic fibrosis in patients with hepatitis C: a prospective repeat liver biopsy study. *Gut* 2004;**53**:451-5.
- 55 **Perumalswami P**, Kleiner DE, Lutchman G, et al. Steatosis and progression of fibrosis in untreated patients with chronic hepatitis C infection. *Hepatology* 2006;**43**:780-7.
- 56 **Camma C**, Bruno S, Di Marco V, et al. Insulin resistance is associated with steatosis in nondiabetic patients with genotype 1 chronic hepatitis C. *Hepatology* 2006;**43**:64-71.
- 57 **Li Z**, Soloski MJ, Diehl AM. Dietary factors alter hepatic innate immune system in mice with non-alcoholic fatty liver disease. *Hepatology* 2005;**42**:755-8.
- 58 **Noto H**, Raskin P. Hepatitis C infection and diabetes. *J Diabetes Complications* 2006;**20**:113-20.
- 59 **Shintani Y**, Fujie H, Miyoshi H, et al. Hepatitis C virus infection and diabetes: direct involvement of the virus in the development of insulin resistance. *Gastroenterology* 2004;**126**:840-8.
- 60 **Weinman SA**, Belalcázar LM. Hepatitis C: a metabolic disease. *Gastroenterology* 2004;**126**:917-9.
- 61 **Sanchez-Alcázar JA**, Schneider E, Martinez MA, et al. Tumour necrosis factor- α increases the steady-state reduction of cytochrome b of the mitochondrial respiratory chain in metabolically inhibited L929 cells. *J Biol Chem* 2000;**275**:13353-61.
- 62 **Tomita K**, Tamiya G, Ando S, et al. Tumour necrosis factor α signalling through activation of Kupffer cells plays an essential role in liver fibrosis of non-alcoholic steatohepatitis in mice. *Gut* 2006;**55**:415-24.
- 63 **Kern PA**, Saghizadeh M, Ong JM, et al. The expression of tumour necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. *J Clin Invest* 1995;**95**:2111-19.
- 64 **Schaffner A**, Scholmerich J, Buchler C. Mechanisms of disease: adipocytokines and visceral adipose tissue—emerging role in non-alcoholic fatty liver disease. *Nat Clin Pract Gastroenterol Hepatol* 2005;**2**:273-80.
- 65 **Musso G**, Gambino R, Durazzo M, et al. Adipokines in NASH: postprandial lipid metabolism as a link between adiponectin and liver disease. *Hepatology* 2005;**42**:1175-83.
- 66 **Yoon D**, Lee SH, Park HS, et al. Hypoadiponectinaemia and insulin resistance are associated with non-alcoholic fatty liver disease. *J Korean Med Sci* 2005;**20**:421-6.
- 67 **Pagano C**, Soardo G, Esposito W, et al. Plasma adiponectin is decreased in non-alcoholic fatty liver disease. *Eur J Endocrinol* 2005;**152**:113-8.
- 68 **Asselah T**, Rubbia-Brandt L, Marcellin P, et al. Steatosis in chronic hepatitis C: why does it really matter? *Gut* 2006;**55**:123-30.
- 69 **Hui JM**, Hodge A, Farrell GC, et al. Beyond insulin resistance in NASH: TNF- α or adiponectin? *Hepatology* 2004;**40**:46-54.
- 70 **Bugianesi E**, Pagotto U, Manini R, et al. Plasma adiponectin in non-alcoholic fatty liver is related to hepatic insulin resistance and hepatic fat content, not to liver disease severity. *J Clin Endocrinol Metab* 2005;**90**:3498-504.
- 71 **Petit JM**, Minello A, Jooste V, et al. Decreased plasma adiponectin concentrations are closely related to steatosis in HCV-infected patients. *J Clin Endocrinol Metab* 2005;**90**:2240-3.
- 72 **Targher G**, Bertolini L, Rodella S, et al. Associations between plasma adiponectin concentrations and liver histology in patients with non-alcoholic fatty liver disease. *Clin Endocrinol* 2006;**64**:679-83.
- 73 **Musso G**, Gambino R, Biroli G, et al. Hypoadiponectinemia predicts the severity of hepatic fibrosis and pancreatic beta-cell dysfunction in nondiabetic nonobese patients with non-alcoholic steatohepatitis. *Am J Gastroenterol* 2005;**100**:2438-46.
- 74 **Chitturi S**, Farrell G, Frost L, et al. Serum leptin in NASH correlates with hepatic steatosis but not fibrosis: a manifestation of lipotoxicity? *Hepatology* 2002;**36**:403-9.
- 75 **Uygun A**, Kadayifci A, Yesilova Z, et al. Serum leptin levels in patients with non-alcoholic steatohepatitis. *Am J Gastroenterol* 2000;**95**:3584-9.
- 76 **Liu ZW**, Zhang N, Han QY, et al. Correlation of serum leptin levels with anthropometric and metabolic parameters and biochemical liver function in Chinese patients with chronic hepatitis C virus infection. *World J Gastroenterol* 2005;**11**:3357-62.
- 77 **Patel K**, Muir A, McLutichon JG, et al. A link between leptin and steatosis in chronic hepatitis C? Time to weigh up the fats. *Am J Gastroenterol* 2003;**98**:952-5.
- 78 **Tsochatzis E**, Papatheodoridis GV, Archimandritis AJ. The evolving role of leptin and adiponectin in chronic liver diseases. *Am J Gastroenterol* 2006;**101**:2629-40.
- 79 **Romero-Gomez M**, Castellano-Megias VM, Grande L, et al. Serum leptin levels correlate with hepatic steatosis in chronic hepatitis C. *Am J Gastroenterol* 2003;**98**:1135-41.
- 80 **Kitade M**, Yoshiji H, Kojima H, et al. Leptin-mediated neovascularization is a prerequisite for progression of non-alcoholic steatohepatitis in rats. *Hepatology* 2006;**44**:983-91.
- 81 **Sahai A**, Malladi P, Pan X, et al. Obese and diabetic db/db mice develop marked liver fibrosis in a model of non-alcoholic steatohepatitis: role of short-form leptin receptors and osteopontin. *Am J Physiol Gastrointest Liver Physiol* 2004;**287**:1035-43.
- 82 **Ikejima K**, Okumura K, Lang T, et al. The role of leptin in progression of non-alcoholic fatty liver disease. *Hepatol Res* 2005;**33**:151-4.
- 83 **Aleffi S**, Petrai I, Bertolani C, et al. Upregulation of proinflammatory and proangiogenic cytokines by leptin in human hepatic stellate cells. *Hepatology* 2005;**42**:1339-48.
- 84 **Potter JJ**, Womack L, Mezey E, et al. Transdifferentiation of rat hepatic stellate cells results in leptin expression. *Biochem Biophys Res Commun* 1998;**244**:178-82.
- 85 **Chalasani N**, Crabb DW, Cummings OW, et al. Does leptin play a role in the pathogenesis of human non-alcoholic steatohepatitis? *Am J Gastroenterol* 2003;**98**:2771-6.
- 86 **Giannini E**, Ceppa P, Botta F, et al. Leptin has no role in determining severity of steatosis and fibrosis in patients with chronic hepatitis C. *Am J Gastroenterol* 2000;**95**:3211-17.
- 87 **Angulo P**, Alba LM, Petrovic LM, et al. Leptin, insulin resistance, and liver fibrosis in human non-alcoholic fatty liver disease. *J Hepatol* 2004;**41**:943-9.
- 88 **Muzzi A**, Leandro G, Rubbia-Brandt L, et al. Insulin resistance is associated with liver fibrosis in non-diabetic chronic hepatitis C patients. *J Hepatol* 2005;**42**:41-6.
- 89 **Gordon A**, McLean CA, Pedersen JS, et al. Hepatic steatosis in chronic hepatitis B and C: predictors, distribution and effect on fibrosis. *J Hepatol* 2005;**43**:38-44.
- 90 **Serin E**, Ozer B, Gumurdulu Y, et al. Serum leptin level can be a negative marker of hepatocyte damage in non-alcoholic fatty liver. *J Gastroenterol* 2003;**38**:471-6.
- 91 **Rubbia-Brandt L**, Quadri R, Abid K, et al. Hepatic steatosis is a cytopathic effect of hepatitis C virus genotype 3. *J Hepatol* 2000;**33**:106-15.
- 92 **Neely D**. Liver enzymes, fatty liver and type 2 diabetes. *Ann Clin Biochem* 2005;**42**:167-9.
- 93 **Smellie WS**, Forth J, Ryder S, et al. Best practice in primary care pathology: review 5. *J Clin Pathol* 2006;**59**:1229-37.
- 94 **Galambos JT**, Wills CE. Relationship between 505 paired liver tests and biopsies in 242 obese patients. *Gastroenterology* 1978;**74**:1191-5.
- 95 **Zelber-Sagi S**, Nitzan-Kaluski D, Halpern Z, et al. Prevalence of primary non-alcoholic fatty liver disease in a population-based study and its association with biochemical and anthropometric measures. *Liver Int* 2006;**26**:856-63.
- 96 **Mofrad P**, Contos MJ, Hague M, et al. Clinical and histologic spectrum of non-alcoholic fatty liver disease associated with normal ALT values. *Hepatology* 2003;**37**:1286-92.
- 97 **Bedogni G**, Miglioli L, Masutti F, et al. Prevalence of and risk factors for non-alcoholic fatty liver disease: the Dionysos Nutrition and Liver Study. *Hepatology* 2005;**42**:44-52.
- 98 **Skelly MM**, James PD, Ryder SD. Findings on liver biopsy to investigate abnormal liver function tests in the absence of diagnostic serology. *J Hepatol* 2001;**35**:195-9.
- 99 **Saverymuttu SH**, Joseph AE, Maxwell JD. Ultrasound scanning in the detection of hepatic fibrosis and steatosis. *BMJ* 1986;**292**:13-5.
- 100 **Mathiesen UL**, Franzen LE, Aselius H, et al. Increased liver echogenicity at ultrasound examination reflects degree of steatosis but not of fibrosis in asymptomatic patients with mild/moderate abnormalities of liver transaminases. *Dig Liver Dis* 2002;**34**:516-22.
- 101 **Pinto HC**, Camilo ME. Non-alcoholic fatty liver disease/non-alcoholic steatohepatitis (NAFLD/NASH): diagnosis and clinical course. *Best Pract Res Clin Gastroenterol* 2004;**18**:1089-104.
- 102 **Hickman IJ**, Jonsson JR, Prins JB, et al. Modest weight loss and physical activity in overweight patients with chronic liver disease results in sustained improvements in alanine aminotransferase, fasting insulin, and quality of life. *Gut* 2004;**53**:43-9.
- 103 **Ueno T**, Sugawara H, Sujaku K, et al. Therapeutic effects of restricted diet and exercise in obese patients with fatty liver. *J Hepatol* 1997;**27**:103-7.
- 104 **Kugelmas M**, Hill DB, Vivian B, et al. Cytokines and NASH: a pilot study of the effects of lifestyle modification and vitamin E. *Hepatology* 2003;**38**:413-9.
- 105 **Nomura F**, Ohnishi K, Ochiai T, et al. Obesity-related non-alcoholic fatty liver: CT features and follow-up studies after low-calorie diet. *Radiology* 1987;**162**:845-7.
- 106 **Petersen KF**, Dufour S, Befroy D, et al. Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycaemia by moderate weight reduction in patients with type 2 diabetes. *Diabetes* 2005;**54**:603-8.
- 107 **American Heart Association**. Dietary guidelines for healthy American adults: a statement for physicians and health professionals by the nutrition committee. *American Heart Association Circulation* 1998;**77**:721A-724A.
- 108 **Hill D**, Sheldofsky S, McClain C, et al. Cytokines and liver disease. In: Remick D, Friedland J, eds. *Cytokines in health and disease*, 2nd edn. New York: Marcel Dekker, 1997:401-25.
- 109 **Fujikawa K**, Ohata K, Honda T, et al. Nonalcoholic steatohepatitis with improved hepatic fibrosis after weight reduction. *Intern Med* 2004;**43**:289-94.
- 110 **Brown AJ**, Tendler DA, McMurray RG, et al. Polycystic ovary syndrome and severe non-alcoholic steatohepatitis: beneficial effect of modest weight loss and exercise on liver biopsy findings. *Endocr Pract* 2005;**11**:319-24.

- 111 **Huang MA**, Greenon JK, Chao C, *et al*. One-year intense nutritional counseling results in histological improvement in patients with non-alcoholic steatohepatitis: a pilot study. *Am J Gastroenterol* 2005;**100**:1072–81.
- 112 **Stratopoulos C**, Papakonstantinou A, Terzis I, *et al*. Changes in liver histology accompanying massive weight loss after gastroplasty for morbid obesity. *Obes Surg* 2005;**15**:1154–60.
- 113 **Jaskiewicz K**, Raczynska S, Rzepko R, *et al*. Nonalcoholic fatty liver disease treated by gastroplasty. *Dig Dis Sci* 2006;**51**:21–6.
- 114 **Busetto L**, Tregnaghi A, De Marchi F, *et al*. Liver volume and visceral obesity in women with hepatic steatosis undergoing gastric banding. *Obes Res* 2002;**10**:408–11.
- 115 **Dixon JB**, Bhathal PS, Hughes NR, *et al*. Nonalcoholic fatty liver disease: improvement in liver histological analysis with weight loss. *Hepatology* 2004;**39**:1647–54.
- 116 **Clark JM**, Alkhuraishi AR, Solga SF, *et al*. Roux-en-Y gastric bypass improves liver histology in patients with non-alcoholic fatty liver disease. *Obes Res* 2005;**13**:1180–6.
- 117 **Barker KB**, Palekar NA, Bowers SP, *et al*. Non-alcoholic steatohepatitis: effect of Roux-en-Y gastric bypass surgery. *Am J Gastroenterol* 2006;**101**:368–73.
- 118 **Kral JG**, Thung SN, Biron S, *et al*. Effects of surgical treatment of the metabolic syndrome on liver fibrosis and cirrhosis. *Surgery* 2004;**135**:48–58.
- 119 **Harrison SA**, Fincke C, Helinski D, *et al*. A pilot study of orlistat treatment in obese, non-alcoholic steatohepatitis patients. *Aliment Pharmacol Ther* 2004;**20**:623–8.
- 120 **Sabunca T**, Nazliqul Y, Karaoglanoglu M, *et al*. The effects of sibutramine and orlistat on the ultrasonographic findings, insulin resistance and liver enzyme levels in obese patients with non-alcoholic steatohepatitis. *Rom J Gastroenterol* 2003;**12**:189–92.
- 121 **Daskalopoulou SS**, Mikhailidis DP, Elisaf M. Prevention and treatment of the metabolic syndrome. *Angiology* 2004;**55**:589–612.
- 122 **Clark JM**. Weight loss as a treatment for nonalcoholic fatty liver disease. *J Clin Gastroenterol* 2006;**40**:S39–43.
- 123 **Fan JG**, Zhong L, Xu ZJ, *et al*. Effects of low-calorie diet on steatohepatitis in rats with obesity and hyperlipidaemia. *World J Gastroenterol* 2003;**9**:2045–9.
- 124 **Fan JG**, Zhong L, Tia LY, *et al*. Effects of ursodeoxycholic acid and/or low-calorie diet on steatohepatitis in rats with obesity and hyperlipidaemia. *World J Gastroenterol* 2005;**11**:2346–50.
- 125 **Pessayre D**, Berson A, Fromenty B, *et al*. Mitochondria in steatohepatitis. *Semin Liver Dis* 2001;**21**:57–69.
- 126 **Capron JP**, Delamarre J, Dupas JL, *et al*. Fasting in obesity: another cause of liver injury with alcoholic hyaline? *Dig Dis Sci* 1982;**27**:265–8.
- 127 **Andersen T**, Gluud C, Franzmann MB, *et al*. Hepatic effects of dietary weight loss in morbidly obese subjects. *J Hepatol* 1991;**12**:224–9.
- 128 **Brown RG**, O'Leary JP, Woodward ER. Hepatic effects of jejunoileal bypass for morbid obesity. *Am J Surg* 1974;**127**:53–8.
- 129 **Ames FC**, Copeland EM, Leeb DC, *et al*. Liver dysfunction following small-bowel bypass for obesity. *JAMA* 1976;**235**:1249–52.
- 130 **Luyckx FH**, Desai C, Thiry A, *et al*. Liver abnormalities in severely obese subjects: effect of drastic weight loss after gastroplasty. *Int J Obes* 1998;**22**:222–6.
- 131 **Lin HZ**, Yang SQ, Chuckaree C, *et al*. Metformin reverses fatty liver disease in obese, leptin-deficient mice. *Nat Med* 2000;**6**:998–1003.
- 132 **Marchesini G**, Marzocchi R, Agostini F, *et al*. Metformin in nonalcoholic steatohepatitis. *Lancet* 2001;**358**:893–4.
- 133 **Schwimmer JB**, Middleton MS, Deutsch R, *et al*. A phase 2 clinical trial of metformin as a treatment for non-diabetic paediatric non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 2005;**21**:871–9.
- 134 **Bugianesi E**, Gentilecore E, Manini R, *et al*. A randomised controlled trial of metformin versus vitamin E or prescriptive diet in nonalcoholic fatty liver disease. *Am J Gastroenterol* 2005;**100**:1082–90.
- 135 **Reynaert H**, Geerts A, Henrion J. Review Article: The treatment of non-alcoholic steatohepatitis with thiazolidinediones. *Aliment Pharmacol Ther* 2005;**22**:897–905.
- 136 **Morita Y**, Ueno T, Sasaki N, *et al*. Nateglinide is useful for non-alcoholic steatohepatitis (NASH) patients with type 2 diabetes. *Hepatogastroenterology* 2005;**52**:1338–43.
- 137 **Igoudjil A**, Begriche K, Maisonneuve C, *et al*. Mitochondrial and metabolic disturbances induced by high dose of stavudine in mice are alleviated by coadministration of L-carnitine. Possible role of PPAR- α . *J Hepatol* 2005;**42**(Suppl):247.
- 138 **Begriche K**, Igoudjil A, Abbey-Toby A, *et al*. A thymine catabolite, β -aminoisobutyric acid, reduces hepatic dysfunction and metabolic disorders in overweight or obese mice. *J Hepatol* 2005;**42**(Suppl 2):243.
- 139 **Savage DB**, Choi CS, Samuel VT, *et al*. Reversal of diet-induced hepatic steatosis and hepatic insulin resistance by antisense oligonucleotide inhibitors of acetyl CoA carboxylases 1 and 2. *J Clin Invest* 2006;**116**:817–24.
- 140 **de Oliveira CP**, Simplicio FI, de Lima VM, *et al*. Oral administration of S-nitroso-N-acetylcysteine prevents the onset of non alcoholic fatty liver disease in rats. *World J Gastroenterol* 2006;**12**:1905–11.
- 141 **Gulbahar O**. Treatment of nonalcoholic steatohepatitis with N-acetylcysteine (abstract). *Gastroenterology* 2000;**118**:A1444.
- 142 **Koppe SWP**, Sahai A, Malladi P, *et al*. Pentoxifylline attenuates steatohepatitis induced by the methionine choline deficient diet. *J Hepatol* 2004;**41**:592–8.
- 143 **Adams LA**, Zein CO, Angulo P, *et al*. A pilot trial of pentoxifylline in non-alcoholic steatohepatitis. *Am J Gastroenterol* 2004;**99**:2365–8.
- 144 **Melov S**, Schneider JA, Day BJ, *et al*. A novel neurological phenotype in mice lacking mitochondrial manganese superoxide dismutase. *Nat Genet* 1998;**18**:159–63.
- 145 **Laurent A**, Nicco C, Tran Van Nhieu J, *et al*. Pivotal role of superoxide anion and beneficial effect of antioxidant molecules in murine steatohepatitis. *Hepatology* 2004;**39**:1277–85.
- 146 **Serviddio G**, Pereda J, Pallardo FV, *et al*. Ursodeoxycholic acid protects against secondary biliary cirrhosis in rats by preventing mitochondrial oxidative stress. *Hepatology* 2004;**39**:711–20.
- 147 **Okan A**, Astarcioglu H, Tankurt E, *et al*. Effect of ursodeoxycholic acid on hepatic steatosis in rats. *Dig Dis Sci* 2002;**47**:2389–97.
- 148 **Ersoz G**, Günsar F, Karasu Z, *et al*. Management of fatty liver disease with vitamin E and C compared to ursodeoxycholic acid treatment. *Turk J Gastroenterol* 2005;**16**:124–8.
- 149 **Vendemiale G**, Altomare E, Trizio T, *et al*. Effects of oral S-adenosyl-L-methionine on hepatic glutathione in patients with liver disease. *Scand J Gastroenterol* 1989;**24**:407–15.
- 150 **Bykov I**, Jarvelainen H, Lindros K. L-carnitine alleviates alcohol-induced liver damage in rats: role of tumour necrosis factor- α . *Alcohol Alcohol* 2003;**38**:400–6.

Access a vast information database with Toll-Free linking

“Toll-free” linking gives you immediate access to the full text of many of the cited articles in a paper’s reference list—FOR FREE. With the support of HighWire’s vast journal catalogue, a huge reference library is now open to you. If HighWire hosts the journal, you can view the full text of the referenced article, completely free of charge by following the Free Full Text links in the references.